Spatial and Temporal Patterns in Concentrations of Perfluorinated Compounds in Bald Eagle Nestlings in the Upper Midwestern United States

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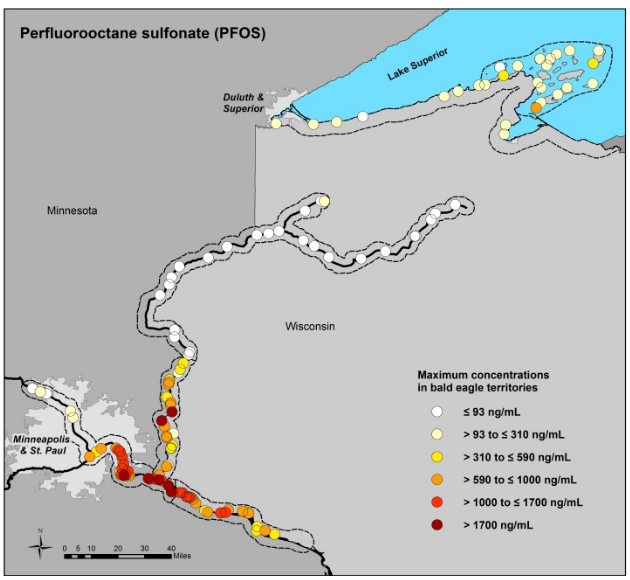

TOC Figure.

Abstract

Perfluorinated chemicals (PFCs) are of concern due to their widespread use, persistence in the environment, tendency to accumulate in animal tissues, and growing evidence of toxicity. Between 2006 and 2011 we collected blood plasma from 261 bald eagle nestlings in six study areas from the upper Midwestern United States. Samples were assessed for levels of 16 different PFCs. We used regression analysis in a Bayesian framework to evaluate spatial and temporal trends for these analytes. We found levels as high as 7370 ng/mL for the sum of all 16 PFCs (ΣPFCs). Perfluorooctane sulfonate (PFOS) and perfluorodecane sulfonate (PFDS) were the most abundant analytes, making up 67% and 23% of the PFC burden, respectively. Levels of ΣPFC, PFOS, and PFDS were highest in more urban and industrial areas, moderate on Lake Superior, and low on the remote upper St. Croix River watershed. We found evidence of declines in ΣPFCs and seven analytes, including PFOS, PFDS, and perfluorooctanoic acid (PFOA); no trend in two analytes; and increases in two analytes. We argue that PFDS, a long-chained PFC with potential for high bioaccumulation and toxicity, should be considered for future animal and human studies.

Introduction

Perfluorinated compounds (PFCs) have been in worldwide production and use since the 1950s. They have the unique properties of repelling both water and oil making them useful in a variety of products, including the production of polymers for non-stick coatings in cookware and food packaging, water and stain repellants for textiles, and in the manufacture of drugs, anesthetics, flame retardants, pesticides, and refrigerants. The first reports of their presence in human blood were published in the late 1960s and 1970s [1, 2] with indications of significant environmental contamination by the late 1970s [3]. At this same time laboratory studies of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in primates indicated a wide range of deleterious effects including hepatotoxicity, cancer, and death at relatively low dose rates [4]. Industry researchers began to express concern by the late 1970s [5, 6] leading to studies on the health effects in occupationally exposed workers [7]. PFCs are now ubiquitous across the globe and many studies have documented their persistence, rates of accumulation in animals, and effects on human health [8], including child behavior [9]. Scientists, regulators, and managers are therefore in need of information on the spatial patterns and temporal trends in PFCs.

Monitoring PFC concentrations in wildlife has proven helpful in estimating distributions [10], long-term trends [11], and routes of human exposure [12, 13]. In particular, sentinel species such as the bald eagle (*Haliaeetus leucocephalus*), which feeds high on the trophic food web, are excellent sentinels for PFC contamination [14, 15]. Eagles provide several tissues (e.g., blood, feathers and eggs) that are relatively easy to obtain [16, 17] and nestlings are particularly useful for monitoring local pollution [17]. Garrett et al. [18] reviewed several studies and estimated home range size of nesting bald eagles varied from 1.5 km^2 to 21.7 km^2. Hence contaminants found in their young are indicative of contamination within 2.6 km of the nesting site.

The bald eagle ranged across most of North America but declined in the late 1950s and 60s due to indiscriminant killing, habitat loss, and chemical contamination. Their populations increased after DDT (dichlorodiphenyltrichloroethane) was banned in the U.S. in 1972 and Canada in 1989. They were removed from the U.S. List of Endangered and Threatened Wildlife in 2007 [19]. Concern remains for this species, however, due to its vulnerability to persistent pollutants. In this study we report on PFC concentrations in blood plasma of bald eagle nestlings from the upper Midwest. Our objectives were to evaluate the spatial patterns and temporal trends of 16 PFC analytes in this region and to suggest implications of exposure to humans and wildlife.

Materials and Methods

Field Collections. From 2006 to 2011 we collected blood samples from occupied bald eagle nests in six study areas: the Apostle Islands National Lakeshore (APIS); Wisconsin's Lake Superior South Shore (LSSS); the upper St. Croix National Scenic Riverway (U-SACN); the lower St. Croix National Scenic Riverway (L-SACN); the Mississippi National River and Recreation Area (MISS); and a portion of Pools 3 & 4 of the Mississippi River (Pools 3&4) (Figure 1). From mid-May through early June each year, when nestlings were five to nine weeks old, we climbed to occupied nests, hand-captured the nestlings, and brought them to the ground for sampling. Nestlings were weighed, aged, banded, sampled, and placed back in the nest. All measurements were consistent with other investigators in the upper Midwest [20-22]. Nestling age was determined by the length of the 8th primary feather [23] and sex was determined by PCR-based genetic analysis [24].

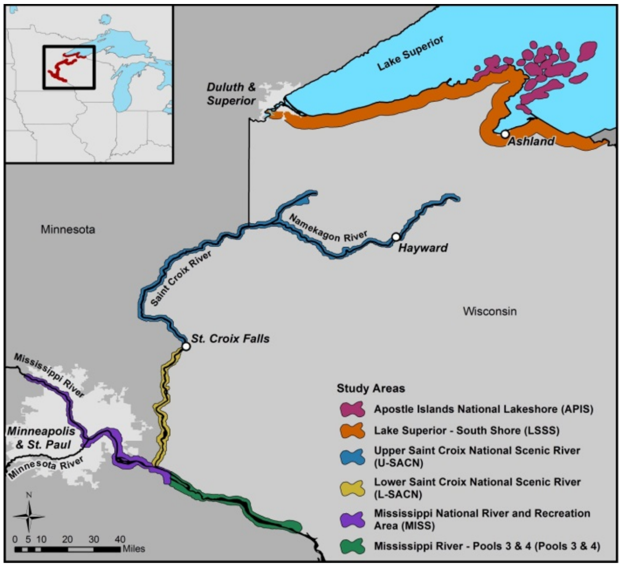

FIGURE 1. Location of six study areas in the upper Midwestern United States where bald eagle nestlings were sampled for perfluorinated compounds, 2006-2011.

We took $\leq$11 mL of blood from the brachial vein of nestlings using a sterile, 10-mL polypropylene syringe, and 20-gauge needle. The blood sample was immediately transferred to a sterile 10-mL vacutainer. Within 12 hours of collection, samples were centrifuged at 1200 rpm for 10-12 minutes to separate plasma from red blood cells. A sterile glass pipette was used to transfer $\leq$ 1.0 mL of plasma to a polypropylene vial for analysis. Glass pipettes were previously baked at 650°F (343°C) to remove chemical residues. A sample of syringes, needles, vacutainers, and vials were tested by the 3M Environmental Laboratory, Maplewood, MN and verified free of PFCs. Plasma samples were kept frozen until delivered to an analytical laboratory.

All nestlings (1-4) were sampled from each nest unless they were too young or old to handle. A single sample was chosen from each nest for analyses in this study (arbitrarily in 2006, randomly thereafter). The remaining samples were archived frozen (-20 °C) for future analyses.

The Wisconsin State Laboratory of Hygiene (WSLH) in Madison, WI was the primary analytical laboratory. To measure inter-laboratory variability we conducted blind studies with the

U. S. Environmental Protection Agency (USEPA) lab in Research Triangle Park, NC and the 3M Environmental Laboratory in Maplewood, MN. Each lab worked independently, was unaware of results from other labs, and did not know the location of sampled nestlings.

Laboratory Procedures. Each laboratory used high performance liquid chromatography/tandem mass spectrometry and gradient elution chromatography to measure concentrations of up to 16 PFCs, including: perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluorodecanesulfonate (PFDS), perfluoroheptane sulfonate (PFHpS), perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorotetradecanoic acid (PFTeDA), perfluoropentanoic acid (PFPA), perfluorotrideconoic acid (PFTrDA), and perfluoroundecanoic acid (PFuDA). The 16 analytes were selected because they were measurable using standard laboratory procedures and believed to be present in the region.

Each laboratory used slightly different procedures and had different limits of quantification (LOQ) (see Supporting Information (SI) Table SI1). All three labs had previous experience with PFC analyses, used matrix spikes of known concentrations to assess accuracy, and surrogate spikes to evaluate extraction efficiency. Results from each laboratory are known only to the study authors.

Statistical Analyses. Twelve of the 16 PFCs were detected by two or more labs at levels above their respective LOQ in $\geq$59% of their samples and these were used for all analyses. Four analytes (PFBS, PFHpA, PFHxA, and PFPA) were included in the ΣPFC, but were omitted from further analyses because they were detected in <25% of the samples. These four contributed <1% of the total PFC burden.

We estimated the spatial distribution and temporal changes in PFCs using regression analysis in a Bayesian statistical framework [25, 26] to account for variability in labs, missing values, potential differences due to age and sex, and for measurements that were below a lab's LOQ. We accounted for the observed PFC levels from the three labs by formulating log-transformed PFC levels (Y) for each individual nest (i) and each lab (l) as a multivariate normal distribution. The multivariate normal is formulated in WinBUGS [27] as $Y_{i,l} \sim MVN(\mu_{i,l}, \Omega)$; where Ω is the precision matrix for the vector of random components, i.e. the values from each of the three labs. The prior for Ω is a Wishart distribution. For values below LOQ we bounded the upper limit of $\mu_{i,l}$ by the lowest LOQ from the lab.

PFC levels were modeled as a function of a spatial correlation term, a time effect, a time by space effect, an effect of eaglet sex, an effect of eaglet age, and a fixed effect of lab (weight was highly correlated with age so was not included). Priors for the estimates of covariate coefficients were based on a non-informative normal distribution $\beta \sim \mathcal{N}(0,0.001)$. To account for spatial correlation between eagle territories we used the correlated autoregressive model, car.normal, in WinBUGS [28]. We considered all Lake Superior nests, and the nearest upstream and downstream nests on rivers, as "neighbors" with spatial weights of one; all other nests were weighted zero. The time by territory effect was formulated as a random variable $\mathcal{N}(\mu,\tau)$ where μ is the mean value of the random effect and τ is the precision parameter $1/\sigma$. We ran three chains for 50000 iterations and discarded the first 25000 as burn-in. Every 10^{th} value in the chains was used to estimate posterior distributions of the estimated parameter coefficients. We assessed convergence using the Gelman-Rubin statistic and R-hat values using library "coda" [29]. We conducted a Goodness of Fit analysis on estimates from the hierarchical model against residuals of observed PFOS values from all labs as a check on model sensitivity (R^2 = 0.81).

The Bayesian framework allowed us to interpret the credible intervals (CI) as true probabilities of change in levels of an analyte. We concluded there was “strong evidence of change” if $\geq$90% of the posterior estimates were above or below zero, “moderate evidence of change” if >80% but <90% of the estimates were above or below zero, “weak” evidence if >70% but <80% were above or below zero, and “no evidence” of change if <70% were above or below zero. An analyte was determined to be either increasing or decreasing if >70% of the posterior estimates were above or below zero respectively.

Results and Discussion

Sample Collection. From 2006 through 2011 we collected blood plasma from 261 bald eagle nestlings in six study areas (Table 1). The number of samples for each area varied yearly due to size of the eagle population, nest occupancy, and funding. All samples were analyzed by the primary laboratory, samples from 2006 through 2008 were analyzed by two laboratories (n=114), and samples from 2009 were analyzed by three laboratories (n=39).

Effects of Laboratory and Nestling Age. For each of 12 analytes we calculated correlation coefficients (CC) between paired labs, the intra-class correlation coefficient (ICC) between all three labs, and intra-lab coefficients of variability (CV) (Table SI2). Those analytes found in high concentrations were reproducible and consistent between laboratories. PFOS and PFDS made up >90% of the PFC burden and they, along with the sum of all 16 PFCs, hereafter ΣPFC, had CCs and ICCs >0.80 and within-lab CVs <26. The less abundant analytes varied more widely with CCs and ICCs ranging from -0.12 to 0.97 and CVs of 12.33 to 71.55. These results are similar to other inter-laboratory comparisons where large differences have been found for analytes found at extremely low concentrations [30]. However, most CCs in our study (68%) were above 0.80 indicating that, while some lab measurements differed in magnitude, they trended in the same direction.

TABLE 1. Distribution across study areas and years for bald eagle nestlings sampled and analyzed for PFC analytes.

study area[a]	2006[b]	2007[b]	2008[b]	2009[c]	2010	2011	totals
APIS	8	6	5	0	9	9	37
LSSS	0	6	4	0	0	1	11
U-SACN	11	8	0	0	11	12	32
L-SACN	3	4	7	9	13	13	49
MISS	11	11	15	18	23	20	98
Pools 3&4	0	0	15	12	4	2	34
totals	33	35	46	39	51	57	261

[a] = 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, Pools 3&4= portions of pools 3 and 4 on the Mississippi River.
[b] = Two way blind lab analyses; all samples analyzed by labs 1 and 2.
[c] = Three way blind lab analyses; all samples analyzed by labs 1, 2, and 3.

We found strong evidence (the 95% CI did not include zero) that levels of three analytes were affected by nestling age: the estimated coefficient for age for PFNA was 1.17 (95% CI = 1.02-1.35) and for PFUnA was 1.23 (95% CI = 1.00-1.27) indicating an increase with age for these two analytes while the coefficient for PFBA was 0.77 (95% CI = 0.60-1.00) indicating a decline with age in this analyte. Other PFCs appeared unaffected by age. The mechanisms involved in age-related differences likely include the bioaccumulative properties of each analyte, exposure time, and the development of excretory organs and processes in the growing nestling. We found no effect on PFC levels due to nestling sex.

PFC Concentrations and Spatial Patterns. The geometric mean (GM) of the sum of ΣPFCs was highest at Pools 3&4 (941 ng/mL) followed by L-SACN (644 ng/mL), MISS (607 ng/mL), APIS (552 ng/mL), and LSSS (490 ng/mL), with the lowest concentrations occurring on the remote U-SACN (163 ng/mL) (Table 2). This pattern of higher ΣPFC concentrations near urban centers has been found in a range of environmental matrices [31-33]. In a state-wide assessment of PFCs in Minnesota fish, five PFCs were found at higher levels near Minneapolis/St. Paul, MN than in more rural areas of the state [33]. Similarly, 10 PFCs in surface water of the Cape Fear River Basin, NC were found at higher levels near known or suspected sources in urban areas [31], and in East Asia, studies have shown higher levels of PFCs in air above industrialized regions [34].

There are several sources of PFCs in this region [35] but most notable is the 3M Cottage Grove facility (Figure 2, A) which used electrochemical fluorination (ECF) to make perfluorooctane sulfonyl fluoride (PSOF) until 2002 [36]. PSOF was the starting material for production of PFOS and other PFC analytes. The ECF process is relatively unrefined and other PFCs may have been unintentional byproducts. Contamination around this [37] and other PFC production facilities has been well documented in soil, water, sediments, and fish [14, 38, 39]. Two bald eagle territories downriver of this 3M facility (at 8.6 km and 13.8 km) had the highest mean concentrations of ΣPFCs over the six year study. Moreover, in 2011, one nestling in this area of river had 7370 ng/mL ΣPFC, the highest level we are aware of in bald eagles.

TABLE 2. Estimated geometric mean and range (ng/mL) in concentrations of 12 PFCs in blood plasma of bald eagle nestlings.

	geometric mean and (range) PFC concentration (ng/mL)[a]					
analyte	APIS	LSSS	U-SACN	L-SACN	MISS	Pools 3&4
ΣPFC[b]	552 (139-1420)	490 (109-472)	163 (14.5-205)	644 (60.8-3450)	607 (62.2-7370)	941 (579-2930)
PFOS	265 (71.0-830)	425 (75.5-290)	77.5 (6.56-180)	429 (10.0-2400)	421 (45.0-4200)	800 (414-1400)
PFDS	13.6 (LOQ-100)	13.7 (0.65-7.80)	3.95 (LOQ-20.0)	131 (13.0-1090)	79.8 (1.90-4100)	265 (130-1400)
PFDA	12.6 (0.06-77.0)	11.3 (3.92-29.0)	8.49 (1.10-5.19)	11.3 (2.95-30.0)	11.3 (2.20-85.0)	12.3 (LOQ-37.0)
PFUnA	17.5 (20.4-110)	11.8 (10.9-81.9)	7.79 (1.30-10.4)	9.46 (2.17-19.0)	10.44 (1.70-49.6)	9.55 (2.30-65.0)
PFDoA	7.03 (3.54-27.0)	5.81 (1.98-16.2)	3.18 (0.04-1.90)	5.47 (1.10-18.0)	6.10 (0.90-33.0)	5.54 (2.11-31.0)
PFNA	8.13 (21.0-160)	4.93 (5.57-83.0)	4.15 (0.65-8.39)	4.31 (0.29-12.0)	4.62 (LOQ-19.0)	4.18 (LOQ-11.0)
PFTrDA	3.87 (9.0-63.0)	3.01 (4.40-48.0)	2.18 (0.13-5.80)	2.68 (0.64-14.0)	2.73 (0.48-14.0)	2.59 (0.94-12.0)
PFHpS	1.88 (0.58-5.40)	1.97 (0.48-1.80)	1.11 (LOQ-2.90)	1.97 (0.19-4.40)	1.96 (0.23-16.0)	2.60 (1.76-11.0)
PFHxS	2.25 (LOQ-8.60)	1.80 (LOQ-8.55)	2.73 (LOQ-9.10)	1.81 (LOQ-8.30)	1.43 (LOQ-46.7)	0.77 (LOQ-26.0)
PFTeDA	1.49 (0.84-19.0)	1.42 (0.43-16.0)	1.22 (LOQ-2.40)	1.43 (0.24-14.0)	1.41 (0.28-310)	1.95 (LOQ-14.0)
PFOA	1.01 (LOQ-14.0)	0.64 (LOQ-5.30)	0.37 (LOQ-1.49	0.34 (LOQ-10. 0)	0.52 (LOQ-9.90)	0.49 (LOQ-14.6)
PFBA	0.31 (LOQ-22.0)	0.43 (LOQ-0.78)	0.50 (LOQ-0.90)	0.47 (LOQ-32.0)	0.55 (LOQ-78.0)	0.47 (LOQ-5.60)

[a] = Means are modeled from independent measurements by three laboratories; min and max values are the actual highest and lowest value measured by any one lab.

[b]= ΣPFC is the sum of all 16 PFC analytes measured by the independent laboratories; four analytes that made up <1% of the sample volume and were below LOQ >25% of the time were excluded from summary statistics.

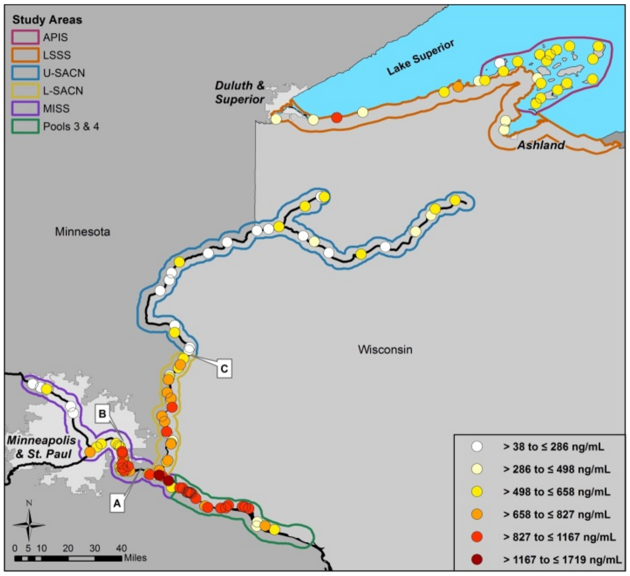

FIGURE 2. Geometric mean concentrations of ΣPFCs in bald eagle nestlings, 2006-2011. Values are modeled from measurements by three independent laboratories. Categories were selected using natural breaks in Arc GIS. A = location of 3M PFC production facility; B = Minneapolis/St Paul WWTP and St. Paul Downtown Airport; C = the communities of St Croix Falls, WI and Taylors Falls, MN. See FIGURE 1 for study area acronyms.

Concentrations of PFCs in nestlings were high along the Mississippi River even upstream from the 3M facility, however. Compared to upstream samples, we observed a near doubling of ΣPFC concentrations in nestlings beginning near the Minneapolis/St Paul waste water treatment plant (WWTP) where treated effluent is discharged to the river (Figure 2, B). Lee et al. [40] found high levels of other organic compounds downstream from this same WWTP where metal plating industries, known for using PFCs, contribute to the influent. Also in this area is the St. Paul Downtown Airport, which is within the flood plain of the river. Other investigators have documented PFCs in surface water near airports where they are used in fire-fighting foams [41]. Determining the source of PFCs to the river is further complicated, however, by the presence of landfills where 3M disposed of PFC waste since the 1950s. Four groundwater aquifers below these landfills are known to be contaminated with PFCs [42] and may release them to the river.

We found a similar pattern on the St. Croix River where ΣPFCs increased sharply immediately downstream from the communities of Taylors Falls and St. Croix Falls (Figure 2, C). This section of river is subjected to effluent from several WWTPs serving communities and industry along the lower St. Croix valley.

The APIS and LSSS study areas are comparatively remote, yet nestlings there had moderately high ΣPFC levels. We suspect this is due in part to Lake Superior's physical characteristics. Lake Superior has a 31700 mi^2 (82103 km^2) surface area that absorbs airborne contaminants from global and regional sources and, though sparsely populated, there are numerous WWTPs from municipalities along its 2700 mile (4385 km) shoreline with >200 tributaries that serve many communities. Moreover, the 191 year residence time for water in Lake Superior results in ample time for bio-concentration (re-suspension of contaminated sediments and recycling through the food web). Moreover, bald eagles on Lake Superior have been shown to feed on gulls and other piscivorous birds at higher rates than inland eagles, which further biomagnifies contaminants [43]. This slow removal and bioaccumulation of persistent contaminants from Lake Superior's food web has been demonstrated with DDE (dichlorodiphenyldichloroethylene) and PCBs (polychlorinated biphenyl), which remain at relatively high levels in bald eagle nestlings more than three decades after being banned in North America [44].

Patterns of Individual Analytes. The 95% credible intervals (in Bayesian statistics these are analogous to traditional confidence intervals) overlap for all PFCs in all study areas (Table SI3). Nonetheless, five general patterns emerge (Table 2): (1) PFOS was found in all sampled nestlings and was the most abundant PFC (GM = 77.5 – 800 ng/mL) in all study areas, contributing 67% of the total PFC burden; (2) PFDS was the second most abundant PFC (GM =

3.95 – 265 ng/mL), accounting for 23% of burden, and highest in the urbanized, riverine study areas (L-SACN, MISS, and Pools 3&4 were more than 5-fold higher than APIS and LSSS, and 20-fold higher than U-SACN); (3) PFOA was generally at low concentrations (GM = 0.34 – 1.01 ng/mL) but highest in Lake Superior study areas (APIS & LSSS); (4) The Lake Superior study areas had higher levels of more analytes than other study areas (highest or second highest for 9 of 12 PFCs); and (5) The U-SACN had the lowest mean concentrations of nearly all PFCs (9 of 12).

We found PFOS at the highest mean concentrations at Pools 3&4 (GM = 800 ng/mL), though the highest recorded value for an individual nestling was at MISS (4200 ng/mL) and second highest at L-SACN (2400 ng/mL). Geometric means for MISS, L-SACN, and LSSS were similar (421-429 ng/mL), APIS was moderately high at 265 ng/mL, and the remote U-SACN study area had the lowest concentrations (77.5 ng/mL). The only comparable study on bald eagle nestlings in the region [45] reported measureable PFOS plasma concentrations in 32 of 33 nestlings from the Great Lakes between 1990-1993 with an arithmetic mean of 330 ng/mL (SE = 126). However, the spike recovery of PFOS was only 17% in that study, suggesting concentrations may have been much higher. Nonetheless, this and the current investigation make it clear that eagles on the Great Lakes have had high burdens of PFOS at least since 1990. Moreover, PFOS is the predominant PFC found throughout the aquatic food web in the upper Midwest. Elevated levels have been found in water, benthic organisms, fish, turtles, mink, and tissue from moribund bald eagles from the Great Lakes [46, 47]. Similar to our findings, high levels have been reported in water, sediments, invertebrates, and fish from the Mississippi River below Minneapolis and St. Paul, MN with the highest concentrations occurring below the 3M Cottage Grove facility [33, 37, 48, 49].

Newsted [50] calculated a toxicity reference value (TRV) of 1700 ng PFOS/mL blood-plasma as protective of a level IV fish-eating bird such as an eagle irrespective of sex and reproductive status. We found GM concentrations to be below this TRV in all of our study areas; however, levels for some individual nestlings were higher: 5 of 98 (5.1%) at MISS, and 2 of 21 (9.5%) at L-SACN. We found no effects of PFOS on bald eagle productivity in our study. We did not measure potential sub-lethal pathological, physiological, or behavioral effects at the individual level.

The second most abundant PFC was PFDS, accounting for 23% of the total burden. PFDS was highest in nestlings along the Mississippi and lower St. Croix Rivers (Pools 3&4, MISS, and L-SACN; GM = 80-265 ng/mL), moderate in nestlings from Lake Superior (APIS and LSSS; GM = 13.7-17.5 ng/mL), and lowest on the upper St Croix River drainage (U-SACN; GM = 3.95) (Table 2). The high PFDS levels we found in the urban study areas are potentially significant. Few studies report on PFDS in environmental or human samples. Furdui et al. [47] found PFDS in 89% of lake trout from the Great Lakes and found levels to be correlated with PFOS - both being highest in Lake Erie and lowest in Lake Superior lake trout. However, the National Health and Nutritional Examination Study, which tests a representative sample of 5000 people across the U.S. annually, does not include PFDS [51]. Given that longer-chain PFCs like PFDS tend to be more toxic and prone to bioaccumulation [52], we argue that it should be considered in future animal and human studies.

We found PFOA concentrations to be comparatively low overall and the highest levels were in nestlings from the Lake Superior study areas (Table 2). The low levels in bald eagles is likely due to PFOAs low bioaccumulation properties [46] and the higher levels in Lake Superior nestlings may be due to differences in availability. PFOA is generally found at higher levels than

PFOS in surface waters of the Great Lakes [46], including Lake Superior where Scott et al. found PFOA to be highest among 23 analytes measured in surface water [53]. The authors of this latter study estimated that 35% of the PFOA in surface water was from precipitation and 59% was from tributaries, noting that WWTPs, many which are located on tributaries, concentrated PFOA up to 20-fold that of intake water . By comparison, median concentrations of PFOA was fourth among 13 PFCs tested in surface water of the upper Mississippi River [48].

Other major analytes included PFDA (GM= 8.49-12.6 ng/mL), PFUnA (7.79-17.5 ng/mL), PFNA (4.15-8.13 ng/mL), and PFDoA (3.18-7.03 ng/mL). All other PFCs were found in concentrations < 3.87 ng/mL.

Temporal Trends. Over the six year study we found strong evidence of decline in ΣPFCs and five analytes (*probability* $\geq$90% to 100%), moderate evidence of decline for two analytes (*probability* $\geq$80% to <90%), no evidence of change in three analytes (*probability* >30% to <70%), and evidence of increase in two analytes (*probability of a decline* = 0 and the expected ratio of change >1.10; Table 3). However these trends were not uniform across study areas (Table SI4). For example, the probability that PFHpS declined from 2006 to 2011 was >92% for all study areas except Pools 3&4 where evidence of decline was lacking (*probability* = 53%). Conversely, evidence of PFOS decline was strong at Pools 3&4 (*probability* = 95%), moderate at MISS and L-SACN (*probability* = 83% to 89%), weak at APIS and LSSS (*probability* = 73% to 76%), and lacking at U-SACN (*probability of decline* = 47%). Similar declines in PFOS have been documented by others in water, sediments, and fish along the MISS section of the Mississippi River between 2004 and 2012 [37]. These declines are likely the result of 3M removing many PFCs from the market in 2002. Nonetheless, the food web will continue to contain PFCs for many years. This is due to the extent of groundwater contamination, leaching

TABLE 3. Expected ratio of change and the 95 % credible interval (C.I.) for 12 PFC analytes found in bald eagle nestlings.

analyte[a]	expected ratio of change (95% C.I.)	probability levels are declining[b]
PFOA	0.78 (0.72, 0.86)	100
PFUnA	0.85 (0.77-0.93)	100
PFHxS	0.83 (0.73-0.95)	100
PFDS	0.83 (0.69-1.01)	97
PFDoA	0.91 (0.82-1.01)	96
ΣPFCs	0.92(0.82-1.03)	90
PFHpS*	0.78 (0.57-1.43)	86
PFOS	0.95 (0.86-1.06)	82
PFNA	0.97 (0.87-1.09)	64
PFBA*	1.01 (0.83-1.24)	42
PFDA	1.02 (0.92-1.14)	33
PFTeDA*	2.48 (1.42-5.03)	0
PFTrDA*	1.30 (1.10-1.51)	0

[a] = * indicates analytes with four years (2008-2011) of data rather than 6; n=202 nestlings.
[b] = The probability that levels are declining, calculated as the percentage of trend estimates from the posterior distribution that are below zero.

from PFC-containing products in municipal landfills, and the continued production of PFCs globally. Even in the U.S a limited number of PFCs will be produced when there are no known alternatives [54]. Moreover, re-suspension of PFCs by flooding can make them available to the aquatic food web. For example, in 2011, even after strong evidence of declines, we found the highest concentration of ΣPFCs in a nestling (7370 ng/mL). This followed a spring of flooding on the Mississippi River which was higher and lasted longer (well into the bald eagle nesting period) compared to the prior 30 years (unpublished USGS stream flow data at Anoka, MN).

Trends for PFNA and PFDA also differed among study areas. We found moderate evidence of declines (*probability* = 80% to 90%) for PFNA at Pools 3&4 and U-SACN, no evidence of decline at MISS and LSSS, (*probability* <70%), and evidence of increase at APIS (*probability of increase* = 83%). Similarly, evidence of decline in PFDA was lacking in all study areas except at U-SACN where it showed evidence of increase (*probability of increase* 83%).

We detected strong evidence that PFTeDA and PFTrDA were increasing in nestlings from all study areas (*probability of increase* >90%). The expected ratios of change were >1.0 and the mean expected ratio was 2.5 for PFTeDA indicating that, on average, levels of this contaminant were more than doubling every year (Table 3). These two PFCs made up < 0.5% of the total PFC load in nestlings, however, the increasing levels and lack of knowledge about their source and toxicity suggests a need for increased study.

In summary, this six year study documents extensive contamination of aquatic systems of the upper Midwest by at least 16 different PFC analytes. Bald eagle nestlings served as good biosentinels of local contamination and our results further substantiate findings of others that PFC levels in the environment are linked to effluent from municipal waste water systems and industrial waste. Bayesian modeling provided a robust means of including measurements from independent laboratories and allowed us to estimate the probabilities of increase or decline for each of 12 PFCs at six study areas. We found that most, but not all, PFCs declined during the study.

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Supporting Information Available

Four tables with information on laboratory methods, comparisons among labs, midranges and confidence intervals, and probabilities of declines at each study area are provided in the Supporting Information. This information is available free of charge via the Internet at http://pubs.acs.org/.

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