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PFOS levels in the blood and liver of a small insectivorous songbird near a fluorochemical plant

Tom Dauwe [a,*], Kristin Van de Vijver [b], Wim De Coen [b], Marcel Eens [a]

[a] Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium
[b] Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

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Abstract

Perfluorooctane sulfonate (PFOS) is the stable end product of the degradation of various perfluorinated compounds and is the predominant compound found in the environment and biota. PFOS is a widespread environmental contaminant that is found in a great diversity of wildlife species with more elevated tissue concentrations in animals from populated and industrialized areas. In this study we determined the PFOS accumulation in blood and livers of a small songbird, the great tit (*Parus major*), in the vicinity of a large fluorochemical plant in Antwerp, Belgium. PFOS concentrations ranged from 553 ng/g to 11359 ng/g in liver and ranged from 24 to 1625 ng/ml in blood, which are among the highest ever reported in free-living animals, and exceeded in almost all birds the hepatic benchmark concentrations for the protection of avian species [Beach SA, Newsted JL, Coady K, Giesy JP. Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS). Rev Environ Contam Toxicol 2006;186:133–174]. Although PFOS concentrations in liver and blood decreased significantly within approximately 5.5 km of the plant, differences were smaller than previously described for wood mice (*Apodemus sylvaticus*) and nestling great tits. PFOS concentrations in liver and blood were higher in young birds (<one-year old) than in older birds (>one-year old). No significant sex differences were found. A highly significant correlation between liver and blood concentrations indicates the usefulness of blood as a non-destructive matrix for biomonitoring purposes.

Keywords: Perfluorinated compounds; Biomonitoring; Age and sex effects

1. Introduction

Perfluorinated compounds (PFCs) have been manufactured for over 50 years. They form a very diverse group of chemicals that are used as surfactants, polymers and fire-fighting foams in a variety of industrial and consumer products. Only recently it was discovered that PFCs, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are accumulating in biota throughout the world, even in remote areas such as the Arctic (Kannan et al., 2001a). PFOS is the terminal degradation product of many of the commercially used perfluorinated products and is the predominant PFC found in tissues of wildlife. Similar to organohalogenated compounds, PFOS is extremely persistent and bioaccumulates and biomagnifies in mammals and birds. Most field studies so far have focused on carnivorous and piscivorous animals. Unlike the lipophilic organohalogens however, PFOS strongly binds to blood proteins, such as albumin, and accumulates mainly in the liver and gall bladder (Bossi et al., 2005).

PFCs have been used predominantly in populated and industrial areas and consequently concentrations in wildlife are higher in more populated regions (Giesy and Kannan, 2001). Small songbirds often live in or around urban areas and may thus be suitable sentinel species for local PFOS contamination. To our knowledge however, there are almost no data on PFOS concentrations in songbirds (Hoff et al., 2005). In this study we investigated the accumulation of PFOS in the liver and blood of adult great tits (*Parus major*) near a fluorochemical plant in Antwerp, Belgium, characterized by a high but local contamination with PFOS (Hoff et al., 2004, 2005). Previous

* Corresponding author. Tel.: +32 3 820 22 85; fax: +32 3 820 22 71.
E-mail address: tom.dauwe@ua.ac.be (T. Dauwe).

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studies have illustrated the usefulness of great tits as sentinel species for local heavy metal and organochlorine pollution (Dauwe et al., 2002, 2003; Van den Steen et al., 2006).

2. Materials and methods

2.1. Study site and sampling

The great tit is a small and resident insectivorous songbird that can be found throughout Europe and Northern Africa. It is one of the most common bird species in Europe and can be found in almost any wooded area. Because great tits are hole-nesting species that use nest boxes as roosting and nesting site, they are relatively easy to catch and study, and are increasingly being gused in field ecotoxicological research (Dauwe et al., 2005; Van den Steen et al., 2006). Great tits were studied in an area west of Antwerp (Belgium), characterized by a high contamination with PFOS (Hoff et al., 2004, 2005). The selected study sites were all located within 5.5 km from a large fluorochemical plant (Fig. 1). Great tits were caught in February 2005, while roosting in nest boxes. The sex and age of the great tits were determined. Birds can be classified as either being less than one-year old or more than one-year old on feather color characteristics. After capture 17 birds were killed by decapitation and blood was collected in vials. Livers were immediately incised, stored in microcentrifuge tubes and snap frozen in liquid nitrogen before being transferred to −20 °C.

2.2. PFOS analysis

PFOS extraction and the measurement of PFOS concentrations in liver tissue were done using combined high pressure liquid chromatography–mass spectrometry according to Giesy and Kannan (2001) with minor modifications as previously described by Van de Vijver et al. (2003a) and Hoff et al. (2005). In brief, soft tissue was homogenised on ice with an MSE 150 Watt ultrasonic disintegrator (MSE Scientific instruments, Sussex, UK) with deionized water (1:3, *w/v*). Afterwards, 500 μL of homogenate, 10 μL of internal standard, 1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (Sigma-Aldrich Chemical Company, Milwaukee, WI, USA), 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate solution (TBAS; adjusted to pH 10) and 2 mL of 0.25 M sodium carbonate buffer were thoroughly mixed. Five mL of methyl-*tert*-butyl ether (MBTE) was added and the mixture was shaken for 2 h at 20 °C (250 rpm). The organic and aqueous layers were separated by centrifugation and 5.45 mL was removed from the aqueous layer. After evaporating the solvent under a stream of N_2, the extract was resuspended in 0.5 mL methanol and filtered trough a 0.2 μm nylon mesh filter. High pressure liquid chromatography was done on a CapLC system (Waters, Milford, MA, USA) connected to a Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK). Aliquots of 5 μl were loaded on an Optiguard C18 pre-column (10 mm * 1 mm inner diameter, Alltech, Deerfield, IL, USA). The analysis was performed on a Betasil C18 column (50 mm * 1 mm inner diameter, Keystone Scientific, San Jose, CA, USA) at a flow rate of 40 μl/min. The mobile phase was 2 mM NH_4OAc (A)/CH_3OH (B). A gradient elution was used starting at 45% B and going to 90% B in 3 min. After 5 min initial conditions were resumed. PFOS was measured under negative electrospray ionization using single reactant monitoring (m/z 499→99). The internal standard (1H, 1H, 2H, 2H-perfluorooctane sulfonic acid), was measured under the same conditions (m/z 427→81). The dwell time was 0.1 s. The electrospray-capillary voltage was set at −3.5 kV and the cone voltage was 24 V. The source temperature was 80 °C. The pressure in the collision cell was $3.3 * 10^{-5}$ mm Hg (Ar).

Data quality assurance and quality control protocols included matrix spikes, laboratory blanks and continuing calibration verification for each block of 8 samples. This way, changes in instrument sensitivity could be monitored and matrix effects on ESI suppression/enhancement could be minimized. Recoveries

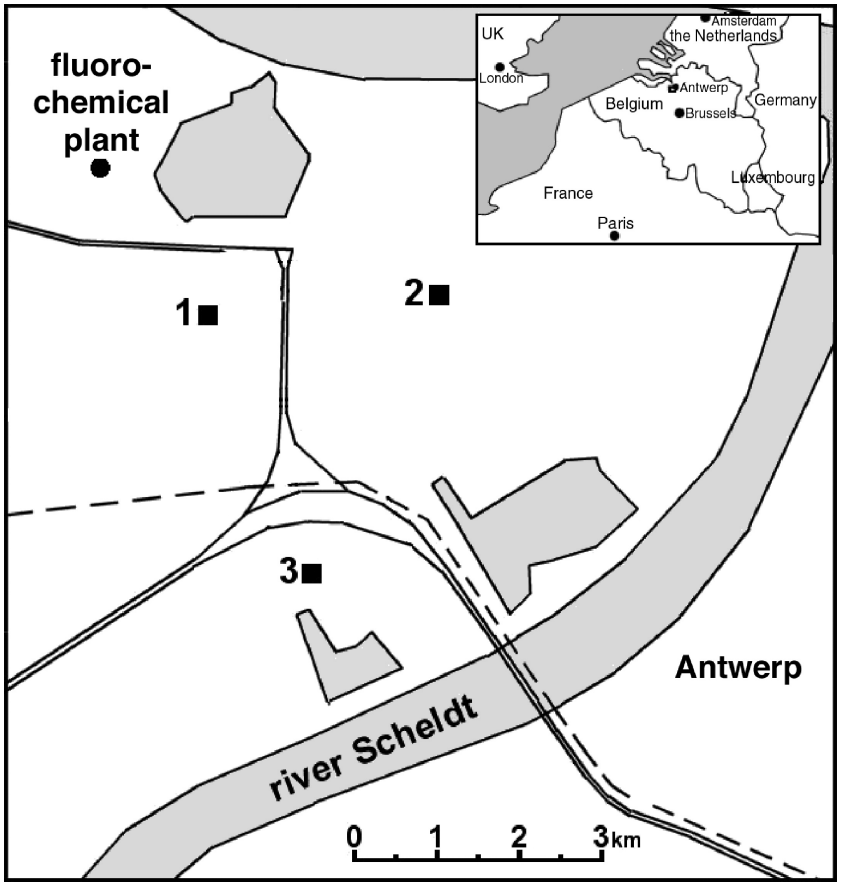

Fig. 1. Location of the three study sites (1. Vlietbos; 2. Rot; and 3. Burchts Weel) in Antwerp, Belgium. Important roads, highways and railways are also indicated.

of spiked samples based on duplicate analysis varied from 78% to 102%. The used standard of PFOS was purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co., Milwaukee, WI, USA). The purity of the standard was 99%. For repeatability testing, three aliquots of a bird liver sample were extracted and analysed. Reproducibility was tested with another bird liver sample. Three aliquots were analysed on different days over a period of 2 months, which included freezing and thawing of the sample homogenate between the different extractions. Samples were run under conditions described above. The percentage relative standard deviation is the standard deviation as a percentage of the mean of the 3 samples analyzed; resulting in percentages of respectively 20% and 14%. The instrumental limit of detection (LOD) for PFOS was determined as three times the signal-to-noise ratio (*S/N*). The LOD of PFOS was 1.5 ng/g wet weight (wet wt). Concentrations were evaluated versus an unextracted standard curve composed of 7 dilutions of a PFOS standard and were not corrected for the recoveries or for the purity of the PFOS standard. The repeatability and reproducibility were done in triplicate and were 80% and 86% respectively.

2.3. Data analysis

All statistical analyses were performed with SPSS (SPSS, 1998). PFOS concentrations in liver and blood were log transformed to fulfill the criteria of normality. Three-way Analysis of Variance (ANOVA) was used to compare PFOS concentrations in liver and blood between sites, sexes and age classes. We started with a model including all main effects and all two-way interactions. Backward elimination of non-significant factors revealed that there were no significant two-way interactions, which were subsequently removed from the final model. We used Pearson correlation to relate PFOS concentrations in blood and liver. Blood concentrations are expressed as ng/ml and liver concentrations as ng/g wet weight (ww).

3. Results and discussion

Concentrations in adult great tits were significantly higher at the site closest to the pollution source, both in liver and in blood (Table 1). Post-hoc comparison revealed that PFOS concentrations in blood differed significantly among all sites (Tukey HSD test, $p<0.05$), while hepatic PFOS concentrations were only significantly higher at Vlietbos compared with Rot and Burchts Weel (Tukey HSD test, $p<0.05$). Young great tits (<one-year old) had higher blood and hepatic concentrations than older great tits (>one-year old; Table 1). Even after elimination of the two individuals (both young females) with the highest PFOS concentrations, this result remained the same. In bottlenose dolphins (*Tursiops truncatus*) and harbor porpoises (*Phocoena phocoena*) concentrations of PFOS were also higher in young animals (Houde et al., 2005; Van de Vijver et al., 2003b). Other studies however have found no (Kannan et al., 2002b) or a positive relationship between PFOS levels and age. In mallards (*Anas platyrhynchos*) for instance, PFOS concentrations increased with age (Sinclair et al., 2006). At present, we have no clear explanation for the observed age effect. We cannot exclude that other factors than age-related differences in accumulation and excretion are possible. For instance, older great tits have been shown to be dominant over younger ones (Carrascal et al., 1998) and in winter may feed more on uncontaminated food from birdfeeders in gardens surrounding the study sites. Nevertheless, PFOS concentrations in nestling birds obtained in the same study area were lower than concentrations found in adult great tits, with the latter accumulating approximately four times more PFOS in liver tissue than nestlings (Hoff et al., 2005). However, we have to point out that nestlings were caught in a different period and year. Considering that the diet of adult great tits in winter (mainly seeds, insects and spiders) is different from the diet during the breeding season (almost exclusively caterpillars and spiders), there may be considerable seasonal variation in PFOS tissue levels, warranting further study.

Table 1
PFOS concentrations in liver (ng/g ww) and blood (ng/ml) of great tits from Antwerp of known sex (M=male, F=female) and age (1=less than one-year old, 2=more than one-year old)

Site	Sex	Age	PFOS liver (ng/g ww)	PFOS blood (ng/ml)
Vlietbos	F	1	11,358	1625.3
	F	1	8705	461.2
	F	2	3897	338.8
	F	1	3819	441.4
	M	2	3527	172.7
	F	2	2174	562.7
	M	2	2034	250.8
Rot	F	1	3022	218.9
	F	1	2420	233.7
	M	2	553	154.1
Burchts Weel	M	1	1775	93.0
	F	1	1584	112.5
	F	1	1336	123.2
	F	2	986	NA
	F	2	929	24.3
	M	1	638	70.1
	M	2	629	66.1

ANOVA table	*F* (*p*)	*F* (*p*)
Site	11.5 (0.002)	37.2 (<0.001)
Age	3.45 (0.069)	14.8 (0.001)
Sex	2.50 (0.14)	0.006 (0.9)

Results of the three-way ANOVA on log-transformed PFOS concentrations (only including the main effects as variables) are also given.

PFOS concentrations in liver and blood did not differ significantly between males and females. Experimental trials have shown that females have a much higher clearance rate of PFOS and subsequently accumulate lower concentrations than males (Newsted et al., 2005). In free-living animals however, most studies have failed to find significant differences between sexes (Houde et al., 2005; Kannan et al., 2002b,c). Sinclair et al. (2006) did find significantly higher concentrations in male common mergansers (*Mergus merganser*) than in females, but this difference was presumable caused by a large sexual size dimorphism. Several other waterfowl species that they studied (with smaller size differences between sexes), showed no sex-related differences in PFOS accumulation (Sinclair et al., 2006).

The PFOS concentrations in livers and blood of great tits from this study are among the highest reported in literature (Table 1). Even in top predators concentrations are in most cases lower. PFOS concentrations in bald eagles (*Haliaeetus leucocephalus*), from the USA ranged from <1 to 2220 ng/ml in plasma (Kannan et al., 2001b) and from 26.5 to 1740 ng/g ww in livers (Kannan et al., 2005). White-tailed sea eagles (*Haliaeetus albicilla*) from Eastern Germany and Poland had PFOS concentrations ranging from <3.9 to 127 ng/g ww (Kannan et al., 2002a). Considering that the insectivorous great tits are low on the food chain, top predators in this area (such as sparrow hawks, *Accipiter nissus*, that feed primarily on great tits and other small birds) could suffer even higher concentrations.

Previous studies in the same study area also showed that it was extremely contaminated with PFOS. In wood mice (*Apodemus sylvaticus*) PFOS concentrations ranged between 470 and 178550 ng/g ww (Hoff et al., 2004). Although, the highest concentration found in wood mice was 10 times higher than the highest concentration in adult great tits, median concentrations did not differ much (*i.e.* wood mouse: 5060 ng/g ww, great tit: 3820 ng/g ww liver). Interestingly, great tits caught approximately 5.5 km from the pollution source (at Burchts Weel), still accumulated very high levels of PFOS in liver and blood (Table 1). Median concentrations were 4 times lower than in the most polluted site. In contrast, hepatic concentrations in wood mice decreased 18 times over a similar distance (Hoff et al., 2004). PFOS concentrations in nestling great tits also decreased sharply with increasing distance from the pollution source (Hoff et al., 2005). The high concentrations of PFOS in adult great tits from Burchts Weel suggest that due to their mobility and extended home range, great tits (and presumably also other passerines) from a larger area surrounding the pollution source may experience negative effects from the local PFOS contamination. Nestling great tits are fed with food items collected within close proximity of the nesting site, so PFOS concentrations will reflect local contamination better than adults.

In their review of several avian toxicological studies, Newsted et al. (2005) concluded that hepatic PFOS concentrations corresponding to the

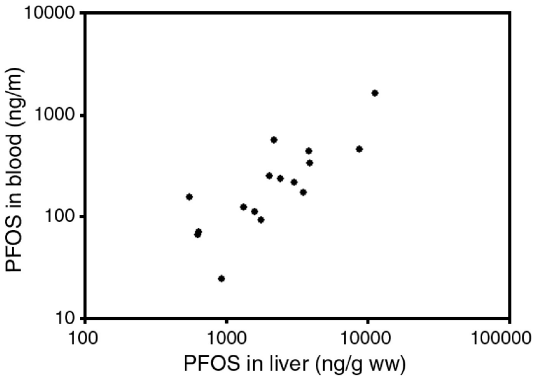

Fig. 2. Relationship between PFOS concentrations in livers and blood of adult great tits (Pearson correlation, $N=16$, $r=0.80$, $p<0.001$).

lowest adverse effect level (LOAEL) from reproductive studies was 9600 ng/g ww for male mallards and was 2900 ng/g ww for male and 4900 ng/g ww for female northern bobwhite quail (*Colinus virginianus*). Some great tits in our study (Table 1), exceeded the LOAEL. If we take into account the effect of long-term exposure to environmental contaminants in the wild and use an acute-to-chronic application factor of 0.1 (Newell et al., 1987; Sinclair et al., 2006), almost all great tits had higher hepatic PFOS concentrations than the reported LOAEL. In a recent review article, the hepatic benchmark concentration for the protection of avian species was set at 600 ng/g ww, although no-effect levels in laboratory studies suggest that actual population-level effects would only occur when hepatic concentrations of 5000 ng/g ww are exceeded (Beach et al., 2006). In this study, only one great tit had hepatic PFOS concentrations lower than the benchmark concentration of 600 ng/g ww. The median hepatic concentration at the most polluted site (3820 ng/g ww) almost reached the PFOS concentration inducing population-level effects. Exposure to PFOS primarily affects the liver and causes vacuolation and hypertrophy of hepatic cells (Seacat et al., 2002). Near the same fluorochemical plant, Hoff et al. (2005) found that PFOS significantly affected alanine aminotransferase, triglycerides and cholesterol levels in plasma of nestling great tits.

PFOS concentrations in blood and liver were highly correlated with each other (Pearson, $N=16$, $r=0.80$, $p<0.001$; Fig. 2). Other studies have also found that PFOS concentrations in blood and liver correlated well (Kannan et al., 2002a). Blood is thus a suitable and non-destructive alternative to tissue sampling for monitoring PFOS exposure. The blood to liver ratio was however very high (median 10.6, range 3.6–38.2) and was higher than reported for mammals and fish (Kannan et al., 2001a,b).

4. Conclusion

PFCs have been used for over 50 years in a variety of industrial and consumer products. Although PFCs have been distributed worldwide, contamination is higher near densely populated and industrialized areas (Giesy and Kannan, 2001). This study shows that small songbirds that live in and near urban areas, such as the great tit, are suitable sentinel species for local PFOS contamination. The concentrations found in liver and blood near a fluoropolymer factory are among the highest reported for birds, and exceeded in almost all individuals the benchmark concentrations for the protection of avian species (Beach et al., 2006). In nestling great tits, alanine aminotransferase, triglycerides and cholesterol levels in plasma were significantly affected at lower hepatic PFOS concentrations than were found in the present study. The high PFOS contamination in this area thus potentially poses a risk to the health of great tits and possibly also other wildlife species and even humans.

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