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Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA

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ABSTRACT

Tree swallow (*Tachycineta bicolor*) samples were collected at a reference lake and a nearby lake (Lake Johanna) in east central Minnesota, USA contaminated with perfluorinated carboxylic and sulfonic acids. Tissues were analyzed for a suite of 13 perfluoroalkyl compounds (PFCs) to quantify exposure and to determine if there was an association between egg concentrations of PFCs and reproductive success of tree swallows. Concentrations of perfluoroocatane sulfonate (PFOS) were elevated in all tree swallow tissues from Lake Johanna compared to tissues collected at the reference lake. Other PFCs, except for two, were elevated in blood plasma at Lake Johanna compared to the reference lake. PFOS was the dominant PFC (>75%) at Lake Johanna, but accounted for <50% of total PFCs at the reference lake. There was a negative association between concentrations of PFOS in eggs and hatching success. Reduced hatching success was associated with PFOS levels as low as 150 ng/g wet weight.

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1. Introduction

Perfluoroalkyl compounds (PFCs) are world-wide contaminants because of their widespread usage as a surface treatment on textiles, leather and carpet, in paper products used for food preparation and storage, and in fire fighting foams, insecticides and floor polishes [1,2]. Elevated concentrations of PFCs have been found in tissues of various species collected near populated, industrialized areas [3] especially near PFC production facilities [2]. A fluorochemical production unit is located in east central Minnesota (Cottage Grove), USA; various waste disposal and fabrication facilities that used PFCs are located in the surrounding metropolitan area of Minneapolis and St. Paul, MN (hereafter Twin Cities). Great blue heron (*Ardea herodias*) eggs collected on the Mississippi River near Cottage Grove had among the highest PFC concentrations ever recorded in bird eggs [4]. Geometric mean concentration of perfluoroocatane sulfonate (PFOS) was 940 ng/g wet wt. in eggs with a maximum concentration of 1878 ng/g.

The Minnesota Pollution Control Agency (MPCA) has been testing fish in Twin Cities' area lakes for the presence of PFCs. In 2007, bluegill (*Lepomis macrochirus*) from Lake Johanna, Ramsey County, MN, had concentrations of PFOS, the major chemical constituent in PFCs that were eight times higher than concentrations found in bluegills sampled from most of the other lakes that year [5]. Only bluegill from Lake Elmo, Washington Co., a lake 23 km ESE of Lake Johanna, had similarly high PFOS concentrations in 2007.

There are limited studies on the effects of PFCs on bird reproduction and the results are equivocal. Some laboratory studies suggest that egg hatchability is relatively insensitive to PFC exposure [6], however, these results should be used with caution because the higher dietary treatments in that study were toxic to some adults prior to any eggs being laid. Egg-injection studies [7–9] are equivocal as well. There was a dose–response relationship between PFOS and hatching success [7,9], but not for three other PFCs [8]. Results from egg injection studies should also be used with caution because they are not able to take into account parental behavior effects, and results can be influenced by a variety of factors such as carrier solution, area of injection, time of injection (developmental stage), and the species used. Newsted et al. [6] found that elevated PFC concentrations caused enlarged livers in female quail (*Colinus virginianus*), but no histological changes were found in the birds. No effects on liver size or histology were observed in male quail or in mallards (*Anas platyrhynchos*) of either sex.

Tree swallows have been used extensively for monitoring exposure and effects of environmental contaminants (see literature cited sections in [10,11] for a complete list). Tree swallows feed on aquatic insects [12] and concentrations of contaminants in eggs and nestling tissues reflect sediment contamination [10,11]. The ability

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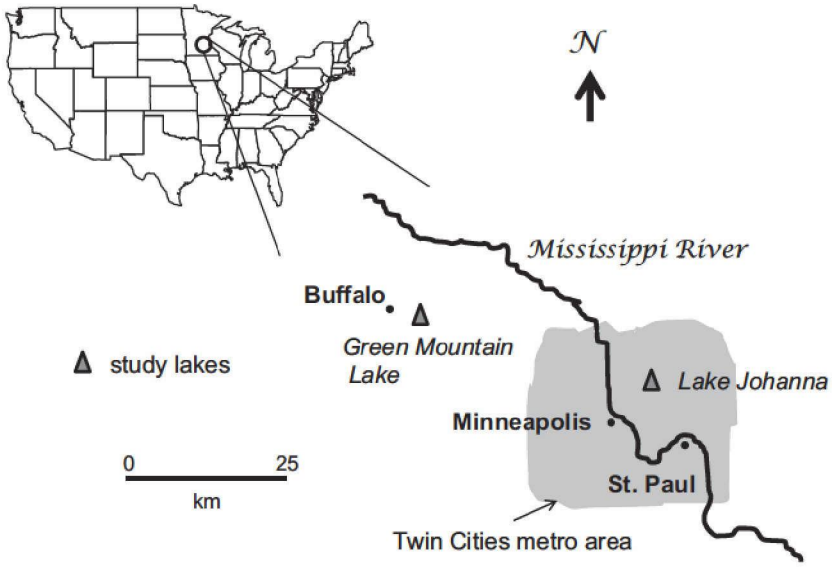

Fig. 1. Map of two study lakes in east central Minnesota, USA in 2008 and 2009.

to attract tree swallows to specific sites with the use of nest boxes; their restricted feeding range (within 1 km of nest box [13]) during the breeding season; and the ability to attract sufficient numbers for statistical testing makes the tree swallow a valuable species for studies of contaminant exposure and effects. Finally, organisms at lower trophic levels, such as the insectivorous tree swallow, might be better indicator species for PFC comparisons and effect studies because PFOS, which covalently binds to proteins in the liver and blood plasma, may not follow the traditional biomagnification processes that lipophilic organic chemicals do [14].

The objectives of this study were to document exposure and possible effects of PFCs on tree swallows nesting on Lake Johanna compared to a nearby reference lake and to assess the utility of various avian tissues for quantifying exposure and effects. Because of the previously reported high concentrations of PFCs in fish from Lake Johanna, we expected concentrations to be sufficiently high to test for possible reproductive and other effects.

2. Methods

Tree swallow nest boxes ($n = 33$) were positioned along the shoreline of Lake Johanna (45°02′38″N, 93°10′15″W), in 2008 and 2009 (Fig. 1). To increase the spatial coverage of the lake, boxes were split between the north (Tony Schmidt Regional Park, Ramsey Co.) and south (Northwestern College) sides of the lake. In 2008 our reference lake was Square Lake (45°09′16″N, 92°47′43″W), Washington Co., MN ($n = 19$ nest boxes), but because no swallows used the nest boxes at that location, our reference lake was changed in 2009 to Green Mountain Lake (45°10′00″N, 93°47′37″W), Wright Co., MN ($n = 18$ nest boxes).

Nest boxes were placed on metal fence posts approximately 20 m apart and protected from predators using metal cylinders. Each nest box was visited approximately once per week beginning in early May each year and the number of eggs laid and the number that hatched were recorded for each nest box during each visit. After the eggs hatched, the number of nestlings present was recorded until they reached 12 days of age (early July). This was called the nestling period and it started (Day 0) when the first egg in the nest hatched.

One egg was randomly collected for contaminant analysis from each clutch after the clutch had been completed. Eggs collected for contaminant analyses were weighed on an electronic pan balance (0.01 g) and the contents emptied into chemically clean jars. The egg contents were weighed, and the stage of embryonic development and whether the embryo was alive (viable) or dead was noted. Egg samples were then frozen (<−30 °C) until chemically analyzed. Any unhatched eggs were collected, opened, and the stage at which embryo death occurred was noted. Eggs that were addled, i.e. with no obvious embryo visible, were not assigned a stage, but classified as infertile eggs, a category which also included those where the embryo died at a very early age (<a few days old). Eggs that disappeared were assumed not to have hatched. In two instances the entire clutch ($n = 4$ and $n = 5$ eggs) was collected when the adult swallow was found dead and the eggs had little chance of hatching. Each egg was analyzed individually to examine within clutch variability.

When nestlings reached 12 days of age, 2 nestlings were collected from each brood unless there were only 2 surviving nestlings and in that case only 1 nestling was collected. The nestlings were decapitated and the blood collected in heparinized tubes. The tubes were centrifuged for approximately 10 min and the plasma decanted for PFC analysis. The plasma from the nestlings was pooled by nest box to provide sufficient volume for chemical analysis. The packed red blood cells were discarded. The liver and contents of the stomach were removed from each nestling carcass, pooled by nest box, and frozen later that day for PFC analysis. The carcass remainders were frozen individually and one carcass from each nest box was analyzed for PFCs.

2.1. Analytical chemistry

Tree swallow samples were analyzed by Axys Analytical Services, British Columbia, Canada for PFCs including PFOS, perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonamide (PFOSA), perfluorooctanoate (PFOA), perfluoroheptanoate (PFHpA), perfluorohexanoate (PFHxA), perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnA), and perfluorododecanoate (PFDoA). Median detection limits (DL) varied by sample matrix and were 0.5 ng/ml in plasma except 1.0 for PFBS, PFHxS and PFOS; 2.435 ng/g wet wt. in carcass remainder except 4.865 for PFBS, PFHxS and PFOS; 7.04 ng/g wet wt. in diet except 14.1 for PFBS, PFHxS and PFOS; 5.88 ng/g wet wt. in egg except 11.8 for PFBS, PFHxS and PFOS; and 4.95 ng/g wet wt. in liver except 9.9 for PFBS, PFHxS and PFOS. The relatively high detection rates for diet, eggs and livers were because of small sample mass for these matrices (ave. mass = 1.01, 1.49, and 2.03 g for the three matrices, respectively). All values provided in tables, text, and figures are ng/g wet wt. except blood plasma which is expressed as ng/ml.

In brief surrogate standards of $^{13}C_4$-PFOS, $^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_2$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, and $^{13}C_2$-PFDoA (Sigma–Aldrich, U.S. and Switzerland, and Chiron, Norway) were added and the sample was extracted by shaking with methanolic potassium hydroxide solution. After centrifugation an aliquot of the supernatant was diluted with water and cleaned up by solid phase extraction (SPE) containing a weak anion exchange sorbent (Oasis®, Waters, Ireland). The eluate was spiked with two recovery standards, $^{13}C_2$-2H-perfluoro-2-decenoicacid and $^{13}C_4$-PFOA (see manufacturers above), and analyzed on a high performance liquid chromatograph (LC, Waters 2690 or Waters 2795) coupled to a triple quadrupole mass spectrometer (MS–MS, Micromass Quattro Ultima). The LC column was a Waters Xtera C_{18} MS reverse phase C_{18}, 2.1 mm × 100 mm with 3.5 μm particles. The MS was operated in negative electrospray ionization ion mode and at unit mass resolution using multiple reaction monitoring. A minimum of six calibration solutions spanning the expected concentrations was used and all were processed through the same SPE procedure. Analyte concentrations were determined by isotope dilution/internal standard method, comparing the area of the quantification ion to that of the ^{13}C-labelled standards, and correcting for response factors. The eight carboxylates were quantified against $^{13}C_2$-PFDA, PFOA against $^{13}C_2$-PFOA, and the sulfonates were quantified against $^{13}C_4$-PFOS. All samples were analyzed in batches of 15–20

samples each. There was one blank and duplicate sample run with each batch for quality assurance purposes. Matrix spikes ($n = 8$) and matrix spike duplicates ($n = 6$) were also analyzed. Percent recovery for the PFCs ranged from 96.5% for PFBA to 113% for PFBS. Percent recovery averaged 103% for PFOS. The overall average percent recovery was 102.7%. Concentrations were not corrected for matrix spike recoveries.

2.2. Histological analyses

Five histomorphological endpoints were measured: prominence of hepatocellular vacuoles containing glycogen, macrophage abundance, adipocyte abundance, proteinaceous fluids, and fibrosis of the liver. For these, a small piece was removed from each freshly dissected liver, placed in a histo-cassette, and fixed in 4% neutral buffered formalin. In the laboratory (HLS), the liver tissue was dehydrated (Jung TP1050 automated tissue processor, Leica, Germany), embedded in paraffin (MICROM EC 350-1, ThermoFisher, USA), sectioned with a Reichert-Jung 2030 microtome (3–4 μm thick and 20 μm apart), and then stained (Leica Autostainer XL, Leica Germany) with a modified haematoxylin and eosin stain following Carson [15]. Between 8 and 12 tissue sections were examined per liver. The slides were graded using a semi-quantitative severity gradient, which reflects the prominence of a histomorphological change in the liver tissue. Grade 0 is assigned to tissue sections not exhibiting the specific histomorphology and Grade 4 to sections dominated by it. Severity grades were assigned to one spatial histomorphological change (prominence of hepatocellular vacuoles containing glycogen) and the abundance of discrete histological structure (macrophages and adipocytes, following [16]). The former has been found in vertebrates to change substantially as a response to either direct toxic insult or, more commonly, indirectly as a response of altered body condition resulting from the stress inflicted on the organism by contaminant exposure [17]. Macrophage abundance is altered in vertebrates as an immunological response, which can be triggered, among other factors, by contaminant exposure [16]. The final Grade used for summarization and statistical analysis was the average Grade for the two nestlings from each box. Additional histopathological changes potentially indicative of a toxic insult such as the presence of proteinaceous fluids or fibrosis of the liver were noted when observed.

2.3. Statistical analyses

Only individual PFCs in matrices that had ≥50% of samples above the detection limit were statistically analyzed. One-half the detection limit was substituted for non-detected values for PFCs with ≥50% but <100% detected. Between year comparisons were done for Lake Johanna plasma, egg, and nestling tissues individually using 1-way analysis of variance (ANOVA). Comparisons of individual PFC congeners between Lake Johanna and Green Mountain Lake were then done using 1-way ANOVAs. Concentrations were log transformed to meet the homogeneity of variance assumptions of ANOVA with geometric means and 95% confidence intervals (CI) provided in tables and text.

The pattern of PFC congeners was compared between lakes with Analysis of Similarity (ANOSIM) which is a multivariate analogue of analysis of variance built on a simple non-parametric permutation procedure and applied to the rank similarity matrix underlying the Bray–Curtis ordination of samples [18]. The test statistic, R, may vary from −1 to +1. An R value close to +1 indicates that there are very clear differences in patterns among the groups being tested, in this case lake. A value near zero means that the distribution of patterns is as similar among the groups as within the groups. An R is considered significant based on its p value (e.g., $p < 0.05$). The proportion that each congener comprised of the total PFCs was used in the ANOSIM; this factored out the influence of concentration differences between lakes. Pearson's correlation coefficient was used to assess the relationship among matrices for PFOS concentrations.

The association between PFOS concentrations in eggs and the hatching success of the remaining eggs in the nest was assessed using logistic regression [10]. Because of the small sample size in 2008 and the lack of between year differences, years were combined. Nests that failed because of depredation or were human caused (automobile collision) were excluded from the analyses because these two factors were presumed not to be contaminant related. Nest success during incubation (probability of at least 1 egg hatching in the nest) and during the nestling period (probability of at least 1 nestling surviving to 12 days of age) was calculated using Mayfield analysis [19–21]. This method uses the concept of nest-days, which is the total number of days that the nests are observed. That total is divided by the total number of losses to calculate the survival rate (probability) per day. Because the probability of successive events is the product of those probabilities, the daily survival rate is raised to the power of the length of the period. For tree swallows, the incubation period, which begins the day after the last egg is laid until the first egg hatches, averages 13 days long. The nestling period is 12 days long and begins (Day 0) on the day the first nestling hatches. The hatching and nestling survival probabilities are merely the percent of the eggs hatching or nestlings surviving in successful nests. The histological data were analyzed with Fisher's Exact tests. The number of samples in each Grade as explained above was tested for differences between lakes for hepatocellular vacuolization and macrophage abundance.

3. Results

3.1. Exposure

Because of the relatively high detection limits, PFPeA, PFHxA, PFHpA and PFBS were not detected in any sample of any matrix (blood plasma, egg, liver, diet or carcass). PFOSA was detected in only 1 plasma and 2 liver samples. PFBA (9 of 24 samples, all 9 from Lake Johanna) and PFOA (23 of 24 samples) were detected in plasma samples, but not in the other matrices. PFNA, PFDA, PFUnA, PFDoA and PFHxS were detected in ≥50% of plasma samples, but <40% of samples of the other matrices. Ninety-two percent of the detections in these other matrices were from Lake Johanna. PFOS was detected in >50% of samples from all matrices.

Because there was no difference in PFOS concentrations between years at Lake Johanna (eggs $p = 0.37$, plasma $p = 0.47$, and carcass $p = 0.73$), data from both years were combined and tested for differences between lakes. PFOS was present in significantly higher concentrations in blood plasma at Lake Johanna compared to Green Mountain Lake ($p < 0.001$, $df = 1,22$, $F = 639.6$) (Table 1). Statistical comparisons were not attempted for the other four matrices because, while PFOS was detected in all samples from Lake Johanna, except for 2 of 14 diet samples, it was either not detected (carcass remainder and diet) or detected in <50% of liver and egg samples from Green Mountain Lake (Table 1). The pattern of higher concentrations in Lake Johanna compared to Green Mountain Lake was consistent in the other four matrices, however. In egg and liver samples, the maximum concentrations were 12.3 and 2.5 times higher at Lake Johanna than the maximum concentrations at Green Mountain Lake. In diet and carcasses, geometric mean concentrations were 8.6 and 1.6 times higher at Lake Johanna than the average detection limit in the non-detected samples from Green Mountain Lake.

In samples collected from Lake Johanna, concentrations of PFOS were roughly equal in plasma and egg samples; however concentrations of PFOS in liver and carcass tissues were half or less the concentrations found in plasma and eggs. Concentrations in blood plasma, liver and carcasses were highly correlated (only Lake Johanna data analyzed) with one another ($r = 0.86$ blood and liver; $r = 0.74$ in blood and carcass; and $r = 0.74$ in liver and carcass). There were approximately six times higher concentrations in blood and eggs compared to diet, and between three and

Table 1
Concentrations of PFOS in tree swallow plasma (ng/ml), tissues and diet (ng/g wet wt.) from Lake Johanna (2008 and 2009) and Green Mountain Lake, MN (2009).

Tissue	Lake Johanna		Green Mountain Lake	
	n	Mean (95% CI) [Min. and max.]	n	Mean (95% CI) [Min. and max.]
Blood plasma	15	137 A[a] (120–157) [75.6–190]	9	9.66 B (7.91–11.8) [6.11–14.5]
Egg	18	141 (112–177) [57.9–285]	10	6ND[b]–23.1[c]
Liver	15	70.6 (60.5–82.3) [32.4–107]	9	8ND–42.2
Carcass	15	41.8 (36.7–47.6) [23.0–62.9]	9	9ND
Diet	14	22.5 (14.9–34.0) [7.0–41.1]	9	9ND

[a] Means not sharing same letter are significantly different between locations.
[b] Number preceding ND is the number of samples below the detection limit. Detection limits are 11.8 ng/g (egg), 9.9 ng/g (liver), 4.87 (carcass), and 14.1 (diet).
[c] The second number is the maximum concentration.

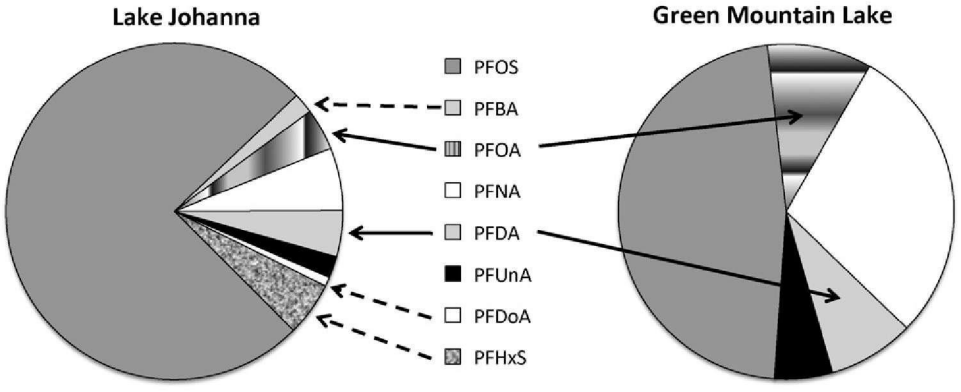

Fig. 2. Proportion that each PFC congener comprised of total PFCs in tree swallow blood plasma at Lake Johanna and Green Mountain Lake, MN, USA in 2008 and 2009. The three congeners identified with dashed arrows were below the detection limit in all samples from Green Mountain Lake.

four times higher concentrations in liver and carcass than in the diet.

Between lake comparisons for four other PFCs (PFOA, PFDA, PFNA and PFUnA) were made only for blood plasma because most samples were below the detection limit at the reference area, Green Mountain Lake (Table 2). For these four PFCs, PFOA ($p = 0.453$, $df = 1,22$, $F = 0.59$) and PFNA ($p = 0.013$, $df = 1,22$, $F = 7.29$) did not differ between the two locations. PFDA and PFUnA were significantly higher at Lake Johanna than at Green Mountain Lake (both $ps < 0.001$). The 0.05 alpha level was corrected for multiple comparisons ($n = 5$), so the effective p-value was 0.01. The geometric mean concentration for total PFCs in blood plasma was 20.8 ng/ml at Green Mountain Lake and 166.3 ng/ml at Lake Johanna.

When the composition (percent that each PFC comprised of the total PFCs) of the eight PFCs present in at least 50% of samples in at least one location was compared in multivariate space, there was nearly complete separation ($R = 0.99$, $p < 0.001$) between the two lakes. PFOS accounted for 76.7% of the total PFCs at Lake Johanna, but only 47.1% at Green Mountain Lake (Fig. 2). The carboxylic acids tended to comprise a larger percentage of the total PFCs at Green Mountain Lake than at Lake Johanna. PFNA accounted for 28.9% of the total at Green Mountain Lake and 6.1% at Lake Johanna and PFOA accounted for 10.1% at Green Mountain Lake and 4.1% at Lake Johanna.

There was considerable variation of PFOS concentrations in eggs within a clutch. We did not know the order in which the eggs were laid, but there was as much as a 4-fold difference in the concentration (Table 3) of PFOS among eggs within the same clutch. The arithmetic mean and standard deviation for PFOS for the two clutches were 289.2 ng/g ± 156.8 (box 730) and 209.3 ng/g ± 87.3 (box 763).

3.2. Reproduction and effects

There were five tree swallow nest attempts at Lake Johanna in 2008 and 15 in 2009. No swallows nested at Square Lake in 2008; there were 11 nest attempts at Green Mountain Lake (reference lake) in 2009. Seventy-nine percent of the eggs hatched (Table 4), both years and lakes combined. Individual egg losses were due primarily to disappearance during incubation and infertility, with fewer losses due to nest abandonment and death of the adult bird (called orphaned in Table 4). Four to five percent of nestlings either died or disappeared during the nestling period.

The probability of nest success during incubation (probability of at least 1 egg hatching in a clutch) ranged between 0.61 (Lake Johanna in 2008) and 0.91 (Green Mountain Lake in 2009, Table 5). Three nests were total failures at Lake Johanna, which does not include the two total failures because of depredation and automobile collision, and one total failure at Green Mountain Lake. The nest at Green Mountain Lake disappeared before eggs could be collected for chemical analysis. Hatching success probability (percent of eggs laid that hatched) ranged from 64% to 98% for both

Table 2
Concentrations of six PFCs in blood plasma (ng/ml) and one PFC in eggs (ng/g wet wt.) of tree swallows nesting at Lake Johanna ($n = 15$) and Green Mountain Lake ($n = 9$) in 2008 and 2009. Sample size was $n = 18$ eggs at Lake Johanna.

PFC congener	Geometric mean (95% CI) [Min. and max.]	
	Lake Johanna	Green Mountain Lake
PFDA	6.81 A[a]	1.53 B
	(5.60–8.29)	(1.18–1.98)
	[3.43–13.3]	[0.94–2.59]
PFNA	3.41 A	5.71 A
	(2.72–4.29)	(3.85–8.47)
	[1.81–7.62]	[2.22–11.5]
PFUnA	2.67 A	1.04 B
	(2.14–3.34)	(0.87–1.23)
	[1.18–5.63]	[0.76–1.23]
PFOA	2.67 A	2.11 A
	(2.06–3.47)	(0.96–4.65)
	[1.52–8.32]	[1ND[b]–7.69]
PFDoA	1.63	9ND
	(1.23–2.17)	
	[0.69–4.33]	
PFHxS	10.37	9ND
	(8.19–12.87)	
	[4.55–19.2]	
PFDA–eggs	5.51	9ND
	(4.01–7.58)	
	[8ND–14.0]	

Detection limits were 0.5 ng/ml for PFOA and PFDoA, 1.0 ng/ml for PFHxS, and 5.88 for PFDA in eggs.

[a] Means not sharing same letter within each chemical are significantly different between locations. A significant p-value of 0.01 was used to accommodate multiple comparisons to yield an overall alpha of 0.05.

[b] Number preceding ND is number of samples below the detection limit.

Table 3
Concentrations of PFCs (ng/g wet wt.) in eggs within two tree swallow clutches from Lake Johanna, MN in 2009.

PFC	Box #	Egg# 1[a]	Egg# 2	Egg# 3	Egg# 4	Egg# 5
PFOS	Box 730	96	169	499	298	434
	Box 763	155	115	284	283	
PFNA	Box 730	ND[b]	4.6	24.3	12.6	15.9
	Box 763	ND	ND	5.37	5.49	
PFDA	Box 730	ND	8.87	22.7	17.5	21.0
	Box 763	4.77	ND	7.22	8.27	

[a] Egg numbers do not denote the order in which they were laid.

[b] ND, not detected.

Table 4
Egg and nestling summaries for tree swallows nesting at Lake Johanna and Green Mountain Lake, MN in 2008 and 2009.

	Number of eggs or nestlings				Percentage
	Lake Johanna		Green Mountain Lake	Total	Losses calculated from remaining eggs
	2008	2009	2009		
Eggs					
Laid	27	78	56	161	
Collected	6	14	10	30	
Remaining	21	64	46	131	
Egg losses					
Reason for losses					
Missing	3	4	3	10	7.6
Infertile	4	2	0	6	4.6
Abandoned	4	0	0	4	3.1
Dead	1	0	0	1	0.8
Orphaned[a]	0	7	0	7	5.3
Total lost	12	13	3	28	21.4
Eggs hatched	9	51	43	103	78.6
Nestlings[b]	9	49	36	94	
Nestling losses					
Dead	0	0	5	5	5.3
Missing	0	2	2	4	4.3
Total lost	0	2	7	9	9.6

[a] Includes eggs from two nests. In one case the adult was killed by a car and in the other the adult was pecked to death inside the nest box.
[b] Nestling period from Day 0 to 12-days of age.

lakes. Nestling survival probability was high and ranged between 0.95 and 1.0.

There was a significant negative association between PFOS concentration in eggs and the hatching success of the remaining eggs in the nest ($p = 0.003$, Fig. 3). Beginning at approximately 150 ng/g wet wt., total hatching failure became obvious and was present in both years of the study. An almost identical result ($p = 0.003$) was obtained when total PFC concentration, rather than PFOS, was regressed against hatching success.

There was no significant difference in the frequency of histological Grades between Lake Johanna and Green Mountain Lake for either hepatocellular vacuoles ($\chi^2 = 0.044$, $p = 0.720$) or macrophages ($\chi^2 = 0.030$, $p = 0.795$) (Table 6). For vacuoles, 100% of the slides were Stage 2 or less at Green Mountain Lake whereas only 81% were Stage 2 or less at Lake Johanna. For macrophages, 64% were Stage 2 or less, and only 22% were Stage 2.5 or higher at Green Mountain Lake. At Lake Johanna 36% was Stage 2.5 or higher. There was a trend towards more adverse liver histology, i.e. more livers with higher Stage scores at Lake Johanna, but it was not statistically significant. The geometric mean PFOS concentration in liver tissue at Lake Johanna with Stage ≥2.5 was 56.8 ng/g, whereas the geometric mean concentration in livers with Stage <2 was 83.8 ng/g

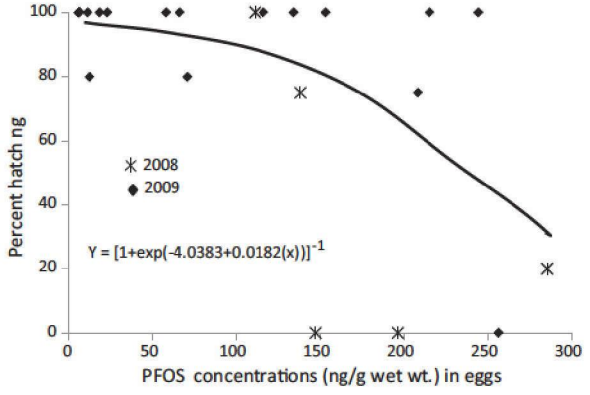

Fig. 3. Logistic regression between hatching success and concentration of PFOS in eggs of tree swallows nesting at Lake Johanna and Green Mountain Lake, MN, USA in 2008 and 2009. Does not include data from nests lost to depredation or human disturbance.

Table 6
Numbers of broods scored for histomorphological changes in each Stage in tree swallow liver cells for abundance of hepatocellular vacuoles and macrophages from Lake Johanna and Green Mountain Lake, MN in 2009.

Metric and Location	Stage					
	≤1.0	1.5	2	2.5	3	4
Hepatocellular vacuoles						
Lake Johanna	4	1	4	2		
Green Mountain	4	2	3	0		
Macrophages						
Lake Johanna		5[a]	2	1	2	1
Green Mountain		3	4	1	1	0

[a] Stage 1 ($n = 2$) combined with Stage 1.5 ($n = 3$) for Fisher's Exact test.

which is the opposite of expected if PFCs were affecting liver histology. Both large sinusoids and proteinaceous fluids were observed, but seemed evenly distributed in samples between the two lakes, six at each location. No adipocytes were observed in either location and fibrosis was observed in one sample from Lake Johanna.

4. Discussion

4.1. Exposure

By sampling nestlings, especially songbird nestlings, an assessment of local contamination is possible because nestlings are only fed from the local environment, and for tree swallows that is generally within 0.5–1 km of the nest box [13]. This contrasts with many other waterbird species which may fly 10–20 km to feed [22,23].

Table 5
Reproductive success including the number of tree swallow nestlings raised to 12 days of age from Lake Johanna and Green Mountain Lake, MN in 2008 and 2009.

Year	Lake	No. of nests with eggs and (nestlings)	Nest success[a]			Hatching probability[b] (C)	Nestling survival probability[c] (D)	Mean clutch size (E)	No. nestlings to 12 days of age ($A \times B \times C \times D \times E$)
			Incubation (A)	Nestling period (B)	Overall nest success ($A \times B$)				
2008	Johanna	5 (3)[d]	0.61	1.00	0.61	0.64	1.0	5.4	2.11
2009	Johanna	13 (12)[e]	0.92[d]	1.00	0.92	0.94[d]	0.96	5.4[d]	4.48
2009	Green Mountain	11 (10)	0.91	0.90	0.82	0.98	0.95	5.1	3.89

[a] Based on days of nest exposure (see Section 2 and [18,19]).
[b] Hatching probability (number hatched/total number of eggs in successful nests [at least one egg hatched]).
[c] Nestling survival probability (number of nestlings surviving to 12 days of age/total number of nestlings in successful nests [at least one nestling lived to 12 days of age]).
[d] First number is number of nest attempts and number in parentheses equals the number of successful nests (at least one egg hatched).
[e] Does not include data from the two nests lost to depredation and human disturbance, so total number of nest attempts = 15.

The roughly equivalent concentrations of PFOS in nestling blood plasma and eggs argues strongly that egg concentrations are also indicative of the local area and there is little carryover from possible exposure during wintering or migration areas.

Although there is a sizable amount of information on PFCs in birds, relatively little data are available for songbirds such as the tree swallow. Nestling great (*Parus major*) and blue tits (*P. caeruleus*), nesting immediately adjacent to a PFC production unit in Antwerp, Belgium, contained an average of 994 and 1055 ng/g wet wt. PFOS in liver tissue [24]. Approximately 10 km away the average liver concentrations had dropped to 146 and 210 ng/g for each species. Both Belgium locations had higher concentrations than were present in Lake Johanna tree swallow nestlings which had geometric mean concentrations of 71 ng/g wet wt. in livers. The liver concentration of PFOS at Lake Johanna, however, was elevated compared to samples collected at Green Mountain Lake where only 1 of 9 livers had detectable concentrations of PFOS. Based on data from the Antwerp [24], we would anticipate that adult tree swallows, which were not sampled at Lake Johanna, would have liver concentrations approximately 2-fold greater than found in nestling livers there.

Concentrations of PFOS in nestling blood plasma of bald eagles (*Haliaeetus leucocephalus*) from the upper Midwest, USA averaged 330 ng/ml [3] which was 2–3 times higher than found in tree swallow nestling plasma (137 ng/ml). This difference between the two species could be age-related; some eagle nestlings were 70 days old when collected compared to 12 days old for swallows; this allowed greater time for accumulation in the eagles. Trophic position may have also played some role in these differences; however, the association with trophic position may not be as straight forward for PFCs as it is for other organic contaminants [14]. PFOS was detected in blood plasma even at our reference lake, geometric mean concentration = 9.7 ng/ml. These concentrations were similar to blood serum concentrations found in 2 albatross species (*Diomedea* sp., mean concentrations = 6.2–14 ng/ml) nesting on Midway Atoll [3], so may represent current background concentrations.

Great blue heron eggs collected in 1993 from Pigs Eye Lake, Twin Cities area, MN, USA which is located just downstream of waste disposal and PFC fabrication facilities, had geometric mean concentrations of 940 ng/g wet wt. PFOS in eggs [4]. This was considerably higher than concentrations in tree swallow eggs (geometric mean = 141 ng/g) from Lake Johanna. This higher concentration could have been because of the heron's higher trophic status, but see discussion above, or because the eggs were collected while PFOS and related chemicals were still being manufactured in the area. Collections of great blue heron and tree swallow eggs from Pigs Eye Lake are ongoing and results from these studies should help resolve this question.

More recently, herring gull (*Larus argentatus*) eggs collected in 2007 from five Great Lakes had arithmetic mean concentrations between 91 and 507 ng/g wet wt. for PFHxS, PFOS and perfluorodecane sulfonate combined (PFSAs) [25]; >90% of the PFSAs was PFOS. Arithmetic mean concentrations in herring gull eggs at 14 of 15 sites exceeded the geometric mean concentration (141 ng/g) in tree swallow eggs perhaps because of their higher trophic level, but also because herring gulls are either resident or short distance migrants and hence are exposure to elevated levels of PFCs in the Great Lakes year-round.

In contrast to the relationship between liver and whole blood PFOS concentrations in adult great tits in Belgium where liver concentrations were 10 times greater than in blood, we found that concentrations in blood plasma were twice as high as in liver tissue. The differences between the tit and swallow data may be partially explained by the sample matrix (whole blood versus plasma), but further testing is needed to fully explain these differences. Kannan et al. [3] found that concentrations were 2–5 times higher in plasma compared to whole blood for a variety of bird species. The degree of correlation between the two tissues, however was similar for tits ($r = 0.8$) as for swallows (r between 0.73 and 0.87). In a laboratory experiment with mallards, the ratio between concentrations of PFOS in liver and serum was 1.6–1.7 at Days 8 and 22 [26] which was similar to the ratio 1.9 (137/70.6 ng/g wet wt.) in tree swallows. The ratio in quail in that study [26], however, was opposite and quite variable, perhaps because of differences in the protein profiles in liver and serum in that species.

It was interesting that about equal concentrations of PFOS were present in blood plasma (137 ng/ml) and eggs (141 ng/g wet wt.) from Lake Johanna. PFOS was detected in all plasma samples from Green Mountain Lake; however it was only detected in 4 of 10 eggs from there. The detection limit for eggs (11.8 ng/g) was 10 times higher than for plasma (1.0 ng/ml) which explains this result. The ability to have a lower detection limit for plasma samples, even though the sample mass was considerably smaller than for most of the other matrices, argues strongly for plasma as the best tissue to use when PFC detection levels are an issue. PFOS was also generally not detected in liver or carcasses in tree swallows from Green Mountain Lake most likely for the same detection limit reason.

The variation of PFOS concentrations among eggs within a clutch was high. There was as much as a 4-fold difference between the highest and lowest concentration of PFOS within a clutch. This level of variability, however, was similar to the amount of variation in other organic contaminants in swallows. For example there was a 3-fold difference in total PCBs within a clutch of tree swallow eggs [27].

The distribution of PFC congeners in tree swallows was similar to common cormorant (*Phalacrocorax carbo*) liver tissue from Sardinia where PFOS and PFOA were detected in all livers, PFHxS was not detected, and PFOSA was detected in only 1 of 12 cormorant liver samples [14]. Detection limits were too high in liver tissue to make this comparison for tree swallows; however this pattern was similar to our results in tree swallow blood plasma. As in the cormorants, PFOA was detected in all Lake Johanna and 7 of 8 Green Mountain Lake plasma samples while PFOSA was rarely detected (only 1 of 24) in swallow samples. In contrast to the cormorant data, PFHxS was detected in all blood plasma samples from Lake Johanna. PFOS vastly dominated the PFC spectrum in eggs of various waterbird species from southern China [28] and the Great Lakes [25] as it did in tree swallows from Lake Johanna, but PFOS did not dominate at Green Mountain Lake. Also in contrast, PFUnA was the secondarily dominant PFC congener in south China waterbirds and it ranked as the first or second most abundant perfluorinated carboxylic acid (PFCA) in the Great Lakes [25], but it ranked fourth in dominance among the PFCAs in swallows. Similar to herring gulls in the Great Lakes [25], where PFNA was one of the co-dominant PFCAs, PFNA either dominated (Green Mountain Lake) or was a co-dominant PFCA (Lake Johanna). Finally, in contrast to the approximately 10-fold difference in PFOA and PFUnA concentrations in Great Lake's herring gull eggs [25], the concentrations in tree swallow blood plasma were similar between these two PFCAs. Gebbink et al. [25] attributed the higher concentrations of PFUnA relative to PFOA to enrichment of the longer-chained PFCAs in eggs compared to livers. As demonstrated by Wang et al. [28], Gebbink et al. [25], and Kannan et al. [14], exposure often differs among areas most likely because of different exposure pathways and sources.

4.2. Effects

There was a significant negative association between hatching success and concentrations of PFOS in swallow eggs in this study. Total hatching failures began to occur at ≥150 ng/g PFOS wet wt.

The sample size was small, however, so these results should be used with caution. Additionally, there is always the possibility with field studies that results may be confounded by unknown or unmeasured causes. This effect level, however, was similar to the dosage administered in an egg injection study of white leghorn chickens (*Gallus domesticus*) in which a significant reduction in percent hatching began at 100 ng/g wet wt. [7,9]. Bustnes et al. [29] did not find an association between PFC concentrations in whole blood of adults and reproductive endpoints in a field study of lesser black-backed gulls (*Larus fuscus*). Although sampling matrices and ages were not directly comparable between that gull study and the current study, it seems that exposures in that study were considerably less than for tree swallows nesting on Lake Johanna. Newsted et al. [6] also found few effects on reproductive endpoints in a study of bobwhite quail and mallard ducks, but birds at the higher dosages died or were euthanized before they could lay eggs so effects at higher egg concentrations could not be assessed. Serum concentrations of PFOS in the 10 μg/g dosage group (8.7 and 16.6 μg/ml in blood serum of female quail and mallard) [6] were considerably higher than a calculated level for adults on Lake Johanna, so reproductive effects might have been expected based on that. As in that same study of mallards and quail, we found few histological pathologies in liver tissue although a trend may have existed towards more abnormalities at Lake Johanna compared to the reference lake. An increased abundance of macrophages could be a sign of inflammation or liver stress.

5. Conclusions

Concentrations of nearly all PFCs were elevated in tree swallow tissues from Lake Johanna compared to the nearby reference lake. PFOS was the dominant PFC (>75%) at Lake Johanna, but accounted for <50% of total PFCs at the reference lake. Because of the variation in postulated effect levels from laboratory and field studies and the relatively small sample size (<25 nests) for the significant logistic regression in this study, we recommend further field studies to validate the field effect levels in tree swallows found in the present study. Similar work is ongoing at swallow study locations on Pool 2 and Pigs Eye Lake along the Mississippi River near putative sources of PFCs so further testing of these results is possible.

Conflict of interest statement

The authors have no conflicts of interest that would have inappropriately influenced the work presented in this manuscript.

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References

[1] Lau C, Butenhoff JL, Rogers JM. The developmental toxicity of perfluoroaklyl acids and their derivatives. Toxicol Appl Pharmacol 2004;198:231–41.
[2] Hoff PT, Van de Vijver K, Dauwe T, Covaci A, Maervoet J, Eens M, et al. Evaluation of biochemical effects related to perfluorooctane sulfonic acid exposure in organohalogen-contaminated great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings. Chemosphere 2005;61:1558–69.
[3] Kannan K, Franson JC, Bowerman WW, Hansen KJ, Jones PD, Giesy JP. Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses. Environ Sci Technol 2001;35:3065–70.
[4] Custer TW, Kannan K, Tao L, Yun SH, Trowbridge A. Perfluorinated compounds and polybrominated diphenyl ethers in great blue heron eggs from three colonies on the Mississippi River, Minnesota. Waterbirds 2010;33:86–95.
[5] MPCA (Minnesota Pollution control Agency). PFCs in Minnesota's ambient environment: 2008 Progress Report. Available from: http://www.pca.state.mn.us/publications/c-pfc1-02.pdf.
[6] Newsted J, Coady KK, Beach SA, Butenhoff JL, Gallagher S, Giesy JP. Effects of perfluorooctane sulfonate on mallard and northern bobwhite quail exposed chronically via the diet. Environ Toxicol Pharmacol 2007;23:1–9.
[7] Molina ED, Balander R, Fitzgerald SD, Giesy JP, Kannan K, Mitchell R, et al. Effects of air cell injection of perfluorooctance sulfonate before incubation on development of the while leghorn chicken (*Gallus domesticus*) embryo. Environ Toxicol Chem 2006;25:227–32.
[8] O'Brien JM, Crump D, Mundy LJ, Chu S, McLaren KK, Vongphachan V, et al. Pipping success and liver mRNA expression in chicken embryos exposed in ovo to C8 and C11 perfluorinated carboxylic acids and C10 perfluorinated sulfonate. Toxicol Lett 2009;190:134–9.
[9] O'Brien JM, Carew AC, Chu S, Letcher RJ, Kennedy SW. Perfluorooctane sulfonate (PFOS) toxicity in domestic chicken (*Gallus gallus domesticus*) embryos in the absence of effects of peroxisome proliferator activated receptor alpha (PPARα)-regulated genes. Comp Biochem Physiol C 2009;149:524–30.
[10] Custer CM, Custer TW, Dummer PM, Munney KL. Exposure and effects of chemical contaminants on tree swallows nesting along the Housatonic River, Berkshire County, Massachusetts, USA, 1998–2000. Environ Toxicol Chem 2003;22:1605–21.
[11] Custer CM, Custer TW, Rosiu CJ, Dummer PM, Melancon MJ, Bickham JW. Exposure and effects of dioxins, furans, and other organochlorine chemicals on tree swallows (*Tachycineta bicolor*) nesting along the Woonasquatucket River, Rhode Island. Environ Toxicol Chem 2005;24:93–109.
[12] Mengelkoch JM, Niemi GJ, Regal RR. Diet of the nestling tree swallow. Condor 2004;106:423–9.
[13] Quinney TE, Ankney CD. Prey size selection by tree swallows. Auk 1985;102:245–50.
[14] Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. Environ Sci Technol 2002;36:3210–6.
[15] Carson FL. Histotechnology. Chicago: ASCP Press; 1996.
[16] Wolf JC, Wolfe MJ. A brief overview of nonneoplastic hepatic toxicity in fish. Toxicol Pathol 2005;33:75–85.
[17] Ferguson HW. Systemic pathology of fish: a text and atlas of comparative tissue responses in diseases of teleosts. Ames: Iowa State University Press; 1989.
[18] Clarke RK, Warwick RM. Nonmetric multivariate analysis in community-level ecotoxicology. Environ Toxicol Chem 1999;18:118–27.
[19] Mayfield H. Nestling success calculated from exposure. Wilson Bull 1961;73:255–61.
[20] Mayfield H. Suggestions for calculating nest success. Wilson Bull 1975;87:456–66.
[21] Hensler GL, Nichols JD. The Mayfield methods of estimating nesting success: a model, estimators and simulation results. Wilson Bull 1981;93:42–53.
[22] Custer TW, Osborn RG. Feeding habitat used by colonially breeding herons, egrets, and ibises in North Carolina. Auk 1978;95:733–43.
[23] Custer CM, Suarez SA, Olsen DA. Feeding habitat characteristics of the great blue heron and great egret nesting along the Upper Mississippi River, 1995–1998. Waterbirds 2004;27:454–68.
[24] Dauwe T, Van de Vijver K, De Coen W, Eens M. PFOS levels in the blood and liver of a small insectivorous songbird near a fluorochemical plant. Environ Int 2007;33:357–61.
[25] Gebbink WA, Hebert CE, Letcher RJ. Perfluorinated carboxylates and sulfonates and precursor compounds in herring gull eggs from colonies spanning the Laurentian Great Lakes of North America. Environ Sci Technol 2009;43:7443–9.
[26] Newsted JL, Beach SA, Gallagher SP, Giesy JP. Pharmacokinetics and acute lethality of perfluorooctanesolfonate (PFOS) to juvenile mallard and northern bobwhite. Arch Environ Contam Toxicol 2006;50:411–20.
[27] Custer CM, Gray BR, Custer TW. Effects of egg order on organic and inorganic element concentrations and egg characteristics in tree swallows, *Tachycineta bicolor*. Environ Toxicol Chem 2010;29:909–21.
[28] Wang T, Yeung LWY, Taniyasu S, Yamashita N, Lam JCW, Lam PKS. Perfluorooctane sulfonate and other fluorochemicals in waterbird eggs from south China. Environ Sci Technol 2008;42:8146–51.
[29] Bustnes JO, Erikstad KE, Lorentsen S-H, Herzke D. Perfluorinated and chlorinated pollutants as predictors of demographic parameters in an endangered seabird. Environ Pollut 2008;156:417–24.