

# Patterns and Trends in Brominated Flame Retardants in Bald Eagle Nestlings from the Upper Midwestern United States

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## Supporting Information

**ABSTRACT:** We report on patterns and trends in polybrominated diphenyl ethers (PBDEs) in the plasma of 284 bald eagle nestlings sampled between 1995 and 2011 at six study areas in the upper Midwestern United States. Geometric mean concentrations of total PBDEs ( $\Sigma$  of nine congeners) ranged from 1.78 ng/mL in the upper St. Croix River watershed to 12.0 ng/mL on the Mississippi River. Lake Superior nestlings fell between these two extremes. Between 2006 and 2011, trends differed among study areas with three declining, two remaining stable, and one increasing. Variation in  $\Sigma$ PBDE trends among study areas was linked to trends in individual congeners. The lower brominated PBDEs (BDE-47, -99, and -100) declined 4–10% while the higher brominated congeners (BDE-153 and -154) increased by about 7.0% annually from 2006 to 2011. This increase was the greatest in nestlings from the St. Croix River and below its confluence with the Mississippi River. Region-wide, our data suggest  $\Sigma$ PBDEs increased in bald eagle nestlings from 1995 through the mid-2000s and then declined by 5.5% annually from 2006 to 2011. These regional trends are consistent with the removal of penta- and octa-PBDEs from the global market.



## ■ INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) have been used since the 1970s as flame retardants in plastics, electronic circuitry, textiles, foams, and many other commercial products. Although PBDEs have reportedly saved lives from accidental fire,<sup>1</sup> there is concern over their persistence, bioaccumulation, and toxicity.<sup>1–6</sup> PBDEs have become ubiquitous in air, sediments, wildlife, and humans around the world.<sup>4,7–9</sup> Moreover, findings from studies on humans and laboratory animals show they can transfer from mother to infant,<sup>10</sup> interfere with immune<sup>2</sup> and thyroid function,<sup>6</sup> and alter human infant behavior.<sup>11</sup>

PBDEs are commercially available in three formulations: penta-, octa-, and deca-BDEs, which are themselves mixtures of 4 to 12 congeners.<sup>12</sup> Our study focuses on congeners in the penta formulation, which are particularly persistent and therefore of concern as environmental pollutants.<sup>13,14</sup> Several governments have now banned or restricted use of penta- and

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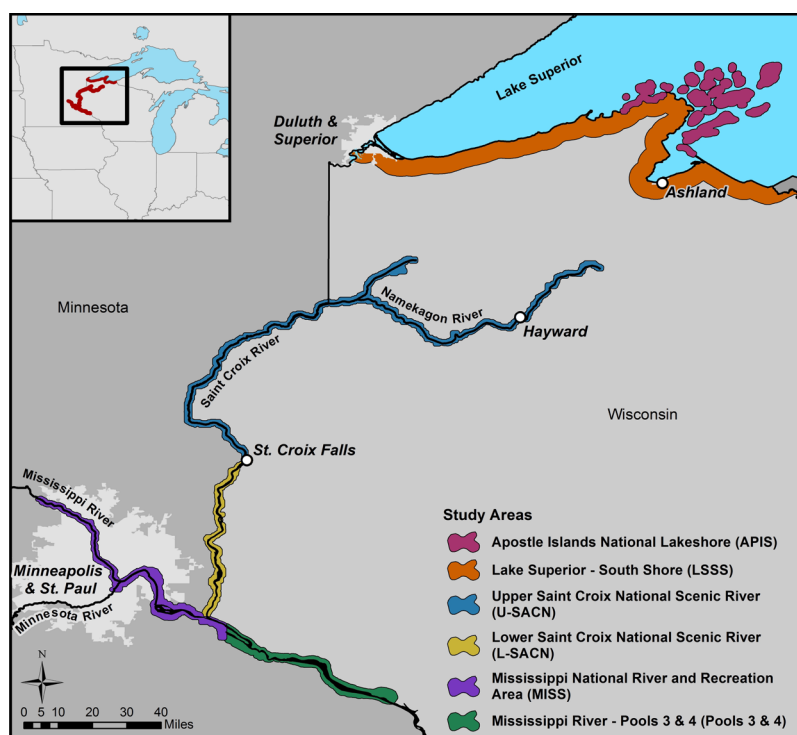


**Figure 1.** Location of six study areas in the upper Midwestern United States where bald eagle nestlings were sampled for polybrominated diphenyl ethers, 1995–2011.

octa-BDEs: the European Union banned manufacture and use in 2004,<sup>1</sup> and production was halted voluntarily in the United States in 2004 followed by restrictions on use and import in 2005.<sup>15</sup> Before these restrictions took place, about 95% of the worldwide production of penta-BDE was consumed in North America.<sup>16</sup> In the Great Lakes region, it has been estimated that the cumulative inventory of penta- and octa-BDEs in manufactured products peaked at 12 000 tons by 2004 and that most of these products will degrade and enter the waste stream by 2020.<sup>17</sup>

Numerous studies have documented the global increase of PBDE concentrations in biota, with more recent work showing some congeners have plateaued or started to decline.<sup>18</sup> For example, on the Great Lakes, penta- and octa-BDE congeners increased in rainbow smelt (*Osmerus mordax*) and lake trout (*Salvelinus namaycush*) in the early 1980s through the mid-1990s, when they began to decline,<sup>19</sup> and these same PBDE congeners plateaued in herring gull (*Larus argentatus*) eggs by 2000.<sup>20</sup> In a summary of studies across North America, Asia, and Europe, it was shown that PBDE concentrations increased dramatically in aquatic birds and marine mammals from the early 1970s until the late 1990s and early 2000s and then stabilized or declined.<sup>21</sup>

Bald eagles (*Haliaeetus leucocephalus*) are effective indicators of environmental contamination because they are high on the aquatic food web and act as integrators of human-made contaminants that enter aquatic systems through direct discharge, aerial deposition, and runoff.<sup>22–24</sup> In particular, the nestlings can reveal local contamination because they are fed from within a territory of 4 to 5 km<sup>2</sup> in size.<sup>25,26</sup> There have been several efforts to monitor environmental contaminants in bald eagle nestlings on the Great Lakes,<sup>27–30</sup> but we are aware of only two that have reported on concentrations of PBDEs. Dykstra et al.<sup>28</sup> reported for the first time on PBDE levels in

plasma from five nestlings on the Wisconsin shore of Lake Superior in 2000 and 2001. They found a geometric mean concentration of 7.9 ng/mL (95% CI = 6.0–10.4). Venier et al.<sup>31</sup> reported ΣPBDE levels in 2005 to range from 0.35 to 29.3 ng/mL (mean 5.7 ng/mL; nondetections omitted) in nestlings from 15 sites across Lakes Michigan, Huron, and Superior.

To better understand the patterns and trends in environmental contaminants, including PBDEs, the U.S. National Park Service (NPS) began monitoring contaminants in bald eagle nestlings in national parks of the upper Midwest in 2006.<sup>32</sup> This program was designed to be consistent with previous work by personnel from the Wisconsin Department of Natural Resources (WDNR) who collected blood samples from bald eagle nestlings in some of the same areas from the late 1980s until 2002.<sup>28</sup> Our objectives in this study are to report on archived samples from WDNR's earlier work and the contemporary samples from NPS to illustrate spatial patterns and temporal trends in PBDE concentrations and to examine their relationship to human land use in the region.

## MATERIALS AND METHODS

**Study Areas.** Blood samples were collected from five- to nine-week-old bald eagle nestlings at six study areas in the upper Midwestern United States (Figure 1). From 2006 through 2011, we sampled at four core study areas: the Apostle Islands National Lakeshore (APIS), the upper St. Croix National Scenic Riverway (U-SACN), the lower St. Croix National Scenic Riverway (L-SACN), and the Mississippi National River and Recreation Area (MISS). The SACN was subdivided because of differences in hydrology and land use.<sup>33,34</sup> When funding allowed, we also sampled along Wisconsin's Lake Superior South Shore (LSSS) and downstream from MISS in Pools 3 and 4 of the Mississippi River (Pools 3&4). Finally, we analyzed samples archived by WDNR

that were collected from nestlings at LSSS and APIS between 1995 and 2002.

**Sample Collection.** Procedures for collecting archived ( $n = 17$ ) and contemporary ( $n = 267$ ) samples were similar and described previously.<sup>28,32,33</sup> Briefly, we used aircraft to map all occupied bald eagle nests in each study area. From mid-May to late June each year, we climbed to nests, hand-captured the nestlings, and brought them to the ground for sampling. Nests were excluded if the tree was unsafe to climb, if the nestling(s) were too old or young to sample, or if access through private property was denied.

Each nestling was weighed (unadjusted for crop content), measured, and examined for abnormalities. Nestlings were aged using the length of the eighth primary,<sup>35</sup> and sex was determined by physical measurements (1995–2002) or by PCR-based genetic analysis<sup>36</sup> (2006–2011). We collected  $\leq 11$  mL of blood from at least one, usually all, nestlings in each nest. Blood was extracted from the brachial vein using a sterile, polypropylene syringe, transferred to a heparinized vacutainer, and placed on blue ice. Within 12 h, the sample was centrifuged, and the plasma was pipetted to a sterile glass vial and frozen at  $-20$  °C until analysis. Plasma from one nestling at each nest was selected (arbitrarily 1995–2006, randomly thereafter) for analysis of PBDEs, and the remaining samples were archived at  $-20$  °C.

Most of the 1995 to 2002 samples ( $n = 12$  of 17) were from frozen archives and had not been previously analyzed for PBDEs. Five samples collected in 2001 and 2002 were previously analyzed and reported<sup>28</sup> and are included here. With the exception of the frozen archives, all samples were analyzed within 20 months of collection. The 12 archived samples were kept frozen for up to 16 years at  $-20$  °C until analysis for this study. The reliability of frozen tissue for measuring PBDE concentrations has been demonstrated. Loss rates for BDE 47, 99, 100, and 153 were 1–2% per year in Great Lakes fish homogenates that had been frozen for 10 years, and the authors concluded that corrections were unnecessary.<sup>19</sup> Similarly, Custer et al. analyzed sample remains from 14 year-old frozen great blue heron (*Ardea herodias*) eggs for PBDEs.<sup>37</sup> They compared PCB profiles in fresh albumin with frozen remainders and found differences were insignificant, concluding the frozen archives were reliable for measuring PBDEs due to their chemical similarity with PCBs (T. Custer, unpublished data).

**Laboratory Analysis and Quantification.** Laboratory analyses for nine PBDE congeners, BDE-28, -47, -66, -85, -99, -100, -138, -153, and -154, were conducted by the Wisconsin State Laboratory of Hygiene, Madison, WI. Five (5) mL of plasma was extracted three times with a hexane/ethyl ether mix. The extract was concentrated for cleanup and fractionation using Florisil and silica-gel and then further concentrated before an internal standard was added. The sample was treated with concentrated sulfuric acid, mixed, and allowed to sit for several hours before 2  $\mu$ L of the supernatant layer was injected into an Agilent 5973 N gas chromatograph–mass spectrometer (GC-MS) operating in the negative chemical ionization mode with methane as reagent gas. The selected-ion-mode was used to monitor for  $m/z$  79 and 81 for PBDEs and  $m/z$  510 for the internal standard. The analytical column was a DB-5HT, 30 m  $\times$  0.25, I.D., and 0.10  $\mu$ m film. For quantification, we used a five point calibration curve with the target analytes and internal standard.

**Quality Assurance.** Quality assurance included matrix spikes of a known quantity of the target analyte added to bovine or chicken serum for each batch of 10 samples to assess recovery. The average recoveries of PBDE congeners were: BDE-28 (91.1%), BDE-47 (83.3%), BDE-66 (90.3%), BDE-85 (82.0%), BDE-99 (87.2%), BDE-100 (88.5%), BDE-138 (82.5%), BDE-153 (86.0%), and BDE-154 (85.9%). The relative standard deviation ranged from 10.6% to 15.7% ( $n = 20$ ). For each batch of approximately 10 samples, a method blank was also analyzed. The limits of quantification (LOQ) for PBDEs were determined by signal/noise ratio and analysis of replicate spikes, modified to account for instrument sensitivity and the presence of interferences.

**Statistical Analyses.** PBDE concentrations were log-transformed before analysis to correct for skew and differences in variance. We found 0.35% of the samples to be <LOQ (see Supporting Information, Table SII), and these were assigned a value of one-half the LOQ to be consistent with past studies in the region. Of the nine PBDE congeners we measured, four (BDE-28, -66, -85, and -138) had concentrations <LOQ in >30% of the samples. These congeners were used in  $\Sigma$ PBDE analyses but omitted from congener-specific analyses. We examined residuals against model results to assess linearity and homogeneity of variance and normal quantile-quantile plots to assess normality of residuals.

Samples collected from nestlings in the same territory in different years were not assumed to be independent because the nestlings may have had the same parents and territories change little from year to year. We used mixed effects models with years and study areas as fixed effects and territories as random effects to account for grouping observations into territories.<sup>38–40</sup> The Tukey-Kramer procedure was used for pairwise comparisons. For the five congeners with sufficient samples above the LOQ, we fitted one model that included a common trend (slope) with separate intercepts for each study area.

Regional means of log-transformed values were estimated directly from the mixed effects models to account for differences in sample size for each territory.<sup>41</sup> Finally, geometric means were computed by back-transforming the log-transformed means.

We used the minimum AIC (Akaike's Information Criterion) to select the model with the best balance between bias and precision.<sup>42</sup> We examined the 95% confidence intervals (CI) for slope estimates and concluded the trend was not significant if it included zero. Computing was carried out using SAS PROC MIXED<sup>43</sup> and R.<sup>44</sup>

## RESULTS AND DISCUSSION

We analyzed 267 plasma samples collected from bald eagle nestlings between 2006 and 2011 to assess contemporary levels of PBDEs (see Supporting Information, Table SI2, for samples/study area/year). Samples were distributed across 129 nesting territories ( $n = 1$ –6 per territory). We also analyzed archived samples from WDNR that were collected between 1995 and 2002 from 17 nestlings in two study areas to examine historical trends. Nestlings averaged 50 days old (range: 19–72 days) and weighed an average of 3.5 kg (range: 2.0–5.6 kg), and the sex ratio of sampled nestlings was not significantly different from parity (143 males/122 females,  $X^2 = 1.664$ ,  $P = 0.1970$ ).

**Contemporary  $\Sigma$ PBDE Levels.** The geometric mean  $\Sigma$ PBDE concentrations for nestling bald eagles sampled in the six study areas between 2006 and 2011 ranged from 1.78 to

**Table 1.** Geometric Means, Confidence Intervals, and Study Area Comparisons for  $\Sigma$ PBDEs Sampled in Nestling Bald Eagle Plasma, 2006–2011

| study area <sup>a</sup> | human density (km <sup>2</sup> ) <sup>b</sup> | n  | geometric mean and 95% CI (ng/mL) | comparisons |   |
|-------------------------|-----------------------------------------------|----|-----------------------------------|-------------|---|
|                         |                                               |    |                                   |             |   |
| MISS                    | 876                                           | 96 | 12.0 (10.8–13.5)                  | A           |   |
| LSSS                    | 29                                            | 11 | 10.2 (7.79–13.4)                  | A           | B |
| Pools 3&4               | 92                                            | 33 | 9.75 (8.25–11.5)                  | A           | B |
| APIS                    | 5                                             | 37 | 7.96 (6.71–9.44)                  |             | B |
| L-SACN                  | 271                                           | 48 | 7.62 (6.53–8.89)                  |             | B |
| U-SACN                  | 22                                            | 42 | 1.78 (1.53–2.07)                  |             | C |

<sup>a</sup>APIS = Apostle Islands National Lakeshore, LSSS = Lake Superior South Shore, U-SACN = upper St. Croix National Scenic Riverway, L-SACN = lower St. Croix National Scenic Riverway, MISS = Mississippi National River and Recreation Area, and Pools 3&4 = portions of pools 3 and 4 on the Mississippi River. <sup>b</sup>Human density was estimated from 2010 U.S. census data by clipping census blocks to the extent of each study area and using their respective proportions to estimate the density for the entire study areas.

12.0 ng/mL (Table 1). Venier et al.<sup>31</sup> reported a wider range of 0.35–29.3 ng/mL for 15 nestlings along the shoreline and drainages of Lakes Superior, Michigan, and Huron in 2005. McKinney et al.<sup>45</sup> reported lower levels in 26 nestlings in British Columbia in 2001–2003 (range = 0.4–8.5 ng/mL) but higher levels in three nestlings on Santa Catalina Island, CA (mean = 30.9 ng/mL).

We found  $\Sigma$ PBDE concentrations to be the highest in nestlings at MISS followed by LSSS, Pools 3&4, APIS, L-SACN, and U-SACN (Table 1, Figure 2). Nestlings on the

**Table 2.** Geometric Means and Study Area Comparisons for Individual PBDE Congeners Measured in Bald Eagle Nestling Plasma, 2006–2011

| study area | n  | geometric means (ng/mL) and area comparisons <sup>a</sup> |                     |                     |                     |                   |
|------------|----|-----------------------------------------------------------|---------------------|---------------------|---------------------|-------------------|
|            |    | BDE-47                                                    | BDE-99              | BDE-100             | BDE-153             | BDE-154           |
| APIS       | 37 | 2.86 <sup>C</sup>                                         | 1.71 <sup>A</sup>   | 1.35 <sup>A,B</sup> | 0.56 <sup>A,B</sup> | 0.75 <sup>A</sup> |
| LSSS       | 11 | 3.96 <sup>B,C</sup>                                       | 1.97 <sup>A</sup>   | 1.89 <sup>A</sup>   | 0.58 <sup>A,B</sup> | 0.72 <sup>A</sup> |
| U-SACN     | 42 | 0.55 <sup>D</sup>                                         | 0.19 <sup>C</sup>   | 0.21 <sup>C</sup>   | 0.14 <sup>C</sup>   | 0.16 <sup>C</sup> |
| L-SACN     | 48 | 4.02 <sup>B,C</sup>                                       | 0.85 <sup>B</sup>   | 0.91 <sup>B</sup>   | 0.38 <sup>B</sup>   | 0.42 <sup>B</sup> |
| MISS       | 96 | 6.58 <sup>A</sup>                                         | 1.60 <sup>A</sup>   | 1.49 <sup>A</sup>   | 0.67 <sup>A</sup>   | 0.64 <sup>A</sup> |
| Pools 3&4  | 33 | 5.44 <sup>A,B</sup>                                       | 1.23 <sup>A,B</sup> | 0.95 <sup>A,B</sup> | 0.47 <sup>A,B</sup> | 0.47 <sup>B</sup> |

<sup>a</sup>Study areas with the same capital letter are not significantly different; mixed effects models,  $P < 0.001$ : BDE-47  $F = 122.5$ , BDE-99  $F = 73.8$ , BDE-100  $F = 43.7$ , BDE-153  $F = 33.8$ , and BDE-154  $F = 52.9$ .

remote U-SACN had the lowest levels of  $\Sigma$ PBDEs, but concentrations increased 4-fold as the St. Croix River entered the L-SACN below the communities of St. Croix Falls, WI, and Taylors Falls, MN (Figure 2A). This section of river is more densely populated by humans and has more industry compared to the U-SACN. Similarly, nestlings associated with the more densely populated MISS study area, where the Mississippi River flows through Minneapolis and St. Paul, MN, had the highest geometric mean  $\Sigma$ PBDE levels. We attribute the increasing downstream pattern of PBDEs in nestlings at MISS and L-SACN (Figure 2) to a concomitant increase in PBDE-laden effluent from wastewater treatment plants (WWTP).<sup>46–48</sup> The contribution of WWTP effluent was most evident at MISS where  $\Sigma$ PBDE concentrations doubled in nestlings sampled immediately below the Minneapolis/St. Paul WWTP (Figure 2B). Henny et al.<sup>49</sup> drew similar conclusions about the

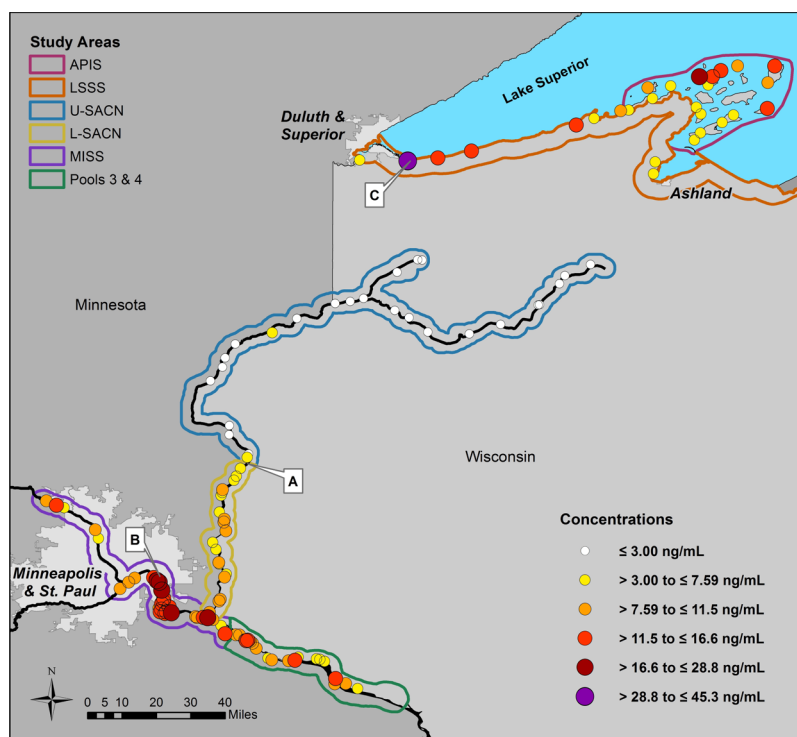


**Figure 2.** Geometric mean concentrations of  $\Sigma$ PBDE in plasma of bald eagle nestlings from 129 nesting territories sampled in six study areas in the upper Midwest, 2006–2011. The concentration categories (colored dots of increasing size) were determined using natural breaks in ArcGIS. (A) The location of the adjacent communities of St. Croix Falls, WI, and Taylors Falls, MN; (B) the location of the Minneapolis/St. Paul WWTP; and (C) the open landfill for the city of Superior, WI.

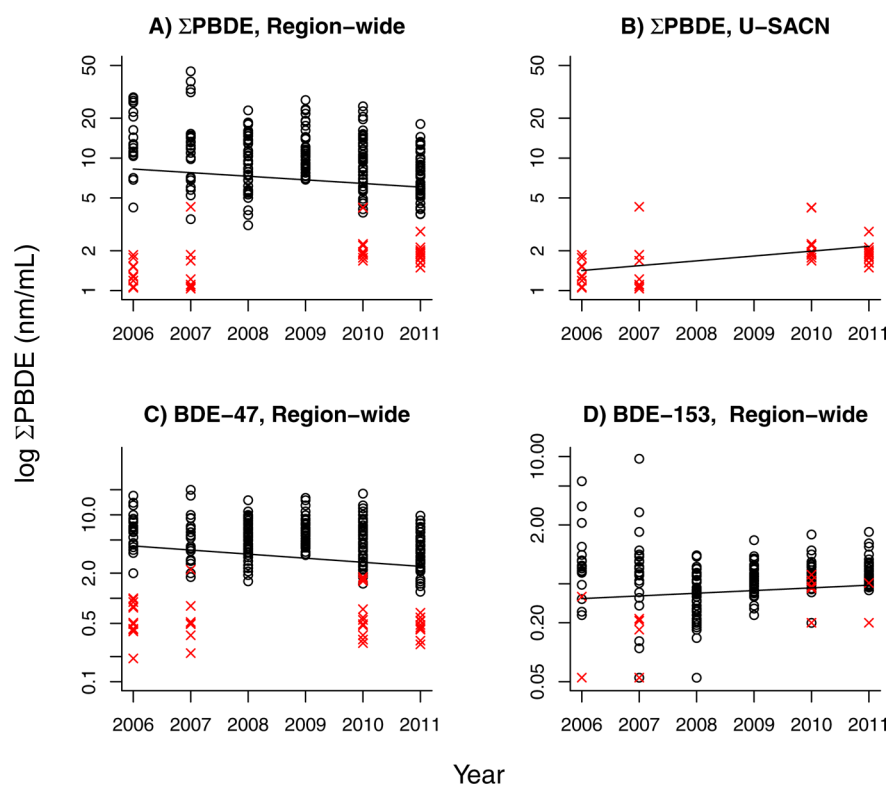


**Figure 3.** Trends in PBDEs measured in bald eagle nestling plasma, 2006–2011. The relative contribution of U-SACN samples to trends is shown as a red × in each panel. (A) 5.5% decline in ΣPBDEs across all six study areas; (B) 7.9% increase in ΣPBDEs at U-SACN; (C) 10.1% decline of BDE-47 across all six study areas; (D) 6.69% increase in BDE-153 across all six study areas.

**Table 3.** Trends in Concentrations of Individual PBDE Congeners Measured in Bald Eagle Nestling Plasma, 2006–2011

| study area <sup>b</sup> | congener trend <sup>a</sup> |        |         |         |         |
|-------------------------|-----------------------------|--------|---------|---------|---------|
|                         | BDE-47                      | BDE-99 | BDE-100 | BDE-153 | BDE-154 |
| APIS                    | -                           | -      | -       | -       | -       |
| LSSS                    | -                           | -      | -       | -       | -       |
| U-SACN                  | -                           | +++    | ++      | +++++   | +++++   |
| L-SACN                  | -                           | -      | -       | +++     | ++      |
| MISS                    | -                           | -      | -       | -       | +       |
| Pools 3&4               | -                           | -      | +++     | +++++   | +++++   |

<sup>a</sup>Each + or - symbol signifies a 0% to 10% increase or decrease in concentrations, respectively. For example, BDE-47 declined by 10% to 20% at APIS and MISS (- -), 20% to 30% at LSSS (- - -), and only 0% to 10% (-) at U-SACN, L-SACN, and Pools 3&4. <sup>b</sup>Sample size for each study area is shown in Table 2.

influence of WWTPs, showing that mean ΣPBDE levels increased in osprey (*Pandion haliaetus*) eggs in direct relation to the volume of WWTP discharge to tributaries of the Columbia River. This association with high human population centers was also shown in osprey eggs from the Chesapeake Bay area,<sup>21</sup> peregrine falcon (*Falco peregrinus*) eggs in California, and peregrine falcon eggs in northeastern and mid-Atlantic states.<sup>50–53</sup>

Concentrations of ΣPBDEs were moderately high in nestlings at APIS and LSSS despite the fact that eagles nested on relatively remote shorelines and islands (Table 1; Figure 2). Concentrations of PBDEs in Lake Superior nestlings are likely influenced by the lake's large (127 700 km<sup>2</sup>) drainage area,

which accumulates wastewater from many communities located along the shore and up its tributaries. The importance of WWTPs and tributaries was illustrated by Melymuk et al.<sup>47</sup> who found that WWTPs and tributaries near Toronto, Canada, contributed about equally to the PBDE loadings to Lake Ontario and that their combined contribution was 90% of total loadings, far exceeding air deposition. We suggest that PBDE levels in Lake Superior nestlings are further influenced by the lake's cold water (mean = 3.9 °C) and long residence time (191 years), which results in slow decomposition of contaminants with ample time for bioconcentration compared to the continual flushing of river systems.

The PBDE distribution patterns in bald eagles can be complicated, however, by the location of individual nesting sites and the type of prey eagles consume. For example, the bald eagle territory where we found the highest ΣPBDE concentration (45.3 ng/mL) was 1.1 km from the landfill for the city of Superior, WI (Figure 2C). This open landfill contains PBDE-laden products, and gulls (*Larus spp*) were observed feeding among the refuse. Adult eagles were also observed pursuing gulls at this landfill, and gull remains were found at the sampled eagle nest. Moreover, gulls are known to accumulate PBDEs,<sup>20</sup> which can be further magnified when eagles feed on the contaminated gulls. We cannot show conclusively that this landfill caused the high PBDE levels at this nest; however, the importance of landfills as a source of PBDEs in the food web has been demonstrated. Eggs of European starlings (*Sturnus vulgaris*) sampled near landfills had significantly higher PBDE concentrations compared to eggs from urban and industrial sites across Canada.<sup>48</sup>

**Congener Levels, 2006–2011.** Five congeners were detected above the laboratories' LOQ in >90% of the samples

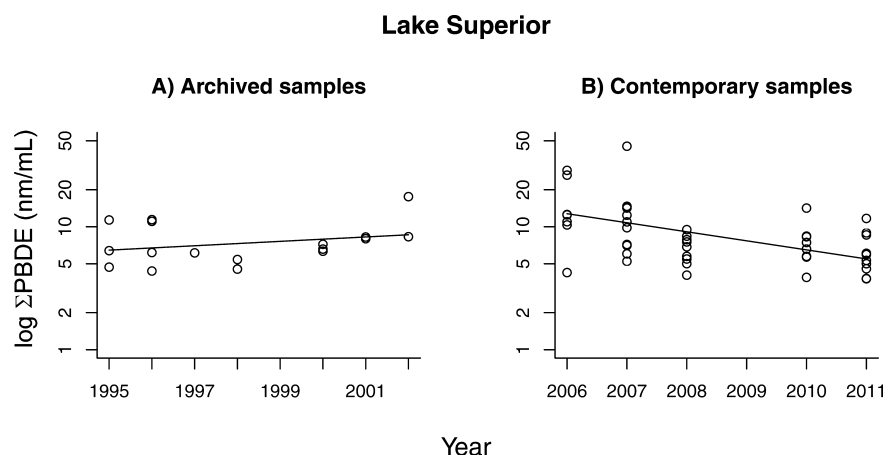


**Figure 4.** Trends in ΣPBDE concentrations in bald eagle nestlings sampled at two study areas on Lake Superior (APIS and LSSS) from 1995 to 2011. (A) Scatter plot and nonsignificant trend (solid line) for archived samples ( $n = 17$ ). (B) Scatter plot and significant decline (solid line) detected from contemporary samples ( $n = 65$ ).

and were used for congener-specific analyses. BDE-47 made up the greatest percentage of the ΣPBDE burden (49.9%), followed by BDE-99 (16.0%), BDE-100 (14.6%), BDE-153 (5.9%), and BDE-154 (6.7%). Mean concentrations for each congener differed significantly among study areas (Table 2). BDE-47 was the highest at MISS, although levels at Pools 3&4 were statistically indistinguishable. LSSS had the highest concentrations of BDE-99 and -100 but did not differ significantly from APIS, MISS, and Pools 3&4. Concentrations of BDE-153 and -154 were low overall but reached their highest levels at LSSS, APIS, and MISS, which were statistically similar. U-SACN had the lowest concentrations of all five congeners.

Overall, congeners BDE-47, -99, and -100 made up 80% of the ΣPBDE burden, which is consistent with other studies on eagles and piscivorous raptors. Venier et al.<sup>31</sup> found that BDE-47, -99, and -100 made up 32%, 20%, and 16% of the PBDE burden in plasma from bald eagle nestlings across three of the Great Lakes. In coastal Pacific Northwest, BDE-47 accounted for approximately half of the ΣPBDE burden in bald eagle nestling plasma, with BDE-99 and -100 the next-most dominant.<sup>45</sup>

**Temporal Trends.** For samples collected between 2006 and 2011, we estimated that ΣPBDE concentrations in nestlings declined across the six study areas by 5.5% annually (Figure 3A; 95% CI = −8.3% to −2.6%). The concentrations declined more steeply at three study areas (not shown): LSSS by 19.6% annually (95% CI = −24.6 to −14.4), APIS by 13.7% (95% CI = −19.1 to −8.1), and MISS declined by 10% (95% CI = −15.5 to −4.0). We did not detect significant trends at L-SACN (95% CI = −10.8 to 1.3) and Pools 3&4 (95% CI = −3.5 to 9.6), though the Pools 3&4 study area was sampled intensively only in 2008 and 2009 (Supporting Information, Table SI2). We found that ΣPBDEs increased by 7.9% annually at U-SACN (Figure 3B; 95% CI = 1.3 to 15.0).

Differences in ΣPBDE trends among the six study areas were linked to trends in individual congeners (Figure 3C, D). Region-wide from 2006 to 2011, the three most abundant congeners declined significantly: BDE-47 by 10.1% annually (Figure 3C; 95% CI = −12.5 to −7.1), BDE-100 by 5.53% (95% CI = −9.97 to −0.88), and BDE-99 by 3.97% (95% CI = −7.8 to −0.01). These congeners largely drove the region-wide decline in ΣPBDEs. In contrast, the higher brominated congeners, BDE-153 and -154, increased: BDE-153 by 6.69%

(Figure 3D; 95% CI = 1.93–11.7), and BDE-154 by 7% (95% CI = 3.52–10.6). The increase in these two congeners contributed to the mixed results we obtained for L-SACN and Pools 3&4 and drove the increase we observed in ΣPBDEs at U-SACN.

Three patterns were evident for the five congeners we analyzed (Table 3). First, the lower brominated congeners (BDE-47, -99, and -100) declined significantly on Lake Superior (APIS and LSSS) and at MISS. Second, the higher brominated congeners (BDE-153 and -154) increased in nestlings from U-SACN, and this trend continued downstream through L-SACN and on to the confluence with Pools 3&4 on the Mississippi River (Table 3; Figure 2). Third, concentrations of all congeners declined in the two Lake Superior study areas, while all congeners except BDE-47 increased on the U-SACN.

We attribute the overall decline in the lower brominated congeners to declines in worldwide production and use of penta- and octa-PBDE formulations. The increases we observed in the higher brominated congeners are more difficult to explain. Other investigators have argued that industry switching to deca-BDEs could be creating a reservoir of higher brominated PBDEs.<sup>54</sup> Moreover, BDE-209, the major component of deca-BDE, can be metabolized by biota to form BDE-154 and -153.<sup>14</sup> Although debromination of deca-BDEs may be occurring, it does not satisfactorily explain why the increases we observed in BDE-153 and -154 were only in nestlings from the St. Croix River drainage (U-SACN and L-SACN) and immediately downriver at Pools 3&4 of the Mississippi River. Potential reasons include differences in PBDE source (e.g., industries using different formulations), differences in trophic status of available prey, and differences in rates of debromination (for example, different species of fish metabolize PBDEs at different rates).<sup>14</sup>

Archived samples from 1995 to 2002 were few ( $n = 17$ ) and limited to APIS and LSSS. As a consequence, we were unable to detect a significant trend in ΣPBDE for this time period (annual change using pooled data = 4.2%, 95% CI = −3.1 to 12.0). Nonetheless, the predicted trend for 1995–2002 combined with the 2006–2011 data (Figure 4) agrees with other studies on a variety of environmental media from the Great Lakes.<sup>12</sup> For example, concentrations of PBDEs in herring gull eggs from the Great Lakes doubled approximately every 5.8 years between 1981 and 2000<sup>55</sup> and then stabilized

through 2006.<sup>56</sup> Concentrations of PBDEs in fish from the Great Lakes rose from the late 1970s through the late 1990s to the early 2000s before beginning to decline.<sup>57,58</sup>

To summarize, we found PBDEs were pervasive in bald eagle nestlings across the upper Midwest but that levels varied widely. Nestlings sampled near large population centers tended to be more contaminated with PBDEs compared to more remote areas, and our data support a growing number of studies that show effluent from wastewater treatment plants is a major source. We also found evidence that landfills can be a source of PBDEs to eagles and argue that the physical and biological characteristics of aquatic systems (e.g., Great Lakes versus flowing rivers) influence the availability of PBDEs to eagles. We found that the lower PBDE congeners declined in most study areas between 2006 and 2011, while the higher brominated congeners increased in the St. Croix River watershed and a stretch of the Mississippi River below their confluence. These higher brominated congeners made up a small portion of the overall PBDE burden in bald eagle nestlings, but the apparent increase in the three connected study areas warrants further investigation.

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Three tables with information on the number of samples that met laboratory limits of quantification; annual sample size for each study area; and congener-specific measurements of central tendency and variability. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

Any use of trade, product, or firm names are for descriptive purposes and do not imply endorsement by the authors or their affiliations.

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