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Perfluoroalkylated acids in the eggs of great tits (*Parus major*) near a fluorochemical plant in Flanders, Belgium☆

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ABSTRACT

Perfluoroalkyl acids (PFAAs) are highly persistent substances which have been detected in wildlife around the world, including birds. Although bird eggs have often been used to determine and monitor PFAAs levels in the marine environment, this has rarely been done in the terrestrial environment. In the present study we examined the concentrations and composition profile of 12 PFAAs (4 perfluoroalkyl sulfonic acids (PFSAs) and 8 perfluoroalkyl carboxylic acids (PFCAs) in the eggs of great tits (*Parus major*) collected at a fluorochemical plant and in three other areas, representing a gradient in distance from the pollution source (from 1 to 70 km), in Antwerp, Belgium.

The PFSA concentrations measured at the site of the fluorochemical plant were among the highest ever reported in eggs with median concentrations of 10380 ng/g (extrapolated), 99.3 ng/g and 47.7 ng/g for PFOS, PFHxS and PFDS respectively. Furthermore, the median concentration of 19.8 ng/g for PFOA was also among the highest ever reported in bird eggs. Although these concentrations decreased sharply with distance from the fluorochemical plant, levels found in the adjacent sites were still high compared to what has been reported in literature. Moreover, based on what is known in literature, it is likely that these concentrations may cause toxicological effects. PFOS was the dominant contributor to the PFSA and PFAAs (63.4–97.6%) profile at each site, whereas for PFCAs this was PFOA at the plant site and the nearest locations (41.0–52.8%) but PFDoA (37.7%) at the farthest location.

Although there is some evidence that PFAAs concentrations close to the plant site are decreasing in comparison with earlier measurements, which may be due to the phase out of PFOS, more research is necessary to understand the extent of the toxicological effects in the vicinity of this PFAAs hotspot.

1. Introduction

Perfluoroalkyl acids (PFAAs) have been produced for more than 50 years. The strength and stability of the C-F binding in combination with the hydrophobic and lipophobic character of PFAAs lead to unique physicochemical properties. PFAAs applications include fire-fighting foams, fast food packaging and surface coatings for carpets (Buck et al., 2011; Kissa, 2001). PFAAs are highly persistent and may enter the environment either directly or indirectly from environmental degradation of precursors (Buck et al., 2011; Prevedouros et al., 2006). The widespread use of PFAAs has resulted in a global presence in the environment, wildlife and even humans as described in many studies (e.g., Butt et al., 2010; D'Hollander et al., 2010; Giesy and Kannan, 2001, 2002; Houde et al., 2006; Miller et al., 2015).

The attention of regulatory agencies and researchers has focused on long chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs), because of their higher bioaccumulative potential compared to their short chain analogues (Buck et al., 2011). They are particularly interested in the two most widely known ones: PFOA ($C_7F_{15}COOH$) and PFOS ($C_8F_{17}SO_3H$).

PFOS, PFOA and related compounds have been phased out by

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3M, the major global manufacturer, in 2002, due to their persistence, potential health effects and global distribution. Furthermore, PFOS was included in the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009. These measures, in most cases, appear to be reducing PFOS environmental levels while levels of other PFAAs are still rising (Ahrens et al., 2011; Filipovic et al., 2015; Miller et al., 2015).

Bird eggs have been used in multiple studies to monitor PFAAs levels in many regions of the world (e.g., Gebbink and Letcher, 2012; Giesy and Kannan, 2001; Holmström et al., 2005; Miller et al., 2015; Yoo et al., 2008). However, the majority of these studies have been performed on aquatic birds, whereas data on terrestrial birds, especially passerine birds, remain scarce (Ahrens et al., 2011; Custer et al., 2012; Holmström et al., 2010; Rüdel et al., 2011; Yoo et al., 2008).

Previous studies conducted near a fluorochemical plant in Antwerp, Belgium, revealed the highest PFOS levels ever found in wildlife (Dauwe et al., 2007; D'Hollander et al., 2014; Hoff et al., 2005; Lopez Antia et al., 2017). Liver PFOS levels measured in great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) from this area were higher than those measured in top predators in other regions worldwide, and were also above the benchmark concentrations for the possible risk levels of avian species (Dauwe et al., 2007). Furthermore, PFOS levels in eggs were among the highest ever reported in bird eggs worldwide (Lopez Antia et al., 2017). These studies conducted nearby the fluorochemical plant in Antwerp have demonstrated that PFOS levels measured in wildlife decreased significantly at relatively short distances from the plant site (from 3 to 10 km) on the one hand, and that levels found at these distances are still very high on the other hand. Monitoring PFAAs levels and composition profile in this hot spot and its surroundings is therefore extremely important.

In the present study concentrations of multiple PFAAs were measured in eggs of a terrestrial songbird, the great tit, at a fluorochemical plant in Antwerp. Additionally eggs from three other areas were analyzed, representing a gradient in distance from the pollution source. It is important to compare the levels and composition profile of PFAAs along this distance gradient to better understand the environmental dynamics of PFAAs. Moreover, the outcome of the present study can be used for further monitoring studies, to investigate temporal changes in PFAAs concentrations using 1) minimally invasive sampling, namely eggs (Furness and Greenwood, 1993), and 2) a species that has demonstrated to be useful as sentinel species for local contamination of Persistent Organic Pollutants (Dauwe et al., 2003, 2007; Van den Steen et al., 2006, 2009). Finally, detected levels were used to assess the potential risk to birds based on the current toxicological benchmark levels.

2. Materials and methods

2.1. Study species and sample collection

Great tits, insectivorous songbirds, are increasingly being used in biomonitoring studies because they readily nest in man-made nestboxes, are abundant and can even be attracted to polluted areas (Eens et al., 1999; Eeva and Lehikoinen, 1995, 1996; Eeva et al., 1998; Dauwe et al., 1999, 2004, 2005; Van den Steen et al., 2006).

During the winter of 2011, nestboxes were placed at four sampling sites. Three locations were situated in the vicinity of a perfluorochemical plant (3 M) in Antwerp, Belgium. These locations were the perfluorochemical plant itself (32 nestboxes), Vlietbos (1 km SE from the plant site; 23 nestboxes) and Rot-Middenvijver (shortly Rot; 2.3 km ESE from the plant site; 16 nestboxes). As a reference site, Tessenderlo-Ham (20 nestboxes), approximately 70 km ESE from the plant site was selected, as it is an area without a known perfluorochemical point source in the direct environment.

Nestboxes were checked weekly or daily just before laying to be able to determine the laying date and clutch size. At each site one egg per clutch was collected randomly by hand from 10 to 12 different nestboxes before the incubation had started (early April).

2.2. Chemical analysis

The used abbreviations of PFAAs are according to Buck et al. (2011). The target analytes included 4 PFSAs (PFBS, PFHxS, PFOS and PFDS) and 8 PFCAs (PFBA, PFHxA, PFOA, PFNA, PFDA, PFDoA, PFTrA and PFTeA). The isotopically mass-labelled internal standards (ISTDs) comprised [1,2-$^{13}C_2$]PFHxA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, [1,2,3,4,5,6-$^{13}C_6$]PFDA, [1,2,3,4,5,6,7-$^{13}C_7$]PFUdA, [1,2,3,4,5,6,7-$^{13}C_7$]PFDoA, $^{18}O_2$-PFHxS and $^{13}C_8$-PFOS and were purchased by Wellington Laboratories (Guelph, Canada). HPLC-grade Acetonitrile (ACN) and water (Acros Organics, New Jersey, USA) were used.

2.3. Sample extraction

After removal of the shell, the content of the egg was homogenized with an Ultra Turrax mixer (T25, Staufen, Germany) in a polypropylene (PP) tube and divided into two parts of approximately 0.5 g.

The extraction procedure was based on a method described by Powley et al. (2005) with minor modifications. Samples were spiked with an internal standard mixture (ISTD, 80 μL, 125 pg/μL), containing 125 pg/μL of each ISTD and mixed thoroughly. Hereafter 10 mL acetonitrile was added, samples were sonicated (3 × 10 min) and left overnight at room temperature on a shaking plate. After centrifugation (4 °C, 10 min, 2400 rpm, Eppendorf centrifuge 5804R), the supernatant was transferred to a 15 mL PP tube and reduced to approximately 0.5 mL by using a rotational-vacuum-concentrator at 20 °C (Martin Christ, RVC 2-25, Osterode am Harz, Germany). The concentrated extract and 2 times 250 μL acetonitrile, which was used to rinse the tubes, were transferred to a PP micro centrifuge tube containing 50 mg graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium) and 70 μL glacial acetic acid merely to eliminate pigments. These tubes were vortex-mixed during at least one minute and centrifuged (4 °C, 10 min, 10 000 rpm, Eppendorf centrifuge 5415R). The cleaned-up supernatants were stored at −20 °C until analysis. Before analyses, 70 μL of extract was diluted with 130 μL 2 mM aqueous ammonium acetate and filtrated through an Ion Chromatography Acrodisc 13 mm Syringe Filter with 0.2 μm Supor (PES) Membrane (Leuven, Belgium) attached into a PP auto-injector vial.

2.4. UPLC-TQD analysis

We analyzed PFAAs by UPLC coupled tandem ES(-) mass spectrometry (ACQUITY, TQD, Waters, Milford, MA, USA) using an ACQUITY BEH C18 column (2.1 × 50 mm; 1.7 μm, Waters, USA), mobile phase: 0.1% formic acid in water(A), 0.1% formic acid in acetonitrile(B), solvent gradient: from 65% A to 0% A in 3.4 min and back to 65%A at 4.7 min, flow rate: 450 μL/min, injection volume: 10 μl. To retain any PFAA contamination originating from the system, we inserted an ACQUITY BEH C18 pre-column (2.1 × 30 mm; 1.7 μm, Waters, USA) between the solvent mixer and the injector. Identification and quantification was based on multiple reaction monitoring (MRM) of the following diagnostic transitions: 213 → 169 (PFBA), 313 → 296 (PFHxA), 315 → 270 ($^{13}C_2$-PFHxA), 413 → 369 (PFOA), 421 → 376 ($^{13}C_8$-PFOA), 463 → 419 (PFNA), 472 → 427 ($^{13}C_9$-PFNA), 513 → 469 (PFDA), 519 → 474 ($^{13}C_6$-PFDA), 613 → 569 (PFDoA), 613 → 319 (PFDoA), 615 → 169 ($^{13}C_7$PFDoA),