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Article

Are Feathers of a Songbird Model Species (The Great Tit, *Parus major*) Suitable for Monitoring Perfluoroalkyl Acids (PFAAs) in Blood Plasma?

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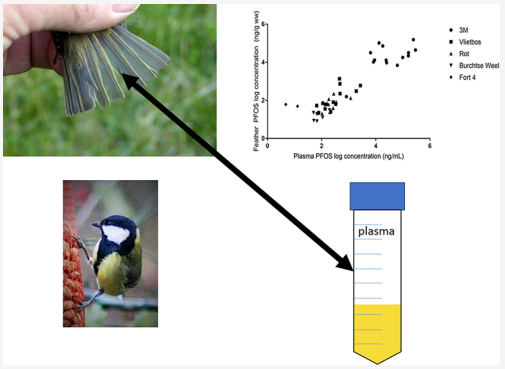
ABSTRACT: Feathers have been shown to be useful in the biomonitoring of environmental contaminants, such as metals and persistent organic pollutants. However, little is known regarding the levels of perfluoroalkyl acids (PFAAs) in feathers and the applicability of these structures for the biomonitoring of these compounds. In the present study, we report the extent to which feathers are suitable for monitoring PFAA concentrations in the blood plasma of an insectivorous songbird model species, the great tit (*Parus major*), settled at and in the vicinity of a fluorochemical plant in Antwerp, Belgium. For most of the target analytes (out of the 15 investigated), the feather PFAA concentrations near the plant are the highest ever reported in free-living birds. As PFAA concentrations did not differ in the adjacent sites, no pollution gradient with distance from the plant was observed. In addition, the PFAA concentrations were not associated with the age and sex of the birds. Perfluorooctanoic acid (PFOA) concentrations were significantly higher in *P. major* feathers than in blood plasma, but for most other PFAAs, these differences were not observed. The concentrations of perfluorooctanesulfonate (PFOS) and PFOA in *P. major* feathers and plasma were significantly and positively correlated when combining data from all sites but often not at individual sites. This result was likely caused by lower sample sizes at the individual sites and the use of matrices that represent different time periods. Our results suggest that *P. major* feathers cannot be used to estimate PFOA and PFOS concentrations in blood plasma, except when there is a great deal of variation in pollutant concentrations among sites/individual birds. Both matrices represent different time frames, providing complementary information on environmental PFAA concentrations, as illustrated by the observation that more PFAA compounds could be detected in *P. major* feathers than in blood plasma.



1. INTRODUCTION

Wild birds have been shown to be important biomonitors of environmental contaminants.[1−5] Because of both ethical and practical aspects, the use of nondestructive or less-destructive sampling methods, including feathers, has increased in research. Although feathers have been used for many decades to monitor environmental metal concentrations,[6−13] they have only been used since the early 21st century to study persistent organic pollutants (POPs).[14−22] In addition, they have only been used during the past decade for the monitoring of perfluoroalkyl acids (PFAAs),[15,23−29] persistent and bioaccumulative anthropogenic chemicals that have been ubiquitously detected in the environment, wildlife, and humans.[30−35]

During feather formation and growth, feathers are connected to the blood circulation, allowing for internal contaminants to be transferred and deposited into the feathers.[14,15] Currently, there is still an ongoing discussion regarding the suitability of feathers for use in the biomonitoring of PFAAs, as the suitability of feathers for monitoring these chemicals is considered to be different than for legislated POPs, such as polychlorinated biphenyls (PCBs) and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE).[36,37] Unlike these POPs, for which the proportions of contaminants in the body to those in the feathers is relatively constant,[36] insufficient information is available to draw any conclusions on the suitability of feathers as a biomonitor for PFAAs, as correlations have been reported for some PFAAs but not for others.[2,37]

Despite the lack of ongoing homeostasis between blood and feather concentrations,[38] several studies have reported significant correlations between the PFAA concentrations in feathers and internal tissues, such as for blood,[15,23,28] the liver[15,23,25,27] or preen oil.[24] Studies examining the correlations between feathers and blood (plasma) all used body feathers,[15,23,28] whereas these correlations have not been tested for tail or wing feathers. Different feather types may

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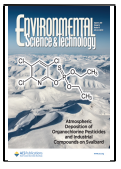

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be exposed to contaminants in different ways, thereby influencing the associations between internal and feather contaminant concentrations.[24,37] In addition, all of these studies focused on raptors, whereas information on other bird species is still lacking. Because raptors have relatively large foraging areas compared to smaller passerine species, they may not be appropriate for monitoring local contamination around point sources.[37] Furthermore, the molt pattern differs between raptors and passerine birds, where molting in songbird species occurs within one year, whereas for larger species, such as raptors, this period can extend over two or more years.[39] Consequently, there is variation among feathers in terms of age, and therefore exposure duration, between the different species used in these studies. Thus, studying other bird species, such as passerines, is necessary and will provide useful information in the ongoing discussion on the suitability of bird feathers for biomonitoring.

In the present study, we aimed to evaluate if tail feathers from free-living adult great tits (*Parus major*), an important songbird model species, present a valid matrix to monitor blood plasma PFAA concentrations by (1) correlating the PFAA concentrations between both matrices and (2) investigating differences in PFAA concentrations and profiles between both matrices. The study area included five areas with increasing distance from a PFAA manufacturing plant that is known for its high PFAA concentrations in the environment and biota. Additionally, we examined whether the PFAA concentrations in feathers decrease with increasing distance from the fluorochemical plant, taking into account the age and sex of the birds.

2. MATERIALS AND METHODS

2.1. Study Species and Sample Collection. Great tits can be considered to be a model species for ecotoxicological field studies, as they have been frequently used in this context for a variety of pollutants.[1,5,40−45] These birds are known to breed and sleep (during winter) in man-made nest boxes, which makes it relatively easy to collect samples. Great tits are also abundant and known to reproduce in polluted areas, making it possible to study associations between pollutants and reproductive parameters in the field.[9,40,46−50] The diet of great tits primarily consists of caterpillars during the breeding season and warmer months, and they supplement the lack of invertebrates during winter by consuming plant materials, such as seeds, nuts, buds, and berries.[51]

During the autumn of 2015, we placed nest boxes (151) at five sampling sites (Figure 1) over a distance gradient from a fluorochemical plant (3M) in Antwerp, Belgium. In addition to the 3M fluorochemical plant (28 nest boxes), which is a known PFAA hotspot that harbors the highest PFOS concentrations ever reported in different abiotic and biotic matrices,[1,44,45,52−56] Vlietbos (24 nest boxes; 1 km SE from 3M), Rot-Middenvijver (further called Rot; 20 nest boxes; 2.3 km ESE from 3M), Burchtse Weel (21 nest boxes; 3 km SE from 3M) and Fort 4 in Mortsel (58 nest boxes; 11 km SE from the plant) were selected as sampling sites. Great tits are highly resident in our nest box populations, with movements between neighboring study sites being extremely limited.

During the winter (February−March, when molting was completed in all individuals) of 2015−2016, the nest boxes were visited after sunset, and roosting birds were captured inside the nest boxes. The outermost tail feathers (two feathers per individual) were collected, as these feathers are easily identifiable and can be distinguished from other tail feathers based on their coloration. In addition, sampling the same feathers from different individuals allowed us to compare the PFAA concentrations in these feathers among individuals and locations, avoiding differences due to the use of different feather types. Tail feathers were sampled from 75 birds (N = 15 for 3M and Vlietbos, N = 14 for Rot and Burchtse Weel, and N = 17 for Fort 4) and stored in PFAA-free polypropylene (PP) tubes in dark conditions at room temperature to protect them from UV radiation.

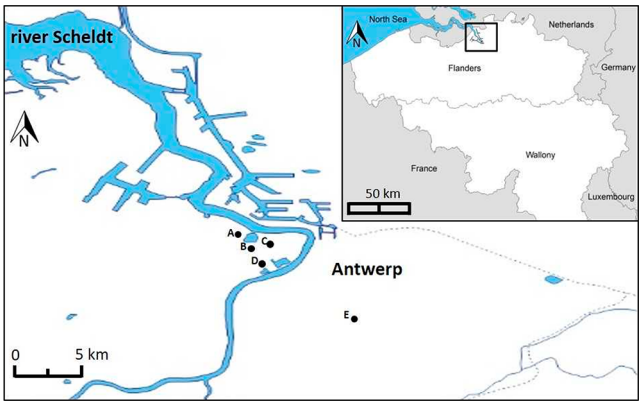

Figure 1. Overview of the study area in Antwerp, Belgium. Sampling locations are indicated as letters: (A) 3M fluorochemical plant, (B) Vlietbos, (C) Middenvijver-Rot, (D) Burchtse Weel, and (E) Fort 4.

For plasma collection, blood samples were taken from 73 birds (N = 14 for 3M, Vlietbos, Rot, and Burchtse Weel, N = 17 for Fort 4) as two birds escaped prior to blood collection. A maximum of 150 μL of blood was collected from the brachial vein using microhematocrit heparinized capillary tubes (Microvettes, Sarstedt, Germany), which were kept refrigerated. The blood samples were centrifuged (10 000 × g, 10 min, 4 °C) to separate the red blood cells from the plasma, which was then transferred to Eppendorf tubes, that were stored at −80 °C.[45] Additionally, the birds were ringed, and the sex and age class (yearling = <1 year old and older = >1 year old) were determined (Table S1).

2.2. Chemicals and Reagents. PFAAs are abbreviated according to Buck et al.[57] The target analytes were selected based on their detection in earlier monitoring studies in the vicinity of the 3M plant in Antwerp[1,5,44,45,54] and consisted of 11 perfluoroalkyl carboxylic acids [PFCAs; perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA)] and four perfluoroalkyl sulfonic acids [PFSAs (perfluorobutanesulfonate (PFBS), PFHxS, PFOS, and perfluorodecanesulfonate (PFDS)]. Isotopically mass-labeled internal standards (ISTDs; Table S2) were purchased from Wellington Laboratories (Canada). In addition, HPLC grade acetonitrile (ACN; LiChrosolv, Merck Chemicals, Belgium), methanol (VWR International, Belgium), ammonium hydroxide (Filter Service N.V., Belgium), and Milli-Q water (18.2 mΩ; TOC = 2.0 ppb; Merck Millipore, Belgium) were used.

2.3. Chemical Extraction. One of the unwashed tail feathers (8.3 ± 2.9 mg) was cut into small pieces (1 mm) using PFAA-free scissors, and placed into a 50 mL PP tube. After adding 10 mL of methanol, the samples were vortexed for 1 min and then left in the dark for 48 h at room temperature. Subsequently, the samples were centrifuged (4 °C, 5 min, 1037

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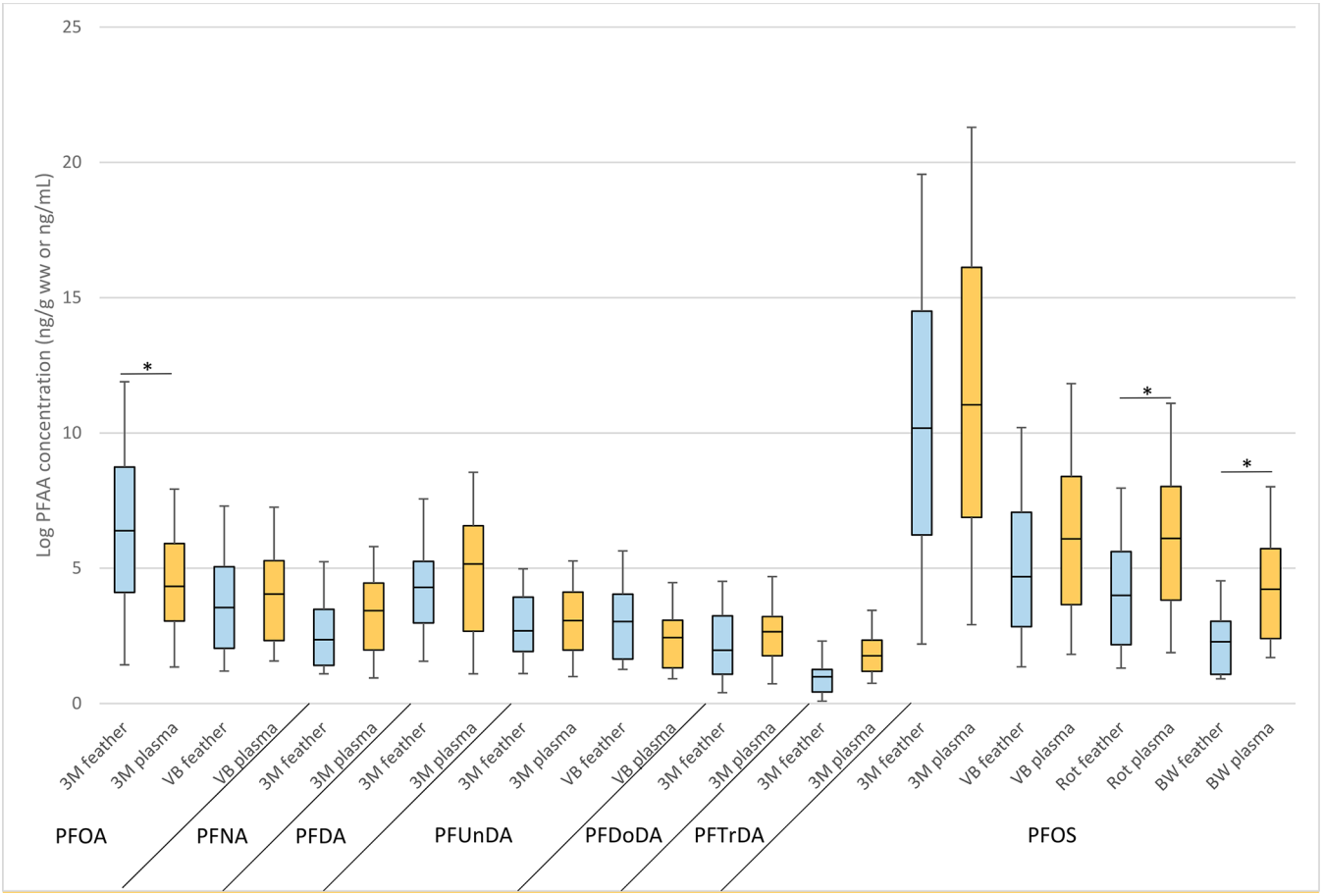

Figure 2. Concentrations (log-transformed) of PFCAs and PFOS in feathers (ng/g ww; blue boxplots) and blood plasma (ng/mL; orange boxplots) at different locations (3M fluorochemical plant, VB = Vlietbos, Rot and BW = Burchtse Weel). Compounds and locations are only included in case where detection frequencies were >50% in both matrices. Significant differences between matrices at a specific location are indicated with an asterisk.

× *g*, Eppendorf centrifuge 5804R, rotor A-4−44), after which the supernatants were transferred to 15 mL PP tubes, spiked with 80 μL of a 125 pg/μL ISTD solution and then dried completely using a rotational vacuum concentrator (Martin Christ, RVC 2−25, Germany). Finally, the samples were reconstituted in 2 mL of a 2% ammonium hydroxide solution in ACN, vortexed, and filtered through an Ion Chromatography Acrodisc 13 mm Syringe Filter with 0.2-μm Supor poly(ether sulfone) (PES) Membrane (VWR International, Belgium) attached to a PP autoinjector vial.

The blood plasma samples were extracted using a weak-anion exchange solid-phase extraction (SPE) technique described by Groffen et al.[58] and were part of a larger data set reported by Lopez-Antia et al.[45] A detailed protocol is presented in the SI. Briefly, to 10 μL of blood plasma, 80 μL of a 125 pg/μL ISTD solution and 10 mL of ACN were added. After sonication, the samples were left overnight on a shaking plate. After centrifugation, the supernatants were transferred to 14 mL PP tubes and loaded on preconditioned and equilibrated Chromabond HR-XAW SPE cartridges. The cartridges were then washed and eluted, after which the eluent was completely dried, reconstituted, and filtered as described above for the feathers.

2.4. UPLC-TQD Analysis and Quantification. We analyzed the target analytes using ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, ACQUITY, TQD, Waters, Milford, MA, USA), with electrospray in negative ion mode [ES(−)]. To separate the analytes, an ACQUITY BEH C18 column (2.1 × 50 mm; 1.7 μm, Waters, USA) was used, and an ACQUITY BEH C18 precolumn (2.1 × 30 mm; 1.7 μm, Waters, USA) was inserted between the injector and the solvent mixer to retain any PFAAs contamination from the system. As mobile phase solvents, we used 0.1% formic acid in water (A) and 0.1% formic acid in ACN, with a flow rate of 450 μL/min and an injection volume of 10 μL. The gradient started at 65% A, decreased to 0% A after 3.4 min and returned to 65% A at 4.7 min. The analytes were identified and quantified using multiple reaction monitoring (MRM) of two diagnostic transitions per analyte. MRM transitions, cone voltages and collision energy of each target analyte, including the ISTDs, are displayed in Table S2 and were validated as described by Groffen et al.[58]

2.5. Quality Assurance. For every batch of 10 samples, one procedural blank (10 mL of methanol for the feathers, 10 mL of ACN for the blood plasma) was added as quality control. The blanks for the feathers contained low concentrations of PFOA (3.4 pg/mL), PFDA (9.8 pg/mL), PFUnDA (20 pg/mL), PFDoDA (1.0 pg/mL), and PFTeDA (0.5 pg/mL), which were subtracted from concentrations in the samples in the same batch. Blood plasma blanks contained no contamination. Method recoveries varied between 60% and 95% in feathers and between 40% and 80% in blood plasma.

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The concentrations of PFHpA in feathers did not exceed the LOQ in any of the samples. The individual limits of quantification (LOQs) for feathers were determined based on a signal-to-noise (S/N) ratio of 10 and are displayed in Table S4. Blood plasma PFAA concentrations in great tits and LOQs were part of a larger data set[45] and are displayed in Table S5.

External contamination is often hypothesized to be an important factor with respect to PFAA pollution on feathers.[15,37] Although feathers are often washed prior to extraction, the contamination of the washing solvent has not been previously tested. Therefore, we performed a small washing-test (with 10 mL of methanol) using the wing feathers of free-range chickens ($N = 2$), which were collected within ±1 km from the 3M fluorochemical plant at a site with a known PFAA contamination history (Table S3). These feathers were collected in the same period as the great tit feathers and thus are of similar age. Furthermore, the molt duration of chickens is similar to that of great tits. As the feathers of both species were relatively young, the degree of external exposure was expected to be limited. On the basis of these tests, we did not expect external contamination to play a major role in the present study, but since the data set was small, we cannot completely rule out external contamination of the feathers.

2.6. Statistical Analyses. Statistical analyses were performed in R Studio (version 3.2.2), and the level of significance was set at $p \leq 0.05$ (adjusted p-values). Normality assumptions of the used statistical models were examined using the Shapiro-Wilk test and running diagnostic plots. The data were log-transformed when needed to meet the normality assumptions of the residuals. The concentrations below the LOQ were given a value of LOQ/2.[59,60]

To investigate the potential of feathers for use in monitoring blood plasma concentrations of PFAAs, we tested for correlations between the PFAA concentrations in both matrices using Spearman's correlations test for all sites together and separately. Furthermore, we compared the plasma and feather PFAA concentrations within sites using paired t tests. In both correlation tests and paired t tests, we only included data for individuals in which concentrations were detected above the LOQ in both matrices. Differences in PFAA concentrations among the locations, sex and age classes were assessed using general linear models followed by a backward elimination (final models for each compound are reported in Table S6), with the location, sex (categorical), and age (categorical) of the bird and the two-way interactions between location, sex, and age used as factors. The general linear models were evaluated using ANOVA and Tukey Post hoc analyses. Locations were excluded from these analyses when the detection frequencies of a specific compound at that location were below 50% (for example, Burchtse Weel and Fort 4 were not included when comparing PFBA concentrations among sites, as their detection frequencies were <50%). For this reason, differences in feather PFAA concentrations among locations were not tested for PFPeA, PFHpA, PFNA, PFTrDA, PFTeDA, PFBS, PFHxS, and PFDS. In blood plasma, we could only compare PFAA concentrations among locations for PFOA, PFUnDA, PFDoDA, and PFOS.

3. RESULTS

3.1. PFAA Concentrations and Detection Frequencies in Feathers and Blood Plasma. The concentrations of the individual PFAA compounds in feathers and blood plasma are displayed in Tables S3 and S4, respectively. The concentrations of compounds that were detected in both the feather and plasma samples at specific sites are illustrated in Figure 2. Significant differences in feather PFAA concentrations among the locations were observed for all target analytes, with the exception of PFUnDA ($F_{4,68} = 0.50$, $p = 0.736$). The concentrations of PFBA ($F_{2,39} = 49.5$, $p < 0.001$), PFHxA ($F_{1,26} = 41.9$, $p < 0.001$), PFOA ($F_{1,26} = 57.0$, $p < 0.001$), PFDA ($F_{4,68} = 16.1$, $p < 0.001$), PFDoDA ($F_{2,41} = 7.13$, $p = 0.002$). and PFOS ($F_{4,68} = 131$, $p < 0.001$) were all significantly higher in feathers at the 3M fluorochemical plant compared to the other locations. Furthermore, the feather PFOS concentrations at both Vlietbos and Rot were significantly higher than those measured at Burchtse Weel and Fort 4 ($F_{4,68} = 131$, $p < 0.001$). Table S1 displays the number of female and male birds collected in this study, as well as the number of yearlings and older birds. Differences in feather PFAA concentrations between sexes were observed for PFDA, with significantly higher concentrations detected in males compared to females ($F_{1,68} = 4.40$, $p = 0.040$) but not for the other compounds ($F = 0.12-2.26$, $p = 0.137-0.731$). In blood plasma, no significant differences were observed between sexes ($F = 0.33-0.65$, $p = 0.422-0.565$). Differences between older and yearling birds and interactions between location, sex, and age were not observed for feathers ($F = 0.03-1.27$, $p = 0.263-0.862$) or blood plasma ($F = 0.02-0.79$, $p = 0.377-0.891$).

The concentrations of PFOS ($F_{3,50} = 72.0$, $p < 0.001$), PFOA ($F_{4,66} = 5.60$, $p < 0.001$), and PFDoDA ($F_{2,37} = 4.73$, $p = 0.015$) in the blood plasma were significantly higher at the 3M fluorochemical plant compared to all other locations. In addition, the blood plasma PFOS concentrations at Vlietbos were higher than those detected at Burchtse Weel ($F_{3,50} = 72.0$, $p = 0.005$). No significant differences were observed in blood plasma PFUnDA concentrations between Vlietbos and the 3M fluorochemical plant ($F_{1,24} = 1.62$, $p = 0.215$).

The detection frequencies of most compounds were generally higher at the 3M fluorochemical plant for both matrices compared to those observed at the other locations (Tables S3 and S4). More compounds could be detected in feathers than in blood plasma (i.e., 14/15 compounds were detected in feathers compared to 10/15 compounds in blood plasma). Only PFHpA was not detected in feathers, whereas PFHpA, PFTeDA, PFBS, PFHxS, and PFDS could not be detected in blood plasma.

3.2. Are Feathers Suitable to Monitor Blood Plasma PFAA Concentrations? When grouping all sites together, highly significantly positive correlations were observed between feather and blood plasma concentrations of PFOA ($N = 40$, $p < 0.001$, $\rho = 0.639$, Figure 3A) and PFOS ($N = 46$, $p < 0.001$, $\rho = 0.930$, Figure 3B). At the individual sites, no significant correlations were observed for PFOA ($N = 14$, $p = 0.502$, $\rho = 0.196$), PFNA ($N = 8$, $p = 0.389$, $\rho = 0.357$), PFDA ($N = 7$, $p = 0.236$, $\rho = 0.536$), PFUnDA ($N = 7$, $p = 0.783$, $\rho = 0.143$), PFDoDA ($N = 8$, $p = 0.752$, $\rho = -0.143$), and PFOS ($N = 14$, $p = 0.186$, $\rho = 0.376$) at the 3M fluorochemical plant. Significantly positive correlations were observed between feather and blood plasma concentrations of PFOA ($N = 10$, $p < 0.001$, $\rho = 0.791$, Figure 3A) and PFOS ($N = 13$, $p = 0.021$, $\rho = 0.618$, Figure 3B) at Vlietbos. At Rot, no significant correlations were observed for PFUnDA ($N = 9$, $p = 0.194$, $\rho = -0.483$), but a significantly positive correlation was observed for PFOS ($N = 10$, $p = 0.028$, $\rho = 0.709$, Figure 3B). Finally, the PFOS concentrations were not significantly correlated at

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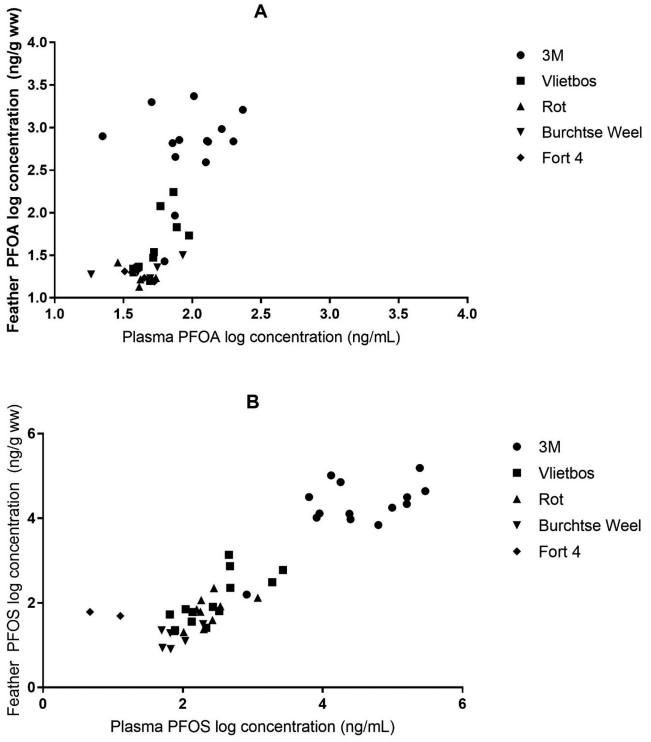

Figure 3. Correlations between feather (ng/g ww) and blood plasma (ng/mL) concentrations (log-transformed) of (A) PFOA (N = 38) and (B) PFOS (N = 44) using data from all the different sites (3M fluorochemical plant, N = 14 for both PFOA and PFOS; Vlietbos, N = 10 for PFOA and N = 13 for PFOS; Rot, N = 5 for PFOA and N = 9 for PFOS; Burchtse Weel, N = 5 for PFOA and N = 6 for PFOS; Fort 4, N = 4 for PFOA and N = 2 for PFOS). Only data for which the detection frequency was >50% for both feathers and blood plasma is included.

Burchtse Weel (N = 12, p = 0.885, ρ = −0.047, Figure 3B), while at Fort 4, we were unable to correlate the PFAA concentrations between blood plasma and feathers, as detection frequencies for individual PFAAs were never >50% in both plasma and feathers.

Significant differences between feather and blood plasma PFAA concentrations at 3M were observed for PFOA (t_{13} = 4.25, p < 0.001), with higher concentrations observed in the feathers than in the blood plasma. The PFDA concentrations exhibited a trend of being higher in the plasma compared to that observed in the feathers (t_6 = −2.28, p = 0.063). No differences between the matrices were observed for PFNA (t_7 = −0.75, p = 0.479), PFUnDA (t_6 = −0.15, p = 0.883), PFDoDA (t_7 = −0.42, p = 0.690), PFTrDA (t_4 = −1.20, p = 0.297), and PFOS (t_{13} = −1.39, p = 0.188). At Vlietbos, a trend was observed in which higher concentrations of PFUnDA were detected in feathers than in plasma (t_5 = 2.40, p = 0.061). The concentrations of PFOS (t_{12} = −1.34, p = 0.204) and PFOA (t_9 = −0.08, p = 0.937) at Vlietbos did not differ between matrices. Finally, the PFOS concentrations were significantly higher in blood plasma than in feathers at Rot (t_9 = −2.28, p = 0.048) and at Burchtse Weel (t_5 = −3.53, p = 0.017). Figure 4 shows the PFCA and PFOS concentrations in both matrices for all locations combined when a detection frequency of >50% was observed for a given compound in both matrices. When analyzing the data in this manner, significantly higher PFOA concentrations were observed in feathers than in blood plasma (t_{23} = 3.43, p = 0.002). In addition, the PFOS concentrations trended higher in blood plasma than in feathers (t_{42} = −1.72, p = 0.092). The, concentrations of PFUnDA did not differ between the matrices (t_{12} = 1.05, p = 0.314).

4. DISCUSSION

4.1. PFAA Concentrations and Detection Frequencies in Feathers and Blood Plasma. With the exception of PFUnDA, the concentrations of PFAAs were generally higher at the 3M fluorochemical plant, whereas they did not differ among the adjacent sites in most cases. Although this result confirms that the 3M fluorochemical plant in Antwerp can be considered a PFAA hotspot, the lack of a distance gradient in this study is different from previous studies, which showed that PFAA concentrations tended to decrease with increasing distance from the plant.[1,44,45,54]

The concentrations of all PFAAs in *P. major* feathers at the 3M fluorochemical plant are the highest ever reported in wild birds. To compare the feather PFAA concentrations with those reported in the existing literature, an overview of PFAA concentrations in bird feathers is provided in Table 1. In previous studies of PFAAs in birds, the highest mean PFOS concentration reported was 247 ng/g dw in gray heron (*Ardea cinerea*) tail feathers,[27] which is slightly lower than that reported at Vlietbos in the present study. However, the PFOS concentrations at the 3M fluorochemical plant are almost 150 times higher than those reported by Meyer et al.[27] Similarly, the mean and median concentrations of all other PFAAs observed in the present study are much higher than the highest PFAA concentrations previously reported in feathers. Feather types may exhibit different PFAA concentrations due to differences in PFAA sources. Herzke et al.[24] reported a positive correlation between the PFOS concentrations in preen oil and body feathers but not wing feathers. They argued that body feathers are preened more and are likely less exposed to air and water, causing them to more closely reflect preen oil PFOS concentrations, whereas wing feathers are likely affected by uptake or adsorption via air, water or airborne particles.[24] Nevertheless, information on the differences in PFAA accumulation by different feather types remains very scarce and should be included in future research on feathers.

Differences between sexes were observed in the present study, as the PFDA concentrations in feathers were higher in males than in females. A review by Sturm and Ahrens[61] showed that most studies on PFAA concentrations in birds do not report differences between sexes, and those that did observe differences always reported higher concentrations in males.[62−66] It is possible that females have lower PFAA concentrations due to maternal deposition in eggs,[45,67] although the extent to which maternal transfer influences concentrations in the mother due to excretion via the eggs is still debated.[65] Studies on mammals have reported that differences in PFAA concentrations between sexes may be the result of differences in elimination half-lives in males and females, which is likely influenced by a hormonal regulation of compound elimination.[68] Furthermore, differences in foraging strategies[69] could explain the potential differences between sexes. Surprisingly, we did not observe any age-related differences in PFAA concentrations. Because age differences in niche use and exploratory behavior, including foraging habits, of male great tits have been previously reported,[70,71] it was expected that older animals would have experienced a higher exposure to PFAAs.

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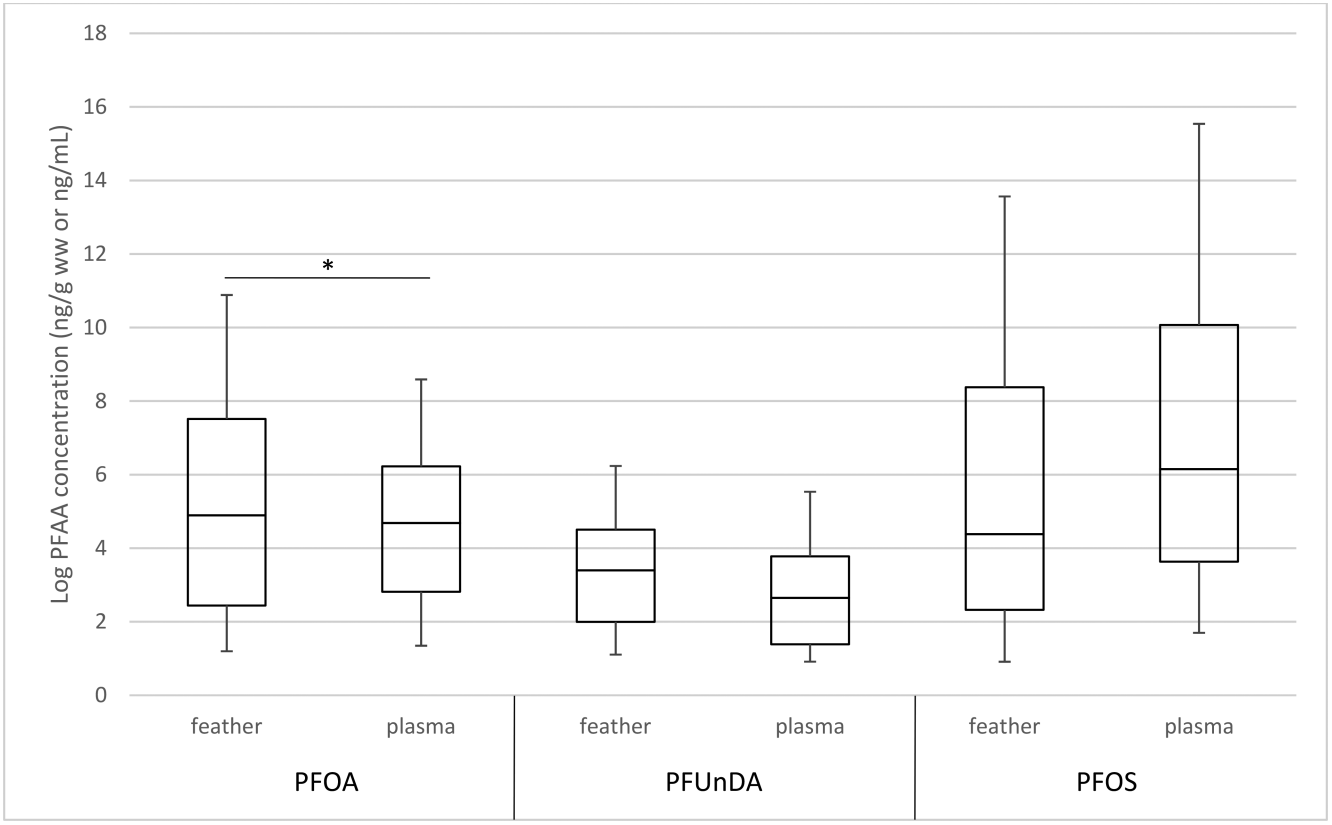

Figure 4. Concentrations (log-transformed) of PFCAs and PFOS in feathers (ng/g ww) or blood plasma (ng/mL) when combining data from all locations. Significant differences between both matrices are indicated with an asterisk.

In blood plasma, the PFOS concentrations decreased with increasing distance from the 3M site, as the concentrations at Vlietbos and Burchtse Weel were significantly lower. Nevertheless, there was a lack of a distance gradient for the other PFAAs examined in this study, which is in agreement with our findings regarding PFAA concentrations in feathers but contrasts with those of previous investigations in this study area on great tit eggs and plasma, soil and isopods.[1,44,45,54] The reported PFAA concentrations in the present study are among the highest ever reported in wildlife and were only surpassed by those observed in bald eagle nestlings.[72] As the blood plasma samples were part of a larger data set, we refer to Lopez-Antia et al.[45] for a more detailed comparison of blood plasma PFAA concentrations with the literature.

Despite the phase-out of the production of PFOS, PFOA and related products by multiple manufacturers[73,74] as well as the addition of both PFAAs to the Stockholm Convention on POPs in 2009 and 2019,[75,76] the PFAA concentrations in the vicinity of the 3M fluorochemical plant in Antwerp are still extremely high in bird feathers and plasma. This might be the result of the degradation of precursors, or the exempted use of PFOS for purposes for which no better alternatives are available.[78−80]

4.2. Are Feathers Suitable for Monitoring Blood Plasma PFAA Concentrations? Feathers have been shown to be suitable for use in monitoring environmental pollutants.[7,8,11,14,16−21] However, there is an ongoing discussion as to whether this is also the case for emerging contaminants, such as PFAAs,[15,37] as the detection frequencies of these compounds are often low, and correlations between feathers and internal organs have only sporadically been observed.[15,24,26,28,40] With regard to PFAAs, significantly positive associations between internal concentrations and feathers have been previously reported for some compounds, but not for others.[37] In the present study, in which higher detection frequencies and concentrations were generally detected compared to those observed in other studies, the PFOA and PFOS concentrations in feathers and blood plasma were strongly and significantly positively correlated when data from all sites were pooled but not for the individual locations in most cases. This result may be due to a small sample size at each individual site, which was also suggested in a study on white-tailed eagle nestlings.[23] Furthermore, the lack of correlations at the individual sites can also be explained by a time difference between the formation and sampling of the feathers. Blood and feather concentrations represent different exposure periods, with blood plasma being a reflection of more recent exposure through diet (or in nestlings through maternal transfer).[46,77] In contrast, feathers represent a more long-term exposure from the moment of molting until the moment of sampling such that feathers may have also been exposed to external sources, such as preen oil or contact with the environment (air, dust and water).[37] Furthermore, given that our study was a short-term study, we cannot be entirely sure that all sampled great tits were at their respective study site during the time of feather formation, which would require long-term studies with individually marked great tits and follow-up observations.

The movements of the birds between the site where feathers were formed/molted and the site where birds finally settled to breed, as well as the different time frames represented by the feathers and plasma, may also explain the significant differences

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Table 1. PFAA Concentrations (ng/g ww) Reported in Bird Feathers in the Literature and at the 3M Fluorochemical Plant in the Present Study[a]

	Northern Goshawk *Accipiter gentilis*	White-tailed eagle *Haliaeetus albicilla* .							*Accipiter sp*	Barn owl *Tyto alba*	Gray Heron *Ardea cinerea*	Herring gull *Larus argentatus*	Eurasian Sparrowhawk *Accipiter nisus*	Eurasian magpie *Pica pica*	Eurasian Collared Dove *Streptopelia decaocto*	Great tit *Parus major*
country	Norway	Norway	Norway[b]	Norway	Norway[b]	Greenland		Greenland	Sweden	China[c]	Belgium[b]	Belgium[c,e,f]					Belgium[b]
year	2015–2016	1971–2015	2014	2015–2016	1997–2009	1997–2009		1984–2013	1968–2011	NM	2008–2009	NM	NM	NM	NM	NM	2016
type	B	B	B	B	T	B	W	B	B	NM	T	T	T	T	T	T	T
PFBS	ND	ND						ND	ND	1.91							134
PFHxS	0.23–5.34	ND	0.05					ND	ND	0.43	<1.90	20	20	30	ND	ND	821
PFOS	0.03–9.38	1.0–43.4	6.18		12.50	8.30	9.40	1.3–7.9	1.0–38.4	4.67	16.90	250	100	80	40	60	21737
PFDS	ND	ND						ND	ND								89
PFBA	ND									1.65							392
PFPeA	ND																50
PFHxA	ND	ND						ND	ND	0.27	128.80[d]						192
PFHpA	ND	ND						ND	ND								ND
PFOA	0.21–0.83	0.1–3.0	0.30					0.1–2.1	0.3–3.5		85.90						698

[a]Only PFAAs that were assessed in the present study are included. Different feather types are abbreviated as follows: body (B), tail (T), and wing (W). NM = not mentioned. [b]Median concentrations. [c]Mean concentrations. [d]Concentration in one sample. [e]Concentrations were not reported in the text but were estimated from bar plots. [f]Concentration in ng/g dw. ND = not detected.

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in the PFOA and PFOS concentrations observed between these matrices at some locations. In particular, juvenile birds may have dispersed to other areas with different PFAA concentrations. Consequently, the feather concentrations will represent the environmental concentrations of both the "old" and "new" habitat. On the other hand, the blood concentrations represent only the concentrations of the new habitat, resulting in differences between both matrices. Furthermore, the different profiles can be explained by differences in binding affinity of the target analytes with keratin. Different PFAAs have different affinities to proteins, which primarily depend on the carbon-chain length and hydrophobicity of the chemical.[81] Therefore, long-chained PFAAs were expected to be primarily present in feathers and to lesser extent in blood plasma.

Positive correlations between feather and internal tissue PFAA concentrations have also been reported in other studies. For example, PFHxS and PFOS concentrations were observed to be positively related between the feathers and livers of Belgian Barn owls (*Tyto alba*).[19] Meyer et al.[27] examined the concentrations of PFHxS, PFOS, PFOA, and PFNA in multiple tissues of five bird species and reported a significant positive correlation between feather and liver PFOS concentrations when they grouped all species. However, the correlations between these matrices were not significant when considering the individual species separately. Positive associations between feathers and plasma of white-tailed eagle nestlings have been reported for PFHxS, PFDA, PFDoDA, and PFTrDA but not for PFOS, PFOA, and PFNA.[17] However, as no correlations were observed for PFUnDA in white-tailed eagles, Løseth et al.[15] suggested prioritizing the use of plasma for PFAAs analyses.

The inconsistencies regarding the observed correlations between internal and feather PFAA concentrations among these studies are likely the result of (1) the use of different feather types, (2) differences in species-specific molting sequences, (3) differences in home range and foraging areas of the bird species, and (4) external contamination. The previous studies that examined the correlations between feathers and blood all used body feathers, whereas these correlations have not been tested for wing feathers. Different feather types may experience different types of exposure, thereby influencing the correlations between internal and feather concentrations.[24,37] The molting sequence of birds is highly species dependent. Great tits molt within one year, whereas the molting of raptors can extend over two or more years.[39] Consequently, raptor feathers can represent longer environmental exposure than those of great tits depending on the time between molts and sampling. Furthermore, as raptors have relatively large foraging areas than the more resident great tits,[37] feather and blood plasma PFAA concentrations may not only represent different time frames but also larger areas, increasing the variation in PFAA concentrations between both matrices and affecting the associations between feathers and blood plasma.

Finally, the external contamination of feathers is often suggested as a primary factor causing the lack of associations between internal and feather PFAA concentrations in raptors,[15,37] but this assertion has never been tested, and species-specific differences may occur. Although limited in terms of sample size, the washing test results obtained using chicken feathers in the present study suggests that the external PFAA contamination of the assayed great tit feathers, which were similar in age to those of the chicken feathers, was rather limited compared to the internal feather PFAA concentrations. Furthermore, in the present study, we collected relatively young feathers that had not been exposed to environmental contamination for long time periods. As mentioned previously, the majority of investigations on PFAA concentrations in feathers were performed on raptors. Because the molting sequence duration is longer for raptors than for great tits, the external contamination of feathers may be more pronounced in raptors as a result of being exposed to external contamination for longer time periods. However, since we cannot validate our results with those of other studies, the effect of the degree of external contamination of feathers on the results cannot be completely ruled out. Thus, the role of external contamination on feathers, including species-specific differences, differences among feather types and among different feather ages, should be further investigated in future studies.

Altogether, our results suggest that feathers and blood plasma are both suitable for monitoring the environmental concentrations of PFAAs, but feathers cannot be used to monitor internal PFAA concentrations. Both matrices provide complementary information on PFAA concentrations and profiles in the environment of the bird. Blood plasma provides a snapshot of recent exposure, whereas feathers, depending on the time period between molts and sampling, provide information on long-term exposure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c00652.

Detailed protocol of the chemical extraction of blood plasma and tables showing metrics for the age and sex of the birds at each location, target PFAA analytes, their acronyms, MRM transitions, and ISTDs used for quantification, PFAA concentrations in washes and chicken feathers to estimate the extent of external contamination, limits of quantifications (LOQs), concentrations, and detection frequencies in feathers, LOQs, concentrations, and detection frequencies in blood plasma, and final models of the general linear models after backward elimination of nonsignificant interactions (PDF)

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Notes

The authors declare no competing financial interest.

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