Temporal trends in PFAS concentrations in livers of a terrestrial raptor (common buzzard; Buteo buteo) collected in Belgium during the period 2000–2005 and in 2021

Temporal trends in PFAS concentrations in livers of a terrestrial raptor (common buzzard; *Buteo buteo*) collected in Belgium during the period 2000 – 2005 and in 2021

Thimo Groffen[a,b,*], Lieven Bervoets[a], Marcel Eens[b]

[a] ECOSPHERE, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

Thimo.Groffen@uantwerpen.be

Lieven.Bervoets@uantwerpen.be

[b] Behavioural Ecology and Ecophysiology Group, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

Marcel.Eens@uantwerpen.be

[*] Corresponding author:

Dr. Thimo Groffen
University of Antwerp, Campus Groenenborger
Groenenborgerlaan 171, 2020 Antwerp, Belgium
Thimo.Groffen@uantwerpen.be
+32 (0) 3 265 8985

Abstract

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemicals that have been globally distributed. Biological time series data suggest variation in temporal PFAS concentrations due to regulations and the phase-out of multiple PFAS analytes. Nonetheless, biomonitoring temporal trends of PFAS concentrations in raptors has only been done sporadically in Europe at a national scale. In the present study, we examined the concentrations of 28 PFAS in livers of common buzzard (*Buteo buteo*) collected in Belgium in the period 2000 – 2005 and in 2021. Despite the regulations and phase-out, the ΣPFAS concentrations remained similar in the livers over the past 20 years. However, over time the abundance of perfluorooctane sulfonate (PFOS), dominant in livers collected in 2000 – 2005, to the ΣPFAS concentration decreased from 46% to 27%, whereas the abundance of perfluorotetradecanoic acid (PFTeDA), dominant in 2021, increased from 19% to 43%. The PFOS concentrations in the present study did not exceed the Toxicity Reference Values (TRVs), which were determined in liver on the characteristics of an avian top predator. The absence of temporal changes in PFAS concentrations is hypothesized to be due to a lagged response in environmental concentrations compared to atmospheric concentrations.

Keywords: Per- and polyfluoroalkyl substances; raptor; temporal variation; PFOS; terrestrial environment; biomonitoring; toxicological implications

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are environmental pollutants that have been used in a wide variety of industrial and consumer products (Cousins et al., 2016). Their production and application have resulted in a global contamination of the environment and wildlife (e.g. Giesy and Kannan, 2001; Miller et al., 2015). Due to their persistence, bioaccumulation and toxicity, long-chained PFAS, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been phased-out and/or regulated (3M Company, 2000; UNEP, 2009, 2020), whereas most emerging PFAS, that also show high toxicity, are not routinely monitored or part of regulatory guidelines (Cao et al., 2019).

Following these regulatory measures, several studies have reported declines in concentrations of PFAS precursors, whereas concentrations of their final degradation products (e.g. PFOS) decreased less consistently (Ahrens et al., 2009; Schultes et al., 2020; Sun et al., 2019). Biological time series data suggest variation in temporal changes in PFAS levels. Because most of these studies focused on legacy perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), it is important to also understand the temporal trends in other, more emerging, PFAS compounds in biota (Spaan, 2020).

Biomonitoring temporal trends of PFAS levels in raptors has only sporadically been done in Europe at a national or regional scale (Bustnes et al., 2022; Faxneld et al. 2016; González-Rubio et al., 2021; Holmström et al., 2010; Sun et al., 2019). As apex predators, common buzzards (*Buteo buteo)* are expected to have particularly high PFAS concentrations compared to their prey species (Androulakakis et al., 2022). Common buzzards are abundant raptors in Europe (Gryz and Krauze-Gryz, 2019) and they are among the most suitable candidates for pollutant quantification in tissues (Badry et al., 2020). They also live in semi-urban areas and are known to forage in industrial sites (Androulakakis et al., 2022). Although they are very adaptable species (Gryz and Krauze-Gryz, 2019), environmental pollution is known to affect their population growth. For example, between the 1950s and the 1970s, the usage of organochlorine

pesticides negatively affected the abundance of common buzzard in Europe (Licata et al., 2012). PFAS may cause reproductive effects in birds (Custer et al., 2012, 2014; Groffen et al., 2019) and consequentially, PFAS pollution may affect the abundance of raptors. The risks that these contaminants pose may increase, considering that common buzzards exist in relatively small numbers in some countries and have slow reproduction rates (Licata et al., 2012). Hence, it is important to monitor this species for these emerging chemicals and their possible threats.

Therefore, we investigated here the temporal changes in the concentrations of 28 PFAS in livers of common buzzard (*Buteo buteo*) collected in Belgium in the periods 2000 – 2005 and 2021. This also enabled us to examine if there was a noticeable effect of the phase-out of some long-chain PFAS in the early 2000s on the concentrations of these analytes.

2. Materials and methods

2.1 Sample collection

In the periods of 2000 – 2005 and the year 2021, carcasses of buzzards, with unknown cause of death, were collected by bird shelters across Belgium and were stored at -20°C. The birds were dissected using stainless steel instruments and livers were sampled and stored in polypropylene (PP) tubes until PFAS extractions in 2021. In total, we collected the livers of 15 birds from 2000 – 2005 and 10 birds from 2021. The number of collected liver samples in 2000 – 2005, however, did not allow us to investigate temporal changes during these five years. Approximately 0.5 g of each liver lobe was taken (the remaining liver parts were required in other studies) and stored at -20°C prior to PFAS extractions. The sex, age, condition and geographical location of the birds were not assessed in this study.

2.2 PFAS extraction and analysis

The liver samples were homogenized using a TissueLyser with stainless steel beads. Subsamples (0.213 ± 0.020 g ww) of the homogenized livers were extracted following a protocol, using granular activated carbon powder (ENVI-Carb), as described by Powley et al. (2005) with minor modifications. The samples were analyzed, targeting 28 analytes, using Ultra Performance Liquid Chromatography coupled Tandem Electrospray Mass Spectrometry (UPLC-ESI-MS/MS). Details on the extraction procedure and instrumental settings as well as the name and abbreviations of the targeted compounds are provided in Appendix A and Table A1.

2.3 QA/QC

Three procedural blanks, which followed the same extraction procedure as the samples, consisting of 10 mL of acetonitrile (ACN) were analyzed and contained no contamination with PFAS. One instrumental blank (100% ACN) was analyzed directly with the UPLC-ESI-MS/MS per two samples. Limits of quantification (LOQ) were calculated in matrix as the concentration corresponding to a signal-to noise ratio of 10 and are displayed in Table A2 for the detected analytes and Table A3 for analytes that were not detected in any of the samples. The recovery of the internal standards (ISTDs) was calculated by comparing the ISTD Area of a directly injected (i.e. non-extracted) standard to the ISTD Area observed in the samples. The recovery varied between 60% (PFHxA) and 99% (PFNA). The target analytes were quantified using the most suitable ISTD (Table A1) based on ionization and extraction efficiency as has been validated by Groffen et al. (2021). For the majority of the analytes, we used two diagnostic transitions to help reduce the incidence of false positives and false negatives (Groffen et al., 2021).

2.4 Statistical analyses

The statistical analyses were performed in statistical software R (version 4.0.2) and in GraphPad Prism (version 9). The significance level for model testing was set at $p \leq 0.05$. The model normality assumptions were evaluated with a Shapiro Wilk test. The F test was used to compare variances of both time periods.

PFAS concentrations that were <LOQ were assigned a replacement concentration following a maximum likelihood estimation method (Villanueva, 2005; de Solla et al., 2012). A two-sample t-test was used to investigate temporal differences in PFAS concentrations. In case of non-normality, data were log(x+1) transformed.

3. Results and discussion

3.1 PFAS concentrations and accumulation

Out of the 28 target analytes, 13 PFAS, i.e. primarily long-chained PFCAs and PFSAs, were detected in at least one sample (Table A2). Since 6:2 FTS was only detected in 1 sample, assignment of a replacement concentration based on the MLE method seemed inappropriate for this analyte. Therefore, we did not include 6:2 FTS in further statistical analyses. The detected PFAS compounds in common buzzard livers were also detected in common buzzards from Germany, the Netherlands and United Kingdom (Androulakakis et al., 2022).

In 2000 – 2005, PFOS was the most abundant PFAS, accounting for 46% of the total levels (Figure 1). This was followed by PFTeDA (19%), PFTrDA (14%) and PFDoDA (8%). In 2021, however, PFTeDA (43%) was more dominant than PFOS (27%). The relative abundance of PFDoDA (t_{23} = -2.21, p = 0.037) and PFTeDA (t_{23} = - 5.83, p < 0.001) has increased significantly in 2021 compared to 2000 – 2005. The opposite, with significantly higher abundance in 2000 – 2005, was observed for PFPeS (t_{23} = 2.44, p = 0.028) and PFOS (t_{23} = 4.32, p < 0.001). The abundance of PFOA showed a trend, with the relative abundance being lower in 2021 compared to 2000 – 2005 (t_{23} = -2.03, p = 0.058). The relative abundance of all other analytes did not differ among periods (p > 0.05; Fig. 1). The dominance of PFOS was in agreement with a study performed on buzzards from the Netherlands and Germany, but not with those from the UK in which 8:8 PFPi (which was not targeted in the present study) was dominant (Androulakakis et al., 2022). In other studies, on other raptor species and using other bird matrices, such as feathers, PFAS accumulation

profiles are generally dominated by PFOS, followed by PFUnDA and PFTrDA (Briels et al., 2019; Chu et al., 2015; Monclús et al., 2022). Androulakakis et al. (2022) compared PFAS concentrations in common buzzards with those in aquatic apex predators and reported that common buzzards were least contaminated. PFAS are likely to end up in the aquatic environment, due to their high water solubility and therefore terrestrial apex predators are less subject to environmental PFAS contamination (Androulakakis et al., 2022). Besides differences in PFAS sources and exposure pathways among bird species, different matrices could also be exposed differently to environmental contamination. For example, feathers can also be contaminated externally through dust particles in addition to internal exposure (Jaspers et al., 2019). Finally, PFAS show different affinities for different tissues and organs (González-Rubio et al., 2021; Pérez et al., 2013), which could explain differences in PFAS dominance among studies, in cases where different matrices were compared.

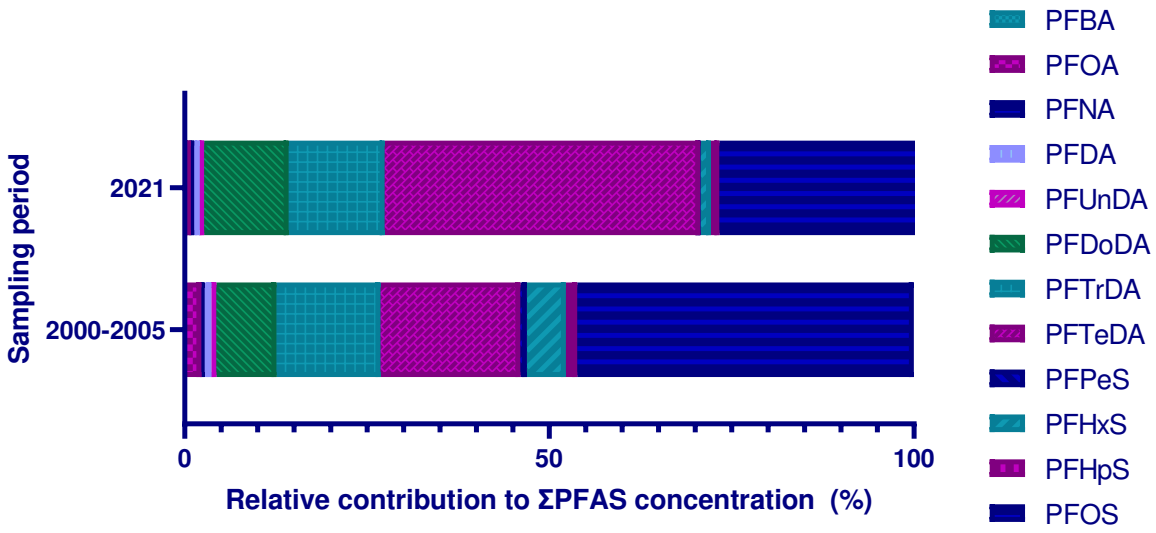

Figure 1. Relative contribution of individual PFAS to the ΣPFAS concentration (%) in common buzzard livers collected in 2000-2005 and in 2021.

Due to legislation, other regulatory measures, and the phase-out of long-chained PFAS, we had expected to observe temporal changes in PFAS concentrations over time. The concentrations of PFDoDA (t_{23} = -1.66, p = 0.023) and PFTeDA (t_{23} = -2.56, p = 0.002) were significantly higher in 2021 compared to 2000 – 2005

(Fig. 2). In addition, PFPeS concentrations were higher in 2000 – 2005 (t_{23} = 2.46, p = 0.028) and there was a trend for higher PFHpS concentrations (t_{21} = 1.87, p = 0.076) in this period. None of the other individual PFASs concentrations, nor the ΣPFAS concentration differed significantly between both time periods (p > 0.05, Fig. 2).

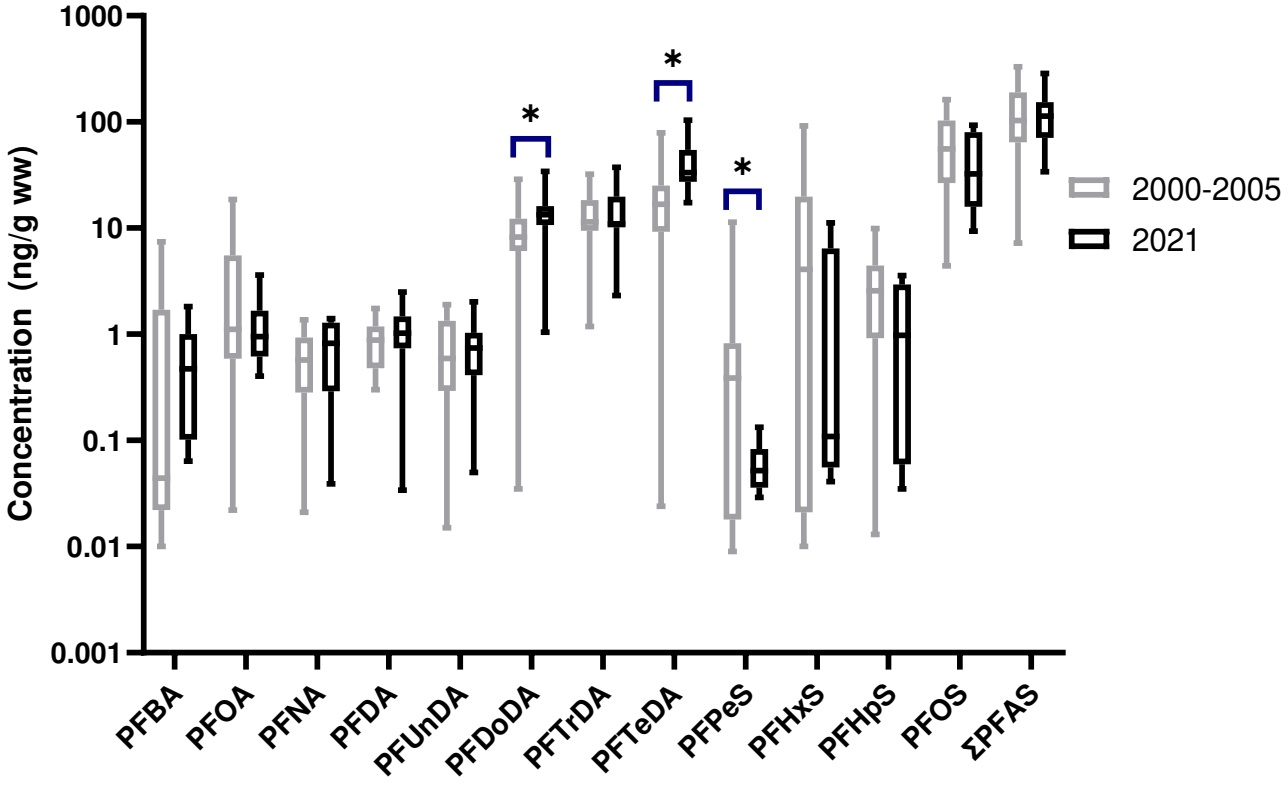

Figure 2. PFAS concentrations (ng/g ww) in livers of common buzzard collected in the period 2000-2005 (N = 15) and 2021 (N = 10). Significant differences between time periods are indicated with an asterisk. Box whiskers represent min-max values.

It is possible that PFDoDA and PFTeDA concentrations increased over time due to atmospheric degradation of their precursor compounds. Atmospheric oxidation from perfluoroalkyl-containing precursors has been reported before (Young and Mabury, 2010). Furthermore, fluorotelomer alcohols may undergo degradation on metal-rich atmospheric particle surfaces (Styler et al., 2013). Similar results have been reported in several studies on PFAS (Land et al., 2018). Bustnes et al. (2022) reported a more

or less linear increase in concentrations of some long-chained PFCAs, including PFDoDA and PFTeDA, in eggs of tawny owls (*Strix alueco*) from central Norway (1986 – 2019). In addition, for the majority of targeted PFAS, they did not observe decreasing concentrations during these 34 years. Although concentrations of perfluorooctane sulfonamide (FOSA), a PFOS precursor, declined rapidly in the atmosphere following the phase-out of PFOS and precursor compounds in the early 2000s, less consistent declines or even increases have been reported for PFOS itself (Ahrens et al., 2009; Schultes et al., 2020; Sun et al., 2019). This implies that there might be a lagged response of ecosystems, including in prey items of buzzards, to changes in chemical production compared to atmospheric concentrations (De Silva et al., 2020). The persistency of many PFAS, in combination with the atmospheric degradation of PFAS precursors, causes PFAS to be still present in the environment despite the termination of production. Consequentially, organisms are still exposed through the same pathways for generations (Land et al., 2018). Generally, PFAS concentrations in biological samples, collected on a global scale, do show mixed patterns and do not appear to be declining after phase outs and regulations (Land et al., 2018).

The absence of many temporal differences in PFAS concentrations might also be due to the opportunistic sampling. Our opportunistic dataset enabled us to determine PFAS concentrations in the livers, but may also have introduced potentially confounding factors related to the life history, and variable age and sex of the birds. This might also explain why Figure 2 shows a large variation in PFAS concentrations. Since we do not have an understanding of their life history, we could not account for differences in such factors in our analyses. For example, the degree of exposure might differ among individuals, as some birds might have lived and foraged in more severely polluted sites than others. PFHxS, for example, is one of the most frequently detected PFAS at military sites with known use of aqueous film-forming-foam (AFFF) use (East et al., 2021).In addition, age or sex differences among the birds may affect the accumulated concentrations as older birds have been exposed to PFAS for longer time periods. The passive maternal deposition of PFAS in eggs has been reported before, and this might reduce the body burden in female

176 birds (Lopez-Antia et al., 2019). Such effects of age and sex have been reported before for other bird
177 species (Bertolero et al., 2015; Blévin et al., 2017; Lopez-Antia et al., 2019; Park et al., 2021). Nonetheless,
178 bias between both sampling periods is expected to be limited, because samples were randomly collected.
179 In addition, the liver samples collected in 2000 – 2005 were only analyzed in 2021, and degradation and
180 freeze-drying of the materials might have started during this long-term storage at -20°C. This could affect
181 concentrations on a wet weight basis, e.g. due to transformation and degradation of PFAS precursors,
182 even if the PFAS themselves have not been degraded. Nonetheless, if PFAS have not degraded, the relative
183 proportions of individual PFAS to the total PFAS concentrations (Fig. 1) would remain similar.

184 Only a few studies have examined PFAS concentrations in livers from European raptors. Kannan et al.
185 (2002) reported PFOS concentrations ranging from < 3.9 to 127 ng/g ww in liver samples of white-tailed
186 eagles collected from 1979 to 1999 in Germany and Poland. Two other studies that were conducted in
187 Belgium reported PFOS concentrations ranging from 47.6 to 775 ng/g ww in Eurasian sparrowhawks
188 (Meyer et al., 2009) and from 42 to 992 ng/g ww in barn owls (Jaspers et al., 2013). The PFOS
189 concentrations in the present study were similar to those reported by Kannan et al. (2002). The studies
190 previously conducted in Belgium sampled individuals in the close vicinity of the city of Antwerp, where a
191 major PFAS chemical plant is located (Jaspers et al., 2013; Meyer et al., 2009). In agreement with this,
192 both studies also reported higher concentrations of PFOA, PFNA and PFHxS compared to the present
193 study.

194 3.2 Toxicological implications

195 Toxicity Reference Values (TRVs) for PFOS have been calculated by Newsted et al. (2005) on the
196 characteristics of an avian top predator. These guidelines were based on acute and chronic laboratory
197 exposure data on the exposure of northern bobwhite quail (*Colinus virginianus*) and mallard (*Anas*
198 *plathyrhynchos*) and are used to protect wildlife. These TRVs are derived from multiple toxicological and

reproductive endpoints, including mortality, growth and histopathology. The lowest observable adverse effect level (LOAEL) was calculated and uncertainty factors, to account for the duration of the exposure, interspecific differences, or the extrapolation of LOAEL to no observed adverse effect levels (NOAEL)) were included to calculate the TRVs for particular bird tissues. The TRVs derived by Newsted et al. (2005) for PFOS in liver of male and female birds are 2400 ng/g ww and 140 ng/g ww, respectively. The TRV for female birds was exceeded only in one sample from the 2000-2005 period, in which PFOS concentrations of 162 ng/g ww were detected. However, we have no information regarding the sex of this specimen. Hence, our results appear to imply that the birds used in this study did not experience adverse effects from PFOS exposure. Exposure to PFAS has not resulted in a population decline in buzzards, because its population has increased during the period 2007 – 2018 in Flanders (Belgium). This increase was mainly due to a better protection of the species and the restriction of several pesticides (Vermeersch et al., 2020).

3.3 Future directions

Overall, the ΣPFAS concentrations remained similar in the livers of common buzzard over the past 20 years. Although it was expected that the impacts of the regulations, and the phase-out of some PFAS in the early 2000s, would be reflected in the accumulated levels, it is hypothesized that accumulated levels in organisms show a lagged response compared to atmospheric concentrations. Hence, a better understanding of the role of precursor compounds and other emerging PFAS, is required in future biological time series studies.

Acknowledgements

The Research Foundation Flanders (FWO) funded this work in terms of a junior postdoctoral grant to TG (grant nr. 12ZZQ21N). Permission to collect the birds was granted to ME by the Royal Belgian Institute of Natural Sciences (KBIN). We would like to thank the bird shelters for providing the bird carcasses, Geert

Eens for collecting and transporting them, Peter Scheys for his help dissecting the birds, and Tim Willems for UPLC-MS/MS analyses. The authors declare no conflict of interest.

Author contributions

Thimo Groffen: Investigation, Validation, Formal analysis, Writing, Visualization, Funding acquisition; Lieven Bervoets: Supervision, Writing; Marcel Eens: Conceptualization, Sample collection, Supervision, Writing.

References

3M Company (2000). Phase-out plan for POSF-based products. USEPA Administrative Record AR 226-0600. Available from: Https://www.regulations.gov as document EPA-HQ-OPPT-2002-0051-0006, Accessed 4th Oct. 2021.

Ahrens L, Siebert U and Ebinghaus R (2009). Temporal trends of polyfluoroalkyl compounds in harbor seals (*Phoca vitulina*) from the German Bight, 1999 – 2008. Chemosphere 76: 151 – 158.

Androulakakis A, Alygizakis N, Gkotsis G, Nika M-C-, Nikolopoulou V, Bizani E, Chadwick E, Cincinelli A, Claßen D, Danielsson S, Dekker RWRJ, Duke G, Glowacka N, Jansman HAH, Krone O, Martellini T, Movalli P, Persson S, Roos A, O'Rourke E, Siebert U, Treu G, van den Brink NW, Anthony Walker L, Deaville R, Slobodnik J and Thomaidis NS (2022). Determination of 56 per- and polyfluoroalkyl substances in top predators and their prey from Northern Europe by LC-MS/MS. Chemosphere 287: 131775.

Badry A, Krone O, Jaspers VLB, Mateo R, García-Fernández A, Leivits M and Shore RF (2020). Towards harmonization of chemical monitoring using avian apex predators: identification of key species for pan-European biomonitoring. Science of the Total Environment 731: 139198.

Bertolero A, Vicente J, Meyer J and Lacorte S (2015). Accumulation and maternal transfer of perfluorooctane sulphonic acid in yellow-legged (*Larus michahellis*) and Audouin's gull (*Larus audouinii*) from the Ebro Delta Natural Park. Environmental Research 137: 208 – 214.

Blévin P, Angelier F, Tartu S, Bustamante P, Herzke D, Moe B, Bech C, Gabrielsen GW, Bustnes JO and Chastel O (2017). Perfluorinated substances and telomeres in an Arctic seabird: cross-sectional and longitudinal approaches. Environmental Pollution 230: 360 – 367.

247 Briels N, Torgersen LN, Castaño-Ortiz JM, Løseth ME, Herzke D, Nygård T, Bustnes JO, Ciesielski TM, Poma
248 G, Malarvannan G, Covaci A, Jaspers VLB (2019). Integrated exposure assessment of northern goshawk
249 (*Accipiter gentilis*) nestlings to legacy and emerging organic pollutants using non-destructive samples.
250 Environmental Research 178: 108678.

251 Bustnes JO, Bårdsen B-J, Herzke D, Bangjord G, Bourgeon S, Fritsch C and Eulaers I (2022). Temporal trends
252 of organochlorine and perfluorinated contaminants in a terrestrial raptor in Northern Europe over 34
253 years (1986 – 2019). Environmental Toxicology and Chemistry 41: 1508 – 1519.

254 Cao X, Wang C, Lu Y, Zhang M, Khan K, Song S, Wang P and Wang C (2019). Occurrence, sources and health
255 risk of polyfluoroalkyl substances (PFASs) in soil, water and sediment from a drinking water source area.
256 Ecotoxicology and Environmental Safety 174: 208 – 217.

257 Chu S, Wang J, Leong G, Woodward LA, Letcher RJ and Li QX (2015). Perfluoroalkyl sulfonates and
258 carboxylic acids in liver, muscle and adipose tissues of black-footed albatross (*Phoebastria nigripes*) from
259 Midway Island, North Pacific Ocean. Chemosphere 138: 60 – 66.

260 Cousins IT, Vestergren R, Wang Z, Scheringer M and McLachlan MS (2016). The precautionary principle
261 and chemicals management: the example of perfluoroalkyl acids in groundwater. Environment
262 International 94: 331 – 340.

263 Custer CM, Custer TW, Schoenfuss HL, Poganski BH and Solem L (2012). Exposure and effects of
264 perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA.
265 Reproductive Toxicology 33: 556 – 562.

266 Custer CM, Custer TW, Dummer PM, Etterson MA, Thogmartin WE, Wu Q, Kannan K, Throwbridge A and
267 McKann PC (2014). Exposure and effects of perfluoroalkyl substances in tree swallows nesting in
268 Minnesota and Wisconsin, USA. Archives of Environmental Contamination and Toxicology 66: 120 – 138.

269 De Silva AO, Armitage JM, Bruton TA, Dassuncao C, Heiger-Bernays W, Hu XC, Kärrman A, Kelly B, Ng C,
270 Robuck A, Sun M, Webster TF and Sunderland EM (2020). PFAS exposure pathways for humans and
271 wildlife: a synthesis of current knowledge and key gaps in understanding. Environmental Toxicology and
272 Chemistry 40: 631 – 657.

273 De Solla SR, de Silva AO and Letcher RJ (2012). Highly elevated levels of perfluorooctane sulfonate and
274 other perfluorinated acids found in biota and surface water down-stream of an international airport,
275 Hamilton, Ontario, Canada. Environment International 39: 19 – 26.

East A, Anderson RH and Salice CJ (2021). Per- and polyfluoroalkyl substances (PFAS) in surface water near US air force bases: prioritizing individual chemicals and mixtures for toxicity testing and risk assessment. Environmental Toxicology and Chemistry 40: 859 – 870.

Faxneld S, Berger U, Helander B, Danielsson S, Miller A, Nyberg E, Persson J-O and Bignert A (2016). Temporal trends and geographical differences of perfluoroalkyl acids in Baltic Sea herring and white-tailed sea eagle eggs in Sweden. Environmental Science and Technology 50: 13070 – 13079.

Giesy JP and Kannan K (2001). Global distribution of perfluorooctane sulfonate in wildlife. Environmental Science and Technology 35: 1339 – 1342.

González-Rubio S, Ballesteros-Gómez A, Asimakopoulos AG and Jaspers VLB (2021). A review on contaminants of emerging concern in European raptors (2002-2020). Science of the Total Environment 760: 143337.

Groffen T, Lasters R, Lopez-Antia A, Prinsen E, Bervoets L and Eens M (2019). Limited reproductive impairment in a passerine bird species exposed along a perfluoroalkyl acid (PFAA) pollution gradient. Science of the Total Environment 652: 718 – 728.

Groffen T, Bervoets L, Jeong Y, Willems T, Eens M and Prinsen E (2021). A rapid method for the detection and quantification of legacy and emerging per- and polyfluoroalkyl substances (PFAS) in bird feathers using UPLC-MS/MS. Journal of Chromatography B 1172: 122653.

Gryz J and Krauze-Gryz D (2019). The common buzzard *Buteo buteo* population in a changing environment, central Poland as a case study. Diversity 11: 35.

Holmström KE, Johansson A-K, Bignert A, Lindberg P and Berger U (2010). Temporal trends of perfluorinated surfactants in Swedish peregrine falcon eggs (*Falco peregrinus*), 1974 – 2007. Environmental Science and Technology 44: 4083 – 4088.

Jaspers VLB, Herzke D, Eulaers I, Gillespie BW and Eens M (2013). Perfluoroalkyl substances in soft tissues and tail feathers of Belgian barn owls (*Tyto alba*) using statistical methods for left-censored data to handle non-detects. Environment International 52: 9 – 16.

Jaspers VB, Covaci A, Herzke D, Eulaers I and Eens M (2019). Bird feathers as a biomonitor for environmental pollutants: prospects and pitfalls. Trends in Analytical Chemistry 118: 223 – 226.

303 Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S and Giesy JP (2002). Perfluorooctanesulfonate and
304 related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the
305 Mediterranean seas. Environmental Science and Technology 36: 3210 – 3216.

306 Land M, de Wit CA, Bignert A, Cousins IT, Herzke D, Johansson JH and Martin JW (2018). Environmental
307 Evidence 7: 4. https://doi.org/10.1186/s13750-017-0114-

308 Licata P, Naccari F, Dugo G, Fotia V, Lo Turco V, Potorti AG and Di Bella G (2012). Organochlorine pesticides
309 and polychlorinated biphenyls in common buzzard (*Buteo buteo*) from Sicily (Italy). Environmental
310 Monitoring and Assessment 184: 2881 – 2892.

311 Lopez-Antia A, Groffen T, Lasters R, AbdElgawad H, Sun J, Asard H, Bervoets L and Eens M (2019).
312 Perfluoroalkyl acids (PFAAs) concentrations and oxidative status in two generations of great tits inhabiting
313 a contamination hotspot. Environmental Science and Technology 53: 1617 – 1626.

314 Meyer J, Jaspers VLB, Eens M and de Coen W (2009). The relationship between perfluorinated chemical
315 levels in the feathers and livers of birds from different trophic levels. Science of the Total Environment
316 407: 5894 – 5900.

317 Miller A, Elliott JE, Elliott KH, Lee S and Cyr F (2015). Temporal trends of perfluoroalkyl substances (PFAS)
318 in eggs of coastal and offshore birds: increasing PFAS levels associated with offshore bird species breeding
319 on the Pacific coast of Canada and wintering near Asia. Environmental Toxicology and Chemistry 34: 1799
320 – 1808.

321 Monclús L, Engvig Løseth M, Dahlberg Persson MJ, Eulaers I, Kleven O, COvaci A, Benskin JP, Awad R,
322 Zubrod JP, Schulz R, Wabakken P, Heggøy O, Jostein Øien I, Steinsvåg MJ, Jaspers VLB and Nygård T (2022).
323 Legacy and emerging organohalogenated compounds in feathers of Eurasian eagle-owls (*Bubo bubo*) in
324 Norway: Spatiotemporal variations and associations with dietary proxies (δ^{13}C and δ^{15}N). Environmental
325 Research 204, Part D: 112372.

326 Newsted JL, Jones PD, Coady KK and Giesy JP (2005). Avian toxicity reference values for perfluorooctane
327 sulfonate. Environmental Science and Technology 39: 9357 – 9362.

328 Park K, Barghi M, Lim J-E, Ko H-M, Nam H-Y, Lee S-I and Moon H-B (2021). Assessment of regional and
329 temporal trends in per- and polyfluoroalkyl substances using the Oriental Magpie (*Pica serica*) in Korea.
330 Science of the Total Environment 793: 148513.

Pérez F, Nadal M, Navarro-Ortega A, Fàbrega F, Domingo JL, Barceló D and Farré M (2013). Accumulation of perfluoroalkyl substances in human tissues. Environment International 59: 354 – 362.

Powley CR, George SW, Ryan TW and Buck RC (2005). Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. Analytical Chemistry 77: 6353 – 6358.

Schultes L, Sandblom O, Broeg K, Bignert A and Benskin JP (2020). Temporal trends (1981 – 2013) of per- and polyfluoroalkyl substances and total fluorine in Baltic cod (*Gadus morhua*). Environmental Toxicology and Chemistry 39: 300 – 309.

Spaan KM, van Noordenburg C, Plassmann MM, Schultes L, Shaw S, Berger M, Heide-Jorgensen MP, Rosing-Asvid A, Granquist SM, Dietz R, Sonne C, Riget F, Roos A and Benskin JP (2020). Fluorine mass balance and suspect screening in marine mammals from the Northern Hemisphere. Environmental Science and Technology 54: 4046 – 4058.

Styler SA, Myers AL and Donaldson DJ (2013). Heterogenous photooxidation of fluorotelomer alcohols: a new source of aerosol-phase perfluorinated carboxylic acids. Environmental Science and Technology 47: 6358 – 6367.

Sun JC, Bossi R, Bustnes JO, Helander B, Boertmann D, Dietz R, Herzke D, Jaspers VLB, Labansen AL, Lepoint G, Schulz R, Sonne C, Thorup K, Tottrup AP, Zubrod JP, Eens M and Eulaers I (2019). White-tailed eagle (*Haliaeetus albicilla*) body feathers document spatiotemporal trends of perfluoroalkyl substances in the northern environment. Environmental Science and Technology 53: 12744 – 12753.

United Nations Environment Programme (UNEP) (2009). SC-4/17: listing of perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride. Conference of the Parties of the Stockholm Convention, United Nations Environment Programma, Geneva. https://www.unep.org/, Accessed 4th Oct. 2021.

United Nations Environment Programme (UNEP) (2020). SC-9/12: listing of perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds. Conference of the Parties of the Stockholm Convention, United Nations Environment Programma, Geneva. https://www.unep.org/, Accessed 4th Oct. 2021.

Vermeersch G, Devos K, Driessens G, Everaert J, Feys S, Herremans M, Onkelinx T, Stienen EWM and T'Jollyn F (2020). Broedvogels in Vlaanderen 2013 – 2018. Recente status en trends van in Vlaanderen

broedende vogelsoorten. Mededelingen van het Instituut voor Natuur en Bosonderzoek 2020 (1), Brussel, 228 p. DOI: doi.org/10.21436/inbor.18794135

Villanueva P (2005). MLE-based procedure for left-censored data excel spreadsheet. Office of Pesticide Programs. U.S. Environmental protection Agency, Washington D.C.

Young CJ and Mabury SA (2010). Atmospheric perfluorinated acid precursors: chemistry, occurrence, and impacts. Reviews of Environmental Contamination and Toxicology 208: 1 – 109.

Appendix

PFAS extraction

Each homogenized sample was spiked with 10 ng of a heavy-labeled perfluoroalkyl carboxylic acid (PFCA) and perfluoroalkyl sulfonic acid (PFSA) mixture (MPFAC-MXA, Wellington Laboratories, Guelph, Canada), containing seven mass-labeled PFCAs and two mass-labeled PFSAs (Table A1). After adding 10 mL of acetonitrile (ACN; Acros Organics BVBA, Belgium), the samples were vortex-mixed and sonicated (Branson 2510) for 3 x 10 min, with vortex-mixing in between periods). Hereafter, the samples were placed overnight on a shaking plate (135 rpm) at room temperature. After vortex-mixing, the samples were centrifuged (4°C, 1037 x g, 10 min, Eppendorf centrifuge 5804R) and the supernatant was transferred to a 15 mL PP tube. These supernatants were dried to approximately 0.5 mL in a rotational-vacuum-concentrator (Eppendorf concentrator 5301) and transferred to polypropylene (PP) Eppendorf tubes that contained 0.1 mL of graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium) and 50 μL of glacial acetic acid. The empty 15 mL tubes, that used to contain the extract, were rinsed twice using 250 μL of ACN and vortex-mixing. This rinse-liquid was then also added to the Eppendorf tubes. After vortex-mixing for at least 1 min, the samples were centrifuged (4°C, 10 min, 9279.4 x g, Eppendorf centrifuge 5415R) and the supernatant was dried completely using the aforementioned vacuum-centrifuge. Finally, the samples were reconstituted with 200 μL of a 2% ammonium hydroxide solution (diluted in ACN), vortex-mixed, and filtered through an Ion Chromatography Acrodisc 13 mm syringe filter with 0.2 μm Supor (polyethersulfone; PES) Membrane (VWR International, Belgium) into a PP auto-injector vial. Procedural blanks followed the same procedure.

UPLC-TQD analysis

Ultra-performance liquid chromatography coupled tandem ES(-) mass spectrometry (UPLC-MS/MS, ACQUITY, TQD, Waters, Milford, MA, USA) was used to analyze the different analytes. As target analytes, we selected eleven PFCAs, six PFSAs, three fluorotelomer sulfonates, sodium

dodecafluoro-3H-4,8-dioxanonanoate (NaDONA), the major and minor components of F-53B (9Cl-PF3ONS and 11Cl-PF3OUdS), GenX (HFPO-DA), three perfluoroether/polyether-carboxylic acids (PF4OPeA, PF5OHxA and 3,6-OPFHpA) and a perfluoroethersulfonate (PFEESA). An ACQUITY BEH C18 column (2.1 x 50 mm; 1.7 μm, Waters, USA) was used to separate the analytes. To retain any PFAS contamination originating from the system, we inserted an ACQUITY BEH C18 pre-column (2.1 x 30 mm; 1.7 μm, Waters USA) between the solvent mixer and the injector. As mobile phase solvents, we used 0.1% formic acid in water and 0.1% formic acid in ACN. The solvent gradient started at 65% of the 0.1% formic acid solution in water. After 3.4 min, this solution decreased to 0% and it returned to 65% at 4.7 min. The flow rate was set at 450 μL/min, with an injection volume of 6 μL (partial loop). Multiple reaction monitoring (MRM) of two diagnostic transitions was used for the majority of analytes to identify and quantify the PFAS. MRM transitions, cone voltages and collision energies of these analytes, including the ISTDs are displayed in Table A1.

Table A1. Full name, abbreviation, MRM transition (precursor and product ion), internal standard (ISTD) used for quantification, cone voltage (V) and collision energy (eV) for the target PFAS and the ISTDs. Mean recovery and standard error (%) are mentioned for the individual ISTDs.

Analyte		**ISTD used for quantification**	**Precursor ion (m/z)**	**Product ion (m/z)**		**Collision energy (eV)**		**Cone voltage (V)**		**Recovery ISTD (%)**
Full name	**Abbreviation**			**Diagnostic product ion 1**	**Diagnostic product ion 2**	**Diagnostic product ion 1**	**Diagnostic product ion 2**	**Diagnostic product ion 1**	**Diagnostic product ion 2**	
Perfluorobutanoic acid	PFBA	$^{13}C_4$-PFBA	213	169	169	19	50	19		
Perfluoropentanoic acid	PFPeA	$^{13}C_4$-PFBA	263	219	219	10	45	15		
Perfluorohexanoic acid	PFHxA	[1,2-$^{13}C_2$]PFHxA	313	269	119	21	65	19		
Perfluoroheptanoic acid	PFHpA	[1,2-$^{13}C_2$]PFHxA	363	319	169	40	30	24		
Perfluorooctanoic acid	PFOA	[1,2,3,4-$^{13}C_2$]PFOA	413	369	169	13	60	22		
Perfluorononanoic acid	PFNA	[1,2,3,4,5-$^{13}C_2$]PFNA	463	419	169	17	20	28		
Perfluorodecanoic acid	PFDA	[1,2-$^{13}C_2$]PFDA	513	469	219	29	29	25		
Perfluoroundecanoic acid	PFUnDA	[1,2-$^{13}C_2$]PFUnDA	563	519	169	30	35	18		
Perfluorododedanoic acid	PFDoDA	[1,2-$^{13}C_2$]PFDoDA	613	569	319	21	30	22		
Perfluorotridecanoic acid	PFTrDA	[1,2-$^{13}C_2$]PFDoDA	663	619	319	21	30	26		
Perfluorotetradecanoic acid	PFTeDA	[1,2-$^{13}C_2$]PFDoDA	713	669	169	21	21	28		
Perfluorobutane sulfonate	PFBS	$^{18}O_2$-PFHxS	299	80	99	65	45	40		

Perfluoropentane sulfonate	PFPeS	[1,2,3,4-$^{13}C_4$]PFOS	349	80	99	40	40	40	35	
Perfluorohexane sulfonate	PFHxS	$^{18}O_2$-PFHxS	399	80	99	30	60	22		
Perfluoroheptane sulfonate	PFHpS	[1,2,3,4-$^{13}C_2$]PFOA	449	80	98.5	47	45	40		
Perfluorooctane sulfonate	PFOS	[1,2,3,4-$^{13}C_4$]PFOS	499	80	99	58	58	60		
Perfluorodecane sulfonate	PFDS	[1,2,3,4-$^{13}C_4$]PFOS	599	80	99	63	63	29		
1H,1H,2H,2H-perfluoro-1-hexanesulfonate	4:2 FTS	[1,2,3,4-$^{13}C_4$]PFOS	327	307	80	25	33	20		
1H,1H,2H,2H-perfluoro-1-octanesulfonate	6:2 FTS	[1,2,3,4-$^{13}C_4$]PFOS	427	407	80	25	33	20		
1H,1H,2H,2H-perfluoro-1-decanesulfonate	8:2 FTS	[1,2,3,4-$^{13}C_4$]PFOS	527	507	81	40	40	36		
Sodium dodecafluoro-3H-4,8-dioxanonanoate	NaDONA	[1,2,3,4-$^{13}C_2$]PFOA	376.8	250.7	84.8	35	32	23		
9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9CL-PF3ONS	[1,2,3,4,5-$^{13}C_2$]PFNA	531	350.5	83	32	37	46	40	
11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11CL-PF3OUdS	[1,2-$^{13}C_2$]PFUnDA	631	451	83	40	35	50	40	
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA (GenX)	[1,2-$^{13}C_2$]PFHxA	285	169		20		30		

Perfluoro-4-oxapentanoic acid	PF4OPeA	[1,2,3,4-$^{13}C_2$]PFOA	228.8	85		20		20		
Perfluoro-5-oxahexanoic acid	PF5OHxA	[1,2-$^{13}C_2$]PFHxA	279	85		20		20		
Perfluoro-3,6,dioxaheptanoic acid	3,6-OPFHpA	[1,2-$^{13}C_2$]PFHxA	201	85		25		30		
Perfluoro(2-ethoxyethane)sulfonate	PFEESA	[1,2-$^{13}C_2$]PFDA	315	135	69	20	55	30	35	
	$^{13}C_4$-PFBA		217	172	172	19	50	19		94.0 ± 3.82
	[1,2-$^{13}C_2$]PFHxA		315	269	119	21	65	19		91.3 ± 8.09
	[1,2,3,4-$^{13}C_2$]PFOA		417	372	172	13	60	22		94.2 ± 1.57
	[1,2,3,4,5-$^{13}C_2$]PFNA		468	423	172	17	20	28		89.4 ± 5.71
	[1,2-$^{13}C_2$]PFDA		515	470	220	29	29	25		93.8 ± 2.60
	[1,2-$^{13}C_2$]PFUnDA		565	520	170	32	35	18		95.8 ± 3.39
	[1,2-$^{13}C_2$]PFDoDA		615	570	320	21	30	22		92.0 ± 3.58
	$^{18}O_2$-PFHxS		403	84	103	30	60	22		82.8 ± 6.82
	[1,2,3,4-$^{13}C_4$]PFOS		503	80	99	58	58	60		86.7 ± 4.41

Table A2. PFAS concentrations (mean and range (between brackets); ng/g ww), Limit of quantification (LOQ; ng/g ww) and frequency of detection (FoD; %) of the analytes that were detected in at least one liver sample from the period 2000 – 2005 (N = 15) or 2021 (N = 10).

Analyte		Time period		LOQ
		2000 – 2005	2021	
PFBA	Concentration	1.14 (<LOQ – 7.44)	0.616 (<LOQ – 1.82)	0.197
	FoD	40	70	
PFOA	Concentration	3.38 (<LOQ – 18.6)	1.26 (0.402 – 3.62)	0.116
	FoD	87	100	
PFNA	Concentration	0.644 (<LOQ – 1.37)	0.788 (<LOQ – 1.40)	0.079
	FoD	93	90	
PFDA	Concentration	0.894 (0.301 – 1.75)	1.14 (<LOQ – 2.50)	0.167
	FoD	100	90	
PFUnDA	Concentration	0.783 (<LOQ – 1.90)	0.78 (<LOQ – 2.01)	0.229
	FoD	80	80	
PFDoDA	Concentration	9.52 (<LOQ – 28.8)	14.5 (1.04 – 34.2)	0.372
	FoD	93	100	
PFTrDA	Concentration	13.1 (1.18 – 32.1)	15.5 (2.32 – 37.4)	0.856
	FoD	100	100	
PFTeDA	Concentration	20.2 (<LOQ – 79.1)	43.6 (17.4 – 104)	0.901
	FoD	87	100	
PFPeS	Concentration	1.23 (<LOQ – 11.4)	<LOQ	0.301
	FoD	60	0	
PFHxS	Concentration	13.1 (<LOQ – 91.9)	3.58 (<LOQ – 11.2)	2.820
	FoD	53	40	
PFHpS	Concentration	3.04 (<LOQ – 9.87)	1.43 (<LOQ – 3.56)	0.585
	FoD	80	60	
PFOS	Concentration	67.1 (4.42 – 162)	41.8 (9.40 – 92.9)	0.181
	FoD	100	100	
6:2 FTS	Concentration	<LOQ	<LOQ (<LOQ – 2.98)	0.731
	FoD	0	10	

Table A3. Limits of quantification (LOQ, ng/g ww) of analytes that were not detected in any of the liver samples.

Analyte	LOQ
PFPeA	0.323
PFHxA	0.284
PFHpA	0.575
PFBS	1.29
PFDS	0.905
11Cl-PF3OUdS	0.348
9Cl-PF3ONS	0.371
4:2 FTS	1.48
8:2 FTS	1.31
NaDONA	0.0870
HFPO-DA	1.84
PFEESA	0.401
PF4OPeA	0.329
PF5OHxA	0.683
3,6-OPFHpA	0.728