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# Flame retardants and organochlorine pollutants in bald eagle plasma from the Great Lakes region

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ABSTRACT

We report measurements of polybrominated diphenyl ethers and of emerging flame retardants in the plasma of nestling bald eagles sampled from early May to late June of 2005. Concentrations of total PBDEs ranged from 0.35 ng $g^{-1}$ ww to 29.3 ng $g^{-1}$ ww (average = 5.7 ± 1.9 ng $g^{-1}$ ww). The most abundant congeners were BDE-47, BDE-99, and BDE-100. The fully brominated congener, BDE-209, was detected in approximately one third of the samples at an average concentration of 1.2 ± 0.72 ng $g^{-1}$ ww. Several emerging flame retardants, such as pentabromoethylbenzene (PBEB), hexabromocyclododecanes (HBCDs), and Dechlorane Plus (DP), were detected in these samples. Polychlorinated biphenyls (PCBs) and organochlorine pesticides were also detected at levels close to those previously published. A statistically significant relationship was found between total PBDE concentrations and total PCB and *p,p′*-DDE concentrations, suggesting that these compounds share a common source, which is most likely the eagle's food.



## 1. Introduction

It came as a shock to the American public when bald eagles, one of the most significant symbols of the United States, were found to be contaminated with persistent organic pollutants (POPs). As a result, bald eagle populations were threatened, and they were listed as an endangered species in the late 1970s in most of the United States and in Canada. Luckily, thanks to legislative efforts to control and reduce persistent pollutants, to protect and restore habitat, and to eliminate harassment of the eagles and their eggs, bald eagle populations have started to recover. Nevertheless, in some regions, including the Great Lakes, this recovery has not been as successful as hoped, and these populations are still experiencing poor reproductive success and poor juvenile and adult survival rates (Elliott and Harris, 2001/2002).

Bald eagles are indigenous to the Great Lakes, and they are top predators of the Great Lakes food web. They feed on fishes, waterfowl, and small mammals, depending on season, location, availability, competition, and other variables. Their position in the food web makes bald eagles susceptible to accumulating high concentrations of environmental contaminants. In fact, bald eagles are excellent biosentinels of Great Lakes water quality, particularly for bioaccumulative halogenated compounds, which are delivered to the Great Lakes largely through atmospheric deposition (Bowerman et al., 2002).

Historically, monitoring programs using eagles and other raptors as sentinels have focused on the bird's eggs as the media for assessing contaminant levels and trends. Despite some obvious advantages, such as ease of collection and proximity between the target chemical and the developing embryo, egg sampling also has some drawbacks. These include the destructive nature of the sampling technique and a high level of nest disturbance that significantly increases the frequency of nest abandonment (Strause et al., 2007). Conversely, using plasma as the sampling medium allows collecting blood without destroying the individual, collecting samples from the same nest over time, and collecting samples from nestlings. This last aspect is especially relevant when trying to use birds as sentinels for a specific area, since nestlings are sedentary and their accumulation of toxic pollutants results mainly from parentally transferred food.

Brominated flame retardants (BFRs) are a broad category of chemicals that include polybrominated biphenyls (PBBs), hexabromocyclododecanes (HBCDs), and polybrominated diphenyl ethers (PBDEs). Flame retardants are added to numerous commercial products to reduce their flammability, and their usage has increased rapidly since the 1980s, probably as a result of more stringent fire safety regulations. As a result of this heavy usage, PBDEs are ubiquitous in the environment, having been detected in air, sediments, biota, and people (Hites, 2004).

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Bowing to environmentalist's pressure, in 2004, two commercial PBDEs mixtures were voluntarily withdrawn from the United States' market (Penta-BDE and Octa-BDE). The other widely used commercial mixture, Deca-BDE, was until recently largely unregulated in the United States and Canada, but it too is being withdrawn from the market, in this case, by the end of 2013 (Hess, 2010). In addition, flame retardant producers have been replacing banned or retired products with unregulated compounds. For example, 1,2-*bis*(2,4,6-tribromophenoxy)ethane (TBE) was reintroduced as a substitute for Octa-BDE; similarly, decabromodiphenylethane (DBDPE) was marketed as an alternative to BDE-209. The persistence of the older brominated flame retardants, together with the market shift towards unregulated compounds, requires a continuous monitoring effort to keep track of their levels in the environment.

The exposure of predatory birds to contaminants such as 2,2-*bis*(4-chlorophenyl)-1,1-dichloroethene (DDE), chlorinated pesticides, and polychlorinated biphenyls (PCBs), caused eggshell thinning, reproductive and developmental challenges, and eventually mortality (Elliott and Harris, 2001/2002). PBDEs and several other halogenated flame retardants are equally persistent and bioaccumulative, and although their toxicity is not completely understood, exposure of rodents to PBDEs has been associated with altered neural development, abnormal endocrine and liver functions, and reproductive failures (Birnbaum and Staskal, 2004). It has been recently reported that decreased plasma thyroxine (T4) and vitamin A levels were observed in American kestrels after a dose of a PBDE mixture *in ovo* and post-hatch (Fernie et al., 2006).

The potential toxicological effects of PBDEs, as well as their ubiquity in the environment, suggests that the concentrations of these compounds should be measured regularly in bald eagles, a species that has already proven to be particularly susceptible to the effects of persistent organic pollutants. This need is supported by a recent study from Dykstra et al. (2005), who showed that the reproductive rate of Lake Superior bald eagles did not increase through the 1990s, despite a general decrease of both DDE and PCB concentrations in biota. This finding may indicate that ecological factors were hindering the bird's productivity, but more importantly, this finding may indicate that other contaminants (i.e. flame retardants) have emerged as possible threats.

In this paper, we report concentrations of PBDEs and emerging brominated and chlorinated flame retardants in the plasma of nestling bald eagles from the Great Lakes region, together with some PCB and pesticide concentrations for context.

## 2. Materials and methods

### 2.1. Sample collection

In this preliminary survey, 15 samples were collected within the Great Lakes watershed in 2005. The sampling locations are given in Fig. 1; the samples were spatially distributed to cover as much area as possible. Since the samples were collected as part of the Michigan Bald Eagle Biosentinel Program, only nests located within the state of Michigan were sampled. Specifically, three samples were collected near the southern shore of Lake Superior; six samples were collected in Lake's Michigan watershed, covering mostly the eastern shore; and six samples were collected on the Michigan side of Lake Huron.

Details of the sampling procedures can be found elsewhere, and only a brief description is given here (Wierda, 2009). Nestling eagles were captured, restrained, processed, and returned to the nest individually. Blood was collected from the brachial vein, and morphological measurements of culmen, hallux claw, and bill depth were collected using a caliper. The length of the eighth primary feather and the spread of the footpad were measured with a ruler. From these data, the sex and the estimated age of the nestling were determined (Wierda, 2009). Nestlings were also weighed.

### 2.2. Materials

Standards for the most common legacy pesticides (25 different compounds including DDTs, endosulfans, chlordanes, HCHs, methoxychlor, and endrin) and PCBs (congeners 8, 18, 28, 44, 52, 66, 77, 101, 105, 110, 118, 128, 138, 153, 156, 170, 180, 187, 195, 206 and 209) were purchased at AccuStandard and Ultra Scientific (Wierda, 2009).

A PBDE standard mixture (BFR-PAR) was purchased from Wellington Laboratories (Guelph, ON). This solution contained the following PBDE congeners: 1, 3, 7, 10, 15, 17, 28, 30, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 139, 140, 153, 154, 156, 169, 171, 181, 183, 184, 191, 196, 197, 201, and 203 to 209. Other compounds included in this standard mixture were: hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 2,2′,4,4′,5,5′-hexabromobiphenyl (BB-153), 1,2-*bis*(2,4,6-tribromophenoxy)ethane (TBE), and decabromodiphenylethane (DBDPE). Hexabromocyclododecane (α-HBCD) from AccuStandard, Dechlorane Plus (DP) from OxyChem, BDE-118 from AccuStandard (New Haven, CT), and $^{13}C_{12}$-BDE-209 from Wellington Laboratories were added individually to the calibration standard.

### 2.3. Analytical procedures

The protocol for extraction, cleanup, and analysis of the pesticides and PCBs is described elsewhere and only a limited description will be provided here (Bowerman et al., 1995). Approximately 1 mL of plasma was weighed, denaturated with 0.5 mL of methanol, extracted with 5 mL of dichloromethane three times, and purified using alumina and silica solid phase extraction. The extracts were then analyzed by dual column gas chromatography with electron capture detection. The internal standard method was used for quantitation with 1-bromo-2-nitrobenzene and PCB-198 as the internal standards.

For the brominated flame retardants, 2.5–5 mL of plasma were weighed, spiked with surrogate standards (BDE-77, BDE-166, and $^{13}C_{12}$-BDE-209), denaturated with 2 mL of HCl and 6 mL of 2-propanol, and extracted with (1:1) hexane/methyl *t*-butyl ether. Lipids were removed using sulfuric acid. The extracts were cleaned on an alumina column capped with anhydrous sodium sulfate using hexane and (4:6) hexane/dichloromethane as eluents. Two procedural blanks were included in every batch of six samples.

The samples were analyzed for the flame retardants on an Agilent 6890 series gas chromatograph coupled to an Agilent 5973 mass spectrometer. The 2 μL injections were made in the pulse splitless mode, with a purge time of 2.0 min. The injection port was held at 285 °C. An Rtx-1614, 15-m long × 250 μm i.d., 0.10-μm phase thickness, fused silica capillary GC column (Restek Corporation, Bellefonte, CA) was used for determination of all the congeners. The GC oven temperature program was as follows: isothermal at 100 °C for 2 min, 25 °C min$^{-1}$ to 250 °C, 3 °C min$^{-1}$ to 270 °C, 25 °C min$^{-1}$ to 325 °C, and held at 325 °C for 11 min. The GC to MS transfer line was held at 280 °C. The mass spectrometer was operated in the electron capture negative ionization mode with selected ion monitoring. Details on the monitoring ions are reported elsewhere (Venier and Hites, 2008). The internal standard method was used for quantitation with BDE-118 as the internal standard.

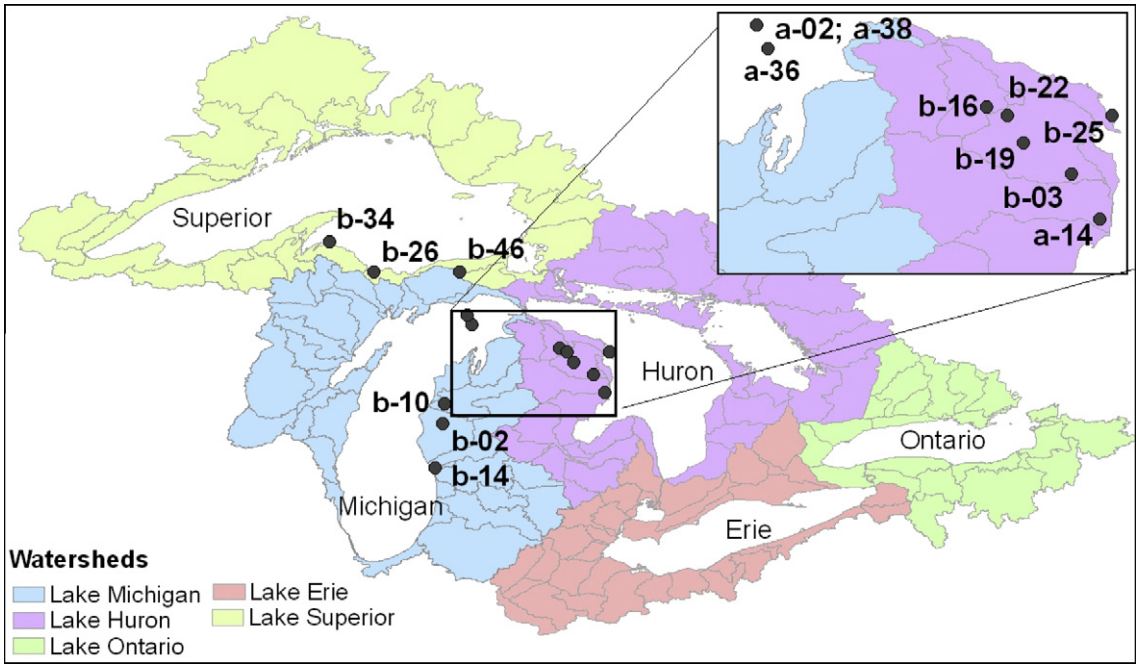


**Fig. 1.** Map showing the location of bald eagle samples.

### 2.4. Quality control

To ensure a correct identification of target compounds, the following criteria were used: (a) the GC retention times matched those of the standard compounds within ±0.1 min; (b) the signal-to-noise ratio was greater than 3:1; and (c) the deviation of the ion intensity ratios was within 20% of the mean values of the calibration standards. Recoveries of the surrogate standards were in the range 40–120%. Samples were not corrected for recoveries. Concentrations of congeners in the blank samples (BDE-47, -99, and -100) were low enough so that it was not necessary to correct the concentration in the samples.

## 3. Results and discussion

The complete data set, including the concentrations (in ng $g^{-1}$ ww) of PBDEs, other flame retardants, selected organochlorine pesticides, and PCBs is given in Table 1.

### 3.1. Polybrominated diphenyl ethers (PBDEs)

As it can be seen from Table 1, the concentration of total PBDEs ranged from 0.35 ng $g^{-1}$ ww to 29.3 ng $g^{-1}$ ww (wet weight), with an average of 5.7 ± 1.9 ng $g^{-1}$ ww. These values are within the range of those recently reported by McKinney et al., who measured PBDEs in nestling bald eagle plasma from British Columbia (range: 0.40–8.5 ng $g^{-1}$ ww) (McKinney et al., 2006). Similarly, an average concentration of 7.9 ng $g^{-1}$ ww (range: 6.0–10.4 ng $g^{-1}$ ww) was reported for PBDEs in bald eagle plasma from Lake Superior (Dykstra et al., 2005). The sum of total PBDEs ranged between 2.49 and 54.4 ng $g^{-1}$ ww in the plasma of Norwegian Arctic herring gulls (Verreault et al., 2007). Bustnes et al. (2008) detected only BDE-47 in the blood of lesser black-backed gulls from northern Norway at a mean concentration of 1.87 ± 0.26 ng $g^{-1}$ ww, which is close to our findings (1.33 ± 0.34 ng $g^{-1}$ ww).

Fig. 2 shows the congener distributions of the PBDEs measured in these bald eagle samples. Congeners 47, 99, and 100 represented, on average, 32%, 20% and 16%, respectively, of the total PBDE levels, and the sum of these three congener concentrations contributed, on average, 67% of the total PBDE concentration measured in these samples. Given its high molecular weight, BDE-209 was expected to have a low bioavailability and to undergo relatively rapid *in vivo* degradation to less brominated compounds (Stapleton et al., 2006). Nevertheless BDE-209 was present in four of these samples at an average concentration of 1.2 ± 0.72 ng $g^{-1}$ ww. BDE-209 represents more than 97% of the Deca-BDE commercial product, which is now one of the most highly used commercial BFR products in North America (Birnbaum and Staskal, 2004). In two extensive studies on Norwegian Arctic glaucus gulls, this congener was reported in only samples collected in 2002 and 2004, at levels ranging between 0.05 and 0.33 ng $g^{-1}$ ww, but it was not in gulls collected in 2006 (Verreault et al., 2005, 2007). BDE-209 was not detected in bald eagle plasma from British Columbia or from California (McKinney et al., 2006). The inconsistency in the detection of BDE-209 could be associated with analytical challenges in the monitoring of this compound.

Other lower brominated congeners that were consistently detected in these samples, although at lower levels, include BDE-28, 49, 66, 85, 153, and 154. For example, BDE-153 was found in all samples at an average level of 0.59 ± 0.31 ng $g^{-1}$ ww. In addition, several hepta- through nona-BDEs (BDE-183, 196, 197, 201, 205, 207, and 208) could be identified and quantitated in several samples. The most abundant heptabrominated congener was BDE-183, which was found in 11 of these samples at an average level of 0.10 ± 0.07 ng $g^{-1}$ ww. BDE-197 was the most abundant of the octabrominated congeners at an average concentration of 0.23 ± 0.14 ng $g^{-1}$ ww ($n$ = 5). Three other hepta- and octa-congeners (BDE-179, 182, and 202) were indentified and confirmed in few samples by comparison with the pure standard. Two of the nona-BDE congeners (207 and 208) were found in a limited number of samples at average levels of 0.29 ± 0.19 and 0.11 ± 0.06 ng $g^{-1}$ ww, respectively. It is known that many of the hepta- to nonabrominated congeners can be formed through metabolic debromination of BDE-209 in several species, including fish and birds (Stapleton et al., 2006; van den Steen et al., 2007). Van den Steen and colleagues also demonstrated that the debromination of BDE-209 may also produce highly bioaccumulative

**Table 1**
Sampling locations and concentration of PBDEs, other flame retardants, PCBs, and pesticides in bald eagles plasma (ng g$^{-1}$ ww). The averages and medians were calculated omitting the non-detects. The following compounds, although included in the analytical methodology, were not detected in any of the samples: HBB, TBE, and DBDPE.

| | a-02 Mich | a-14 Hur | a-36 Mich | a-38 Mich | b-02 Mich | b-03 Hur | b-10 Mich | b-14 Mich | b-16 Hur | b-19 Hur | b-22 Hur | b-25 Hur | b-26 Sup | b-34 Sup | b-46 Sup | Average ± Std. err. | Median |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *PBDEs* | | | | | | | | | | | | | | | | | |
| BDE-17 | 0.01 | 0.01 | ND | ND | ND | ND | 0.02 | 0.01 | ND | ND | ND | 0.01 | ND | 0.01 | ND | 0.01 ± 0.002 | 0.01 |
| BDE-28 | 0.05 | 0.05 | 0.03 | 0.02 | 0.05 | 0.01 | 0.20 | 0.06 | 0.01 | ND | 0.04 | 0.09 | 0.05 | 0.07 | ND | 0.06 ± 0.01 | 0.05 |
| BDE-47 | 0.58 | 1.30 | 0.78 | 0.77 | 2.73 | 0.46 | 5.21 | 0.92 | 0.55 | 0.24 | 0.71 | 0.97 | 2.13 | 2.47 | 0.13 | 1.3 ± 0.3 | 0.78 |
| BDE-99 | 1.24 | 0.38 | 0.47 | 0.28 | 1.06 | 0.57 | 1.55 | 0.27 | 0.47 | 0.20 | 0.29 | 2.99 | 0.73 | 6.96 | 0.06 | 1.2 ± 0.5 | 0.40 |
| BDE-100 | 0.70 | 0.52 | 0.40 | 0.40 | 1.07 | 0.17 | 2.71 | 0.36 | 0.28 | 0.17 | 0.22 | 1.26 | 0.69 | 2.55 | 0.07 | 0.77 ± 0.21 | 0.47 |
| BDE-153 | 0.72 | 0.11 | 0.21 | 0.13 | 0.31 | 0.10 | 0.52 | 0.08 | 0.15 | 0.08 | 0.07 | 1.42 | 0.13 | 4.72 | 0.03 | 0.59 ± 0.31 | 0.13 |
| BDE-154* | 1.68 | 0.42 | 0.33 | 0.22 | 0.57 | 0.10 | 1.35 | 0.11 | 0.19 | 0.08 | 0.15 | 1.23 | 0.37 | 3.19 | 0.04 | 0.67 ± 0.22 | 0.33 |
| BDE-183 | 0.05 | 0.01 | 0.04 | 0.01 | 0.01 | ND | 0.02 | ND | 0.01 | ND | 0.02 | 0.20 | 0.01 | 0.77 | ND | 0.10 ± 0.07 | 0.02 |
| BDE-197 | 0.32 | ND | 0.03 | ND | ND | ND | ND | 0.05 | ND | ND | ND | 0.74 | ND | 0.01 | ND | 0.23 ± 0.14 | 0.05 |
| BDE-207 | 0.01 | ND | 0.04 | ND | ND | ND | ND | ND | 0.01 | ND | ND | 0.41 | ND | 0.99 | ND | 0.29 ± 0.16 | 0.04 |
| BDE-208 | ND | ND | 0.01 | ND | ND | ND | ND | ND | ND | ND | ND | 0.11 | ND | 0.22 | ND | 0.11 ± 0.06 | 0.11 |
| BDE-209 | ND | ND | 0.11 | ND | ND | ND | ND | ND | 0.04 | ND | ND | 1.37 | ND | 3.13 | ND | 1.2 ± 0.7 | 0.74 |
| ΣPBDEs | 5.51 | 3.03 | 2.65 | 1.91 | 6.35 | 1.52 | 12.6 | 2.35 | 1.86 | 0.80 | 1.72 | 11.6 | 4.40 | 29.3 | 0.35 | 5.7 ± 1.9 | 2.7 |
| *Other flame retardants* | | | | | | | | | | | | | | | | | |
| PBEB | 0.10 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.12 | ND | ND | ND | 0.11 ± 0.01 | 0.11 |
| ΣHBCD | ND | 0.03 | ND | ND | ND | ND | 0.05 | ND | 0.02 | ND | 0.05 | 0.14 | 0.05 | 0.56 | ND | 0.13 ± 0.07 | 0.05 |
| Syn-DP | 0.01 | 0.01 | 0.23 | 0.01 | ND | ND | ND | ND | ND | ND | ND | 0.05 | ND | 0.07 | ND | 0.06 ± 0.03 | 0.03 |
| Anti-DP | 0.01 | 0.01 | 0.45 | 0.01 | ND | ND | ND | ND | ND | ND | ND | 0.11 | ND | 0.15 | ND | 0.12 ± 0.07 | 0.06 |
| Total DP | 0.02 | 0.02 | 0.68 | 0.02 | ND | ND | ND | ND | ND | ND | ND | 0.16 | ND | 0.22 | ND | 0.19 ± 0.10 | 0.09 |
| *Pesticides and PCBs* | | | | | | | | | | | | | | | | | |
| ΣPCBs | 129 | 62.9 | 41.5 | 22.6 | 52.8 | ND | 33.4 | 31.2 | 5.46 | ND | 15.5 | 164 | ND | 254 | ND | 73.8 ± 23.2 | 41.5 |
| *p,p′*-DDE | 19.4 | 20.3 | 20.2 | 13.6 | 18.8 | 2.85 | 17.3 | 10.8 | 5.45 | ND | 5.35 | 41.7 | ND | 62.6 | ND | 19.9 ± 4.9 | 18.1 |
| ΣDDT | 29.3 | 27.8 | 20.2 | 13.6 | 24.1 | 2.85 | 18.3 | 18.2 | 5.45 | ND | 5.35 | 52.9 | ND | 73.1 | ND | 24.3 ± 5.9 | 19.3 |
| α-Chlordane | 4.88 | ND | ND | 4.69 | ND | ND | 4.47 | ND | ND | ND | 10.0 | ND | 9.85 | ND | | 6.8 ± 1.1 | 5.9 |
| γ-Chlordane | ND | ND | ND | 4.27 | ND | ND | 4.84 | ND | ND | ND | 5.41 | ND | ND | ND | | 4.8 ± 0.3 | 4.8 |
| Dieldrin | 4.38 | 4.77 | 4.39 | ND | 2.76 | ND | ND | 2.37 | ND | ND | ND | 4.97 | ND | 7.27 | ND | 4.4 ± 0.6 | 4.4 |
| HCB | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 3.42 | ND | ND | 2.51 | ND | 3.0 ± 0.5 | 3.0 |
| Heptachlor epoxide | ND | ND | ND | ND | ND | ND | ND | 2.65 | ND | ND | ND | 2.98 | ND | 3.64 | ND | 3.1 ± 0.3 | 3.0 |

* Includes PBB-153.

hexa-BDEs (i.e. BDE-138, 153, 154), which were also detected in these eagle samples. Overall, the congener patterns we observe suggest that BDE-209 could be degraded *in vivo* to several less brominated congeners. However, from our data, we cannot rule out that some or all of these congeners may be coming from the eagle's food.

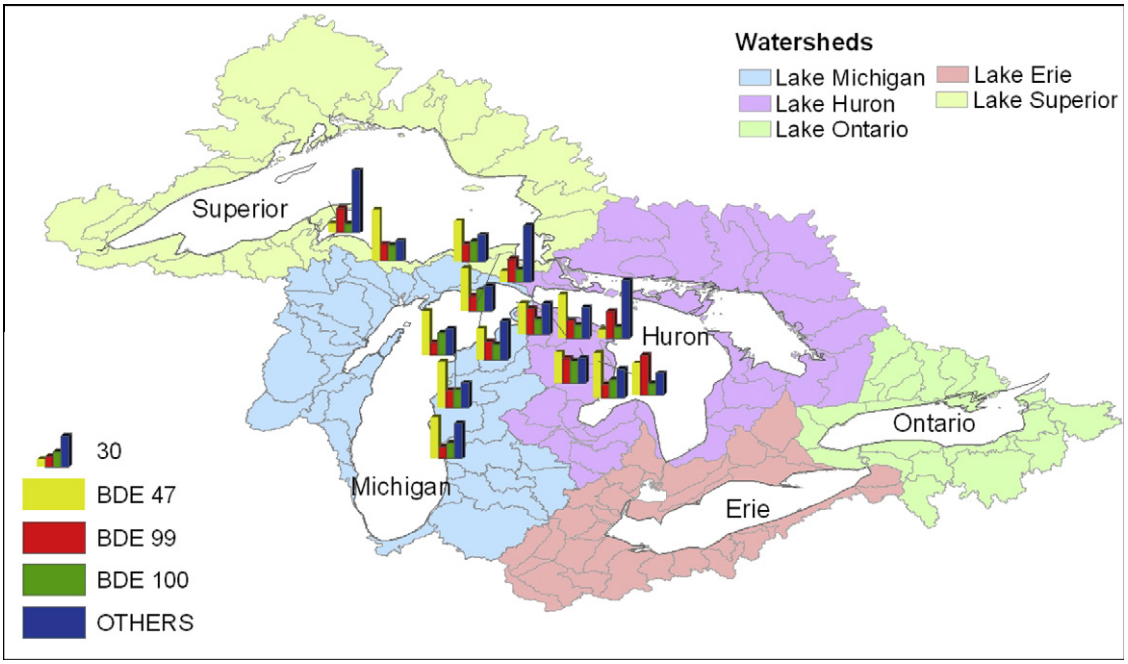


**Fig. 2.** Map showing the congener profiles for PBDEs in bald eagle plasma.

No statistical differences were found in the concentrations of total PBDEs, BDE-47, 99, and 100 when the samples were divided into three groups based on the lake watersheds. This lack of a spatial difference may be a result of the limited number of samples per lake.

### 3.2. Non-PBDE flame retardants

In addition to PBDEs, these samples were also analyzed for several unregulated, current use flame retardants, including hexabromobenzene (HBB), 1,2-*bis*(2,4,6-tribromophenoxy)ethane (TBE), hexabromocyclododecanes (HBCDs), pentabromoethylbenzene (PBEB), decabromodiphenylethane (DBDPE), and Dechlorane Plus (DP). HBB, TBE, and DBDPE were not detected in any of the 15 samples analyzed, even though these are all high production chemicals that show high potential for bioaccumulation.

PBEB was detected in two samples at an average level of 0.11 ± 0.01 ng $g^{-1}$ ww. This compound is closely related to pentabromotoluene (PBT), which was produced in the United States until 1990 and used as an additive flame retardant. The Oslo Paris Commission (OSPAR) added PBEB to its first list of priority chemicals for the protection of the North Atlantic marine environment in 2001, after it was shown to be persistent, bioaccumulative, and toxic (OSPAR, 2001). More recently, PBEB was classified as a low production volume chemical, which is produced in Europe by Albemarle (Harju et al., 2009). Very few studies have reported this compound in biota; for example, Verrault et al. reported a PBEB concentration of 0.23 ng $g^{-1}$ ww in herring gulls egg yolks, but PBEB was not detect it in the bird's plasma (Verreault et al., 2007).

HBCDs, reported here as the sum of the three isomers, were detected in seven samples at an average concentration of 0.13 ± 0.07 ng $g^{-1}$ ww. HBCDs were a widely used additive flame retardant for polystyrene foams, upholstery, and thermal insulation. HBCD isomers are classified as high volume production flame retardants, especially in Europe, and are a possible substitute for the Penta-BDE and Octa-BDE commercial mixtures (Covaci et al., 2006). HBCD was detected in plasma samples of Norwegian Arctic glaucus gulls at an average level of 0.34 ± 0.09 ng $g^{-1}$ ww and 0.32 ± 0.05 ng $g^{-1}$ ww for males and females, respectively (Verreault et al., 2005). HBCD concentrations from the North American continent appear to be lower than the levels in similar samples from Europe, which probably reflects the different market demand for this chemical (Covaci et al., 2006).

DP (as the sum of the two isomers, *syn* and *anti*) was detected in six samples at an average concentration of 0.19 ± 0.10 ng $g^{-1}$ ww. DP is a highly chlorinated flame retardant used in specialized applications. In North America, DP is produced by OxyChem in Niagara Falls, New York. This flame retardant was also reported in herring gulls eggs from the Laurentian Great Lakes at levels ranging from 1.7 to 4.5 ng $g^{-1}$ ww (Gauthier et al., 2007). The levels in these bald eagle samples seem to be much lower than in gull eggs, probably because of differences in the diet of these two species.

### 3.3. Pesticides

*p,p′*-DDE was the detected most frequently and at the highest levels. Its concentration ranged from 2.85 to 62.6 ng $g^{-1}$ ww, with an average of 19.9 ± 4.9 ng $g^{-1}$ ww ($n$ = 12). *p,p′*-DDE was the most abundant compound in the DDT family, representing on average more than 85% of the total. Based on a study of paired egg and plasma samples from the Pacific Northwest, Elliott and Norstrom (1998) determined regression equations for calculating the *p,p′*-DDE concentration in eggs given the *p,p′*-DDE concentration in plasma. Their equation was further improved by Elliott and Harris (2001/2002) by including regional means from the Great Lakes. Using the latter equation and the average concentration of *p,p′*-DDE in plasma found in this study, we predict a bald eagle egg concentration of 3.7 mg $kg^{-1}$. The No Observed Adverse Effect Level (NOAEL) value for *p,p′*-DDE for reproductive effects of eggshell thinning is 3.6 mg $kg^{-1}$ (Wiemeyer et al., 1993), which is slightly lower than the predicted value of 3.7 mg $kg^{-1}$. Nevertheless, it is unlikely that *p,p′*-DDE is causing eggshell thinning in these eagle populations. On the other hand, the concentration of *p,p′*-DDE in these samples is close to the levels that have been shown to cause a significant reduction in reproductive success (Wiemeyer et al., 1993).

The findings reported here compare well with data published previously. Bowerman et al. (2003) reported geometric mean concentrations ranging from 22 to 35 ng $g^{-1}$ ww for *p,p′*-DDE for bald eagle plasma from the Great Lakes region. The mean concentration of *p,p′*-DDE in eagle nestling blood plasma in Green Bay, on Lake Michigan was 53 ng $g^{-1}$ ww (Dykstra et al., 2001). Bustnes et al. (2008) reported a mean of 15.8 ± 3.2 ng $g^{-1}$ ww for lesser black-backed gulls from Northern Norway, which is close to our values. The geometric mean of *p,p′*-DDE for peregrine falcon plasma captured at Padre Island, Texas, in 2004 was 13 ng $g^{-1}$ ww, with the highest value corresponding to 67 ng $g^{-1}$ ww (Henny et al., 2009).

α-Chlordane was detected in six samples at an average level of 6.8 ± 1.1 ng $g^{-1}$ ww. The highest concentration was measured in a sample on Lake Huron and the lowest one on Lake Michigan. γ-Chlordane was detected in only three samples, at an average concentration of 4.8 ± 0.3 ng $g^{-1}$ ww. Dieldrin was found in seven samples at an average concentration of 4.4 ± 0.6 ng $g^{-1}$ ww. HCB was detected in two samples at a level of 3.0 ± 0.5 ng $g^{-1}$ ww, and heptachlor epoxide was detected in three samples at a level of 3.1 ± 0.3 ng $g^{-1}$ ww. As already discussed for PBDEs, no statistical difference between watersheds was found for any of the pesticides, which is most probably due to the small number of samples per watershed.

### 3.4. Polychlorinated biphenyls

The total PCB concentrations in these samples ranged from 5.46 to 254 ng $g^{-1}$ ww and averaged 73.8 ± 23.2 ng $g^{-1}$ ww ($n$ = 11). The corresponding total PCB concentration in eggs, using the same regression procedure as described above for *p,p′*-DDE, is 9.4 mg $kg^{-1}$. The No Observed Adverse Effect Level (NOAEL) value for total PCBs for eggshell thinning is 4.0 mg $kg^{-1}$ (Wiemeyer et al., 1993). Although the plasma-egg equation may not be particularly robust, the plasma concentrations measured in this study are above the "no effect" threshold.

The PCB concentrations found in this study are in good agreement with those reported previously. Bowerman et al. (2003) reported a geometric mean for total PCBs ranging from 127 ng $g^{-1}$ ww for Lake Superior samples to 199 ng $g^{-1}$ ww for Lake Erie. The mean concentration of total PCBs in eagles nestling blood plasma in Green Bay, on Lake Michigan was 207 ng $g^{-1}$ ww, which is not unexpected given that this location was listed as an Area of Concern in the mid 1980's by the United States and Canada under the Great Lakes Water Quality Agreement (Dykstra et al., 2001). Bustnes et al. (2008) measured a mean of 27.5 ± 3.6 ng $g^{-1}$ ww for PCBs in plasma from lesser black-backed gulls from northern Norway.

Although no significant differences could be detected between the concentrations of total PCBs in the three watersheds, one of the three samples (b-34, collected near Lake Superior) showed a particularly high PCB concentration (see Table 1). Although the concentration of total PBDEs was also relatively high for this sample, the difference from the other samples was particularly striking for PCBs. This sample was collected on West Huron Island, on Lake Superior, which is just offshore of the mouth of Little Huron River.

It is not clear why the concentrations of most of the measured chemicals were so high in this sample, especially given that the nest was in the Huron National Wildlife Refuge, a relatively protected area, but this trend of high concentrations of PCBs in plasma from nestling eagles at this location has been consistent since 1986 (data from Bowerman et al. (2003)).

### 3.5. Relationships among PBDEs, PCBs and pesticides

A possible relationship between total PBDE and total PCB concentrations was explored. Although these two classes of compounds have different sources, different uses, and different applications, they do share similar structures and properties. The data showed a positive correlation, which was statistically significant ($r = 0.851$, $p < 0.001$). This behavior has also been observed by Elliott and Norstrom (1998) for bald eagle populations on the Pacific coast of Canada. Similarly, a significant correlation was found between total PBDE and *p,p′*-DDE concentrations ($r = 0.902$, $p < 0.001$) and between total PBDE and dieldrin concentrations ($r = 0.812$, $p < 0.05$). Because bald eagles tend to nest in proximity to water and because they tend to prey on aquatic species (Donaldson et al., 1999), the blood levels of POPs in nestlings reflect the effect of their diet and ultimately the relative contamination of the aquatic ecosystems of the Great Lakes. These correlations suggest that all of these contaminants enter the eagles through the same source and pathway, which most likely is their diet.

## 4. Conclusions

Taken together, these data show that PBDEs are present in all the samples, and they are present at significant levels. Their presence in a species, which is still showing poor reproductive success and poor juvenile and adult survival rates, is a cause for concern. Fernie et al. recently showed that, when American kestrel eggs were exposed to environmentally relevant concentrations of DE-71 (a common brominated flame retardant commercial mixture) and HBCDs, effects such as delayed egg laying, eggshell thinning, and reduced fertility and reproductive success could be observed (Fernie et al., 2009). Considering that these measurements were conducted on a different species (American kestrels vs. bald eagles) and on a different matrix (eggs vs. plasma), it is not possible to directly compare these data. Based on previous studies which compared egg concentrations in bald eagles with plasma concentrations in nestlings from the same area, egg concentrations for PBDEs from these sites would also be expected to be in the mg $kg^{-1}$ range, if their biomagnifications factors are similar to organochlorine pesticides and PCBs based on conversion factors from Elliott and Harris, 2001/2002. While there are no published NOEALs for bald eagles for PBDEs, these studies and our data suggest that further studies on the reproductive effects of PBDEs on eagles are warranted.

In addition to PBDEs, several other new and emerging flame retardants were detected in these samples. The toxicity, sources, bioaccumulation potentials, and environmental fates of these new compounds are not well understood. Further measurements are necessary in order to better understand the environmental behavior of these newer flame retardants.

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