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Evaluation of biochemical effects related to perfluorooctane sulfonic acid exposure in organohalogen-contaminated great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings

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Abstract

A perfluorooctane sulfonic acid (PFOS) biomonitoring survey was conducted on great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings from Blokkersdijk, a bird reserve in the proximity of a fluorochemical plant in Antwerp (Belgium) and Fort IV, a control area. PFOS, together with 11 organochlorine pesticides, 20 polychlorinated biphenyl congeners and 7 polybrominated diphenyl ethers were measured in liver tissue. The hepatic PFOS concentrations at Blokkersdijk (86–2788 and 317–3322 ng/g wet weight (ww) for great and blue tit, respectively) were among the highest ever measured and were significantly higher than at the control area (17–206 and 69–514 ng/g ww for great and blue tit, respectively). The hepatic PFOS concentration was species- and sex-independent and correlated significantly and positively with the serum alanine aminotransferase activity and negatively with the serum cholesterol and triglyceride levels in both species but did not correlate with condition or serum protein concentration. In the great tit, a significant positive correlation was observed between the liver PFOS concentration and the relative liver weight. In the blue tit, the hepatic PFOS concentration correlated positively and significantly with hematocrite values. None of the investigated organohalogen pollutants except for PFOS were suggested to be involved in the observed biological alterations.

Abbreviations: PFOS, perfluorooctane sulfonic acid; ww, wet weight; ALT, alanine aminotransferase; OCP, organochlorine pesticide; PCB, polychlorinated biphenyl; PBDE, polybrominated biphenyl ether; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; OxC, oxychlordane; TN, *trans*-nonachlor; TC, *trans*-chlordane; CC, *cis*-chlordane; *p,p′*-DDE, *p,p′*-dichlorodiphenyldichloroethylene; *p,p′*-DDD, *p,p′*-dichlorodiphenyldichloroethane; *p,p′*-DDT, *p,p′*-dichlorodiphenyltrichloroethane; PLS, partial least square; LOQ, limit of quantitation; PFOSA, perfluorooctanesulfonamide; LOEL, lowest observed effect level; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level.

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1. Introduction

PFOS is a perfluorooctanesulfonylfluoride based chemical that is mainly produced by the 3M Company using electrochemical fluorination of octanesulfonyl fluoride. In 2000, the volume of PFOS and its salts that were commercialized as finished products was less than 91 metric tons. PFOS is also used as a chemical intermediate for the production of PFOS-based chemicals. These chemicals can be divided in three main classes: products for surface treatment applications such as textile, leather and carpet production and aftermarket treatment, products for paper protection for food and non-food applications and performance chemicals such as fire fighting foams, insecticides and floor polishes. The estimated global production volume of these PFOS-based chemicals was 4481 metric tons in 2000 although production volumes are expected to decline because 3 M announced phasing out the production of POSF-derived chemicals in May 2000 (OECD, 2002).

PFOS is a widespread environmental contaminant that is found in a great diversity of wildlife species with more elevated tissue concentrations in populated and industrialized areas (Giesy and Kannan, 2001). PFOS is generally measured at concentrations <1000 ng/g in tissues from a great diversity of aquatic birds species from Japan, Korea, Canada, the USA, the Northern Pacific region and Europe although PFOS concentrations up to 2570 ng/ml (bald eagle plasma, USA) have been reported (Giesy and Kannan, 2001; Kannan et al., 2001a, 2002a,b; Rattner et al., 2004).

Hoff et al. (2004) measured extremely elevated hepatic PFOS concentrations up to 178 550 ng/g wet weight (ww) in wood mice (*Apodemus sylvaticus*) from Blokkersdijk, in the proximity of a fluorochemical production unit (Antwerp, Belgium). Due to the PFOS contamination degree of the wood mice and to the status of Blokkersdijk as an area protected by the European Council directive 79/409/EEC on the conservation of wild birds (1979), we assessed liver PFOS concentrations in great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings from Blokkersdijk and Fort IV, a park presumed to be a reference area for PFOS contamination. Birds in their pre-fledging stage were chosen because they provide clear advantages for local biomonitoring: their mobility is low in contradiction to adult tits which are basically resident but can make eruptive movements over a distance of several hundreds of kilometers and generally return early in spring to establish breeding territories. The food origin of tit nestlings is another factor contributing to the suitability of tit nestlings as biomonitors because their food, predominantly consisting of lepidopterous larvae, is generally captured by the parents within territorial boundaries in which the parents are foraging (≈0.5 ha in deciduous habitats, Cramp and Perrins, 1993). Also, very young wood mice from Blokkersdijk have considerably elevated hepatic PFOS concentrations (Hoff et al., 2004) showing that young animals can be good indicators of PFOS contamination. Common PFOS exposure routes for wood mice and tit nestlings are not excluded because next to plant material, lepidopterous larvae are also food items for wood mice (Corbet and Harris, 1991).

Known in vivo effects of PFOS are increased relative liver weight, induced peroxisomal liver fatty acid β-oxidation and lowered serum cholesterol and triglyceride concentrations (Ikeda et al., 1987; Haughom and Spydevold, 1992; Sohlenius et al., 1993; Seacat et al., 2002, 2003). PFOS exposure also increases the serum alanine aminotransferase (ALT) activity, which is a marker for hepatic damage (Hoff et al., 2003; Seacat et al., 2003). Other in vivo effects are the inhibition of gap junction intercellular communication (Hu et al., 2002), the induction of carboxylesterase expression (Derbel et al., 1996), neuroendocrine effects (Austin et al., 2003) and the occurrence of developmental effects (Lau et al., 2004; Thibodeaux et al., 2003).

In order to evaluate some of these effects for PFOS-contaminated tit nestlings, relationships were evaluated between the hepatic PFOS concentration and the following selected endpoints: relative liver weight, serum cholesterol, triglyceride levels and ALT activity. Also body condition, serum protein concentration and hematocrite were assessed because previous PFOS investigations have demonstrated significant correlations between hepatic PFOS levels and the latter two endpoints (Hoff et al., 2005).

In addition to PFOS, 11 organochlorine pesticides (OCPs), 20 polychlorinated biphenyls (PCBs) and 7 polybrominated biphenyl ethers (PBDEs) were measured in liver tissue for two reasons. Firstly, tits have been demonstrated to be contaminated with organochlorine compounds in the Antwerp region (Dauwe et al., 2003). Secondly, laboratory controlled experiments show that organochlorine contamination of juvenile birds can potentially affect a considerable part of the endpoints under consideration in this study. In American kestrel (*Falco sparverius*) nestlings for example, PCB exposure has been shown to induce the liver weight and the serum ALT activity (Hoffman et al., 1996). In PCB fed broiler chicks, the liver weight, serum cholesterol and triglyceride concentrations were increased while the hematocrite was decreased (Kosutzky et al., 1993; Kosutzky and Scrobanek, 1994). Also OCPs, such

as hexachlorobenzene and chlordane can potentially alter the liver weight, the serum ALT activity and the serum cholesterol concentration as has been shown in rodents (Almeida et al., 1997; Khasawinah and Grutsch, 1989). In humans, serum cholesterol concentrations have been demonstrated to be significantly and positively correlated with tissue DDE concentrations (Laden et al., 1999).

2. Materials and methods

2.1. Sampling

Between May 11 and 24, 2004, 48 great tit and 33 blue tit pre-fledging nestlings (17–20 days old) were collected. Great tit nestlings were collected from nest boxes (M1–4) in the nature reserve Blokkersdijk (Antwerp, Belgium), an artificial sand dune habitat with mainly willow and poplar groves situated next to a fluorochemical plant production site and from three nest boxes (M5–7) in Fort IV (Mortsel, Belgium), a park with loam soil and groves with deciduous trees (mainly beech) 10 kilometers south-east. Blue tit nestlings were taken from two boxes in Blokkersdijk (C1–2) and two in Fort IV (C3–4, Fig. 1). In Blokkersdijk, nest boxes were closely situated to a pond. In Fort IV, the sampled nest boxes were situated within a radius of about 0.5 km. All nest boxes were located in groves with deciduous trees (willow, beech, poplar). The boxes were installed at least six months before the onset of the breeding season. Before decapitation, the birds were weighed with an accuracy of 0.1 g with a Pesola spring balance. After decapitation, blood was collected with glass capillaries and serum was prepared by centrifugation (4000 rpm, 5 min) and stored in liquid nitrogen. For the hematocrite measurement, blood was collected with heparinized capillaries. The liver was immediately excised, weighed and stored in liquid nitrogen. The tarsus length was determined with an accuracy of 0.1 mm with digital callipers. The condition index was calculated as the residual from a linear regression of the tarsus length and body weight and has been shown to predict survival probability relatively well (Merilä, 1997).

2.2. Sex determination

DNA was extracted from the shaft of the tail feathers using the Dneasy Tissue Kit (Qiagen, Venlo, The Netherlands). A PCR reaction amplifying the CHD-W and CHD-Z genes was carried out as described in Griffiths et al. (1998) with small modifications.

2.3. Biochemical assays

The serum alanine aminotransferase activity was determined by the spectrophotometric method of Bergmeyer et al. (1986). Cholesterol concentrations were measured according to Allain et al. (1974) and the triglyceride concentration was measured according to the method of Spayd et al. (1978). The serum protein content was determined with the Bio-Rad Protein Assay (Bio-Rad, Munich, Germany). For the determination of the hematocrite, the relative red blood cell volume was determined after centrifugation of heparinized blood in sealed glass capillaries (2000 rpm, 5 min).

2.4. Determination of liver PFOS concentrations

PFOS extraction and the measurement of PFOS concentrations in liver tissue were done using combined high pressure liquid chromatography–mass spectrome-

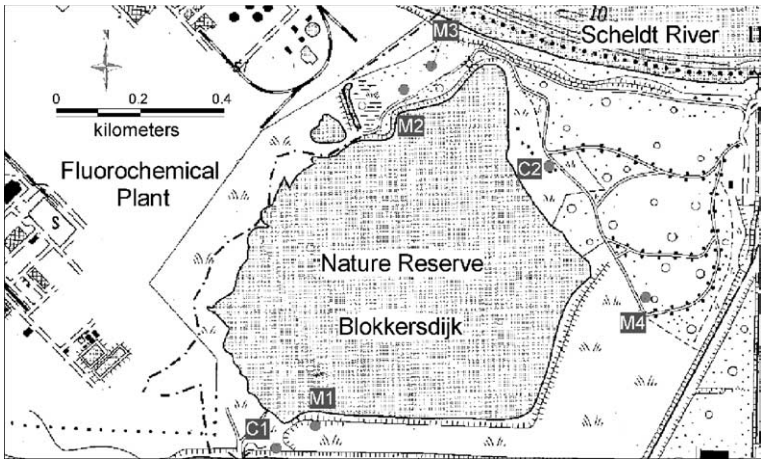

Fig. 1. Area of study and sampling locations. The Blokkersdijk nest boxes are indicated with black squares. Great tit nest boxes are M1–4, blue tit nest boxes are C1–2.

try according to Giesy and Kannan (2001) with minor modifications as previously described by Van de Vijver et al. (2003). In contradiction to Van de Vijver et al. (2003), the internal standard (1H, 1H, 2H, 2H-perfluorooctane sulfonic acid) was added to the liver tissue before homogenization. The analytical procedure (amount of added internal standards, solvent volumes used) was adapted according to the available liver tissue masses. The liver tissue masses used for PFOS extraction ranged between 140 and 300 mg. 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). PFOS concentrations were calculated using an unextracted calibration curve that was constructed using a dilution series of PFOS in MeOH. Internal standard was added to these serial PFOS dilutions at the same concentration as added to the liver tissue samples. The PFOS concentrations were calculated using a linear calibration curve ($R^2 = 0.99$) of a plot of $\log[I(499 \rightarrow 99)/I(427 \rightarrow 81)]$ versus log[PFOS concentration] where "I" is the peak area. Repeatability was 77%.

Internal standard concentrations in unspiked liver samples were not above the limit of quantification in nestlings from Blokkersdijk or Fort IV ($n = 5$ for each location). The LOQ for PFOS was 10 ng/g ww.

2.5. Determination of liver concentrations of polychlorinated and polybrominated pollutants

The OCPs under investigation were α-, β-, γ-isomers of hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), oxychlordane (OxC), *trans*-nonachlor (TN), *trans*-(TC), *cis*-chlordane (CC), *p*,*p*′-dichlorodiphenyldichloroethylene (*p*,*p*′-DDE), *p*,*p*′-dichlorodiphenyldichloroethane (*p*,*p*′-DDD) and *p*,*p*′-dichlorodiphenyltrichloroethane (*p*,*p*′-DDT). The following PCB congeners (IUPAC numbers) were targeted: 28, 52, 74, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 167, 170, 180, 183, 187, 194 and 199. PBDE congeners 28, 47, 99, 100, 153, 154 and 183 were also included. The method used for sample preparation and analysis was described in detail by Dauwe et al. (2003) and Voorspoels et al. (2003). Briefly, the available amount of tissue (150–350 mg) was ground with Na_2SO_4, internal standards were added and the mixture was extracted for 2 h with 75 ml hexane:acetone = 3:1 into a hot Soxhlet manifold. After concentration, the extract was subjected to clean-up on acidified silica and analytes were eluted with 15 ml *n*-hexane followed by 10 ml dichloromethane. The eluate was concentrated to 80 μl and transferred to an injection vial. PCBs were determined on a HP 6890 gas chromatograph (GC)–5793 mass spectrometer (MS, Hewlett Packard, Palo Alto, CA, USA) operated in electron impact ionisation mode and equipped with a 30 m × 0.25 mm × 0.25 μm DB-1 capillary column (J & W Scientific, Folsom, CA, USA). PBDEs and OCPs were determined on a HP 6890 GC–MS operated in negative chemical ionisation and equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE Scientific, Zulte, Belgium). Instrumental operating conditions and quality control were detailed presented by Dauwe et al. (2003) and Voorspoels et al. (2003). Limits of quantification (LOQ) for individual PCB congeners ranged between 0.5 and 1 ng/g ww, while for OCPs and PBDEs, they were 0.2 and 0.1 ng/g ww, respectively.

2.6. Statistics

The liver PFOS concentrations in great and blue tit nestlings from Blokkersdijk and Fort IV were compared with the non-parametric Mann–Whitney U test. This test was also used for comparison of hepatic PFOS concentrations between sexes and species for Blokkersdijk and Fort IV. For each species, differences in liver PFOS concentration between boxes were investigated using the non-parametric Kruskal–Wallis test with Dunn's test as post hoc criterion. The values of the biological endpoints investigated (serum ALT activity, cholesterol, triglyceride and protein concentrations, hematocrite and condition index) were compared between males and females for Blokkersdijk and Fort IV with the non-parametric Mann–Whitney U test. Partial Least Square (PLS) analysis models were constructed to establish relationships between the measured pollutants and the biological endpoints. The nestlings for which at least 60% and 56% (for great tit and blue tit, respectively) of the measured organohalogens were <LOQ and the organohalogens for which the variance was close to zero or the concentration <LOQ were excluded for PLS analysis. Spearman rank correlation analysis was used to establish relationships between the liver PFOS concentration and the relative liver weight, the serum ALT activity, cholesterol, triglyceride, protein concentrations,

hematocrite and condition index. Correlation analysis was also used to investigate the relationships between the other compounds and the latter endpoints if these compounds were quantifiable in >50% of the nestlings.

3. Results

The liver PFOS concentrations in great and blue tit liver were significantly higher in Blokkersdijk compared with Fort IV ($p < 0.001$ for both species). No significant sex or species differences in hepatic PFOS concentrations were observed in Blokkersdijk or Fort IV ($p > 0.05$). In Fig. 2a and b it can be seen that the PFOS concentrations in nestlings from the same location were not significantly different within one species. However, the hepatic PFOS concentrations in nestlings from nestboxes at the eastside of the Blokkersdijk pond (boxes M4 and C2 for great and blue tit, respectively) tended to be lower than at the westside (boxes M1–3 for great tit and box C1 for blue tit) of the pond although the differences were not statistically significant.

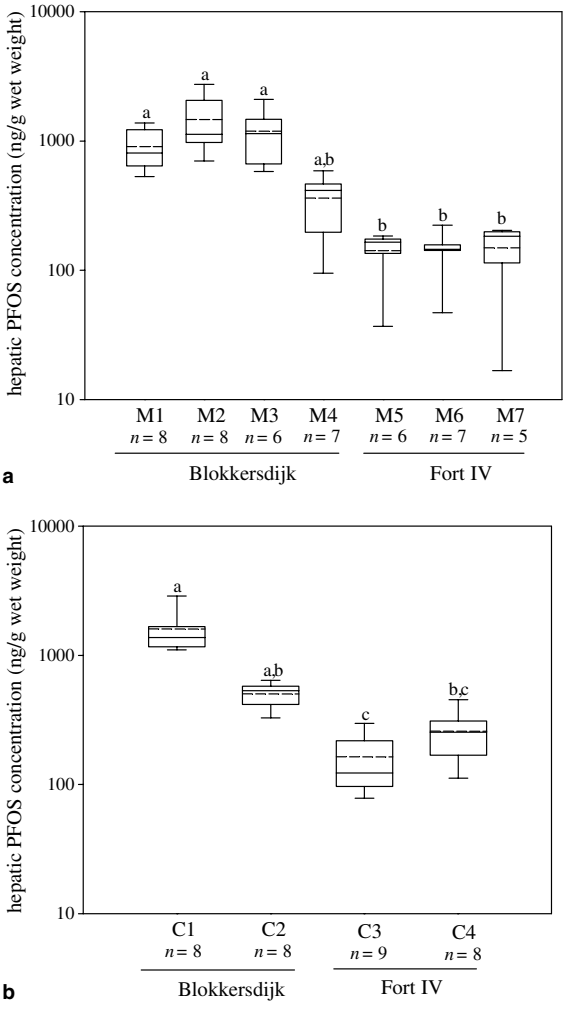

Fig. 2. Hepatic PFOS concentrations in great tit (a) and blue tit (b) nestlings. The straight line is the median and the dotted line represents the mean. The 25th and 75th percentiles define the boxes. The whiskers represent the 10th and 90th percentiles. Boxes having different letters are significantly different ($p < 0.05$). n = number of nestlings.

The concentration ranges of measured organohalogen pollutants are shown in Table 1. PFOS was present in liver tissue of all nestlings measured at higher concentrations than the other organohalogens.

The values of all the biological endpoints did not differ significantly between sexes, neither in Blokkersdijk, nor in Fort IV ($p > 0.05$).

No significant correlations ($p > 0.05$) were found between the hepatic PFOS concentration and the serum protein content ($r = -0.13$, $p = 0.4$, $n = 45$; $r = -0.27$, $p = 0.2$, $n = 26$ for great and blue tits, respectively) or condition index ($r = 0.01$, $p = 0.9$, $n = 45$; $r = 0.02$, $p = 0.9$, $n = 30$ for great and blue tits, respectively). Both in great and blue tits, the liver PFOS concentration correlated significantly with the serum ALT activity and the cholesterol and triglyceride levels. In the great but not in the blue tit, correlation analysis also showed significant correlations between the hepatic PFOS concentration and the relative liver weight. In blue but not in great tit nestlings, the liver PFOS concentration was positively and significantly related to hematocrite (Figs. 3 and 4).

PLS analysis of the relations between the hepatic pollutant concentrations and the biological endpoints did not result in robust models ($Q^2 < 0.10$ for both species).

No significant correlations were observed between biological endpoints and compounds other than PFOS.

4. Discussion

The high PFOS concentrations found in the Blokkersdijk tits confirm that the Blokkersdijk nature reserve is severely PFOS polluted what is in agreement with PFOS measurements in Blokkersdijk wood mice and carps (*Cyprinus carpio*) in which the average liver PFOS concentrations were 2618 and 1241 ng/g ww, respectively (Hoff et al., 2004; Hoff et al., 2005).

The measured liver PFOS concentrations in the Blokkersdijk nestlings were significantly higher than in Fort IV. This suggests that the fluorochemical plant might be a source of PFOS release and/or PFOS precursor release in the environment as recent studies have shown that several perfluorinated compounds can be metabolised to PFOS. Xu et al. (2004) showed that *N*-ethyl-*N*-(2-hydroxyethyl)perfluorooctanesulfonamide can undergo *N*-deethylation to *N*-(2-hydroxyethyl)per-

Table 1
Ranges and mean concentrations (in brackets) expressed in ng/g wet weight for organohalogens measured in liver of great and blue tits nestlings

Compound	Great tit Blokkersdijk (n = 29–30)	Great tit Fort IV (n = 18)	Blue tit Blokkersdijk (n = 16)	Blue tit Fort IV (n = 7–19)
PFOS	86–2788 (994)	17–206 (146)	317–3323 (1055)	69–514 (210)
α-HCH	<0.2	<0.2	<0.2	<0.2
β-HCH	<0.2	<0.2	<0.2	<0.2
γ-HCH	<0.2	<0.2	<0.2	<0.2
p,p′-DDE	2.7–9.1 (5.0)	<0.2–1.6	0.8–2.3 (1.7)	<0.2–1.0
p,p′-DDD	<0.2	<0.2	<0.2	<0.2
p,p′-DDT	<0.2	<0.2	<0.2	<0.2
HCB	<0.2–0.5	<0.2–0.4	<0.2	<0.2–0.3
OxC	<0.2	<0.2	<0.2	<0.2
TN	<0.2	<0.2	<0.2	<0.2
TC	<0.2	<0.2	<0.2	<0.2
CC	<0.2	<0.2	<0.2	<0.2
PCB 28	<1.0	<1.0	<1.0	<1.0
PCB 52	<1.0	<1.0	<1.0	<1.0
PCB 74	<1.0	<1.0	<1.0	<1.0
PCB 99	1.3–5.1 (2.9)	<1.0	<1.0–1.5	<1.0
PCB 101	1.4–5.0 (2.4)	<1.0	<1–2.3	<1.0
PCB 105	<1.0	<1.0	<1.0	<1.0
PCB 110	1.2–5.5 (2.3)	<1.0	<1.0–1.5	<1.0
PCB 118	1.4–13.9 (5.7)	<1.0	1.9–6.3 (3.1)	<1.0–2.9
PCB 128	0.9–3.2 (1.6)	<0.5	<0.5–0.9	<0.5
PCB 138	6.0–35.1 (19.6)	0.8–2.6 (1.5)	5.2–13.2 (8.8)	0.8–2.4 (1.4)
PCB 149	1.5–8.8 (4.9)	<0.5–1.3	1.4–2.8 (1.9)	<0.5–0.9
PCB 153	6.7–38.5 (23.0)	0.8–2.8 (2.0)	6.2–15.8 (10.7)	0.9–2.6 (1.5)
PCB 156	<0.5–2.1	<0.5	<0.5–0.9	<0.5
PCB 167	<0.5–1.5	<0.5	<0.5	<0.5
PCB 170	1.3–8.4 (4.9)	<0.5	<0.5–2.5	<0.5
PCB 180	4.0–23.7 (14.2)	<0.5–1.4	2.7–9.3 (5.6)	<0.5–1.6
PCB 183	1.5–3.0 (2.4)	<0.5	<0.5–1.3	<0.5
PCB 187	2.1–10.7 (6.6)	<0.5–0.7	1.6–3.6 (2.5)	<0.5
PCB 194	<0.5–2.6	<0.5	<0.5	<0.5
PCB 199	<0.5–2.6	<0.5	<0.5	<0.5
PBDE 28	<0.1	<0.1	<0.1	<0.1
PBDE 47	<0.1–1.3	<0.1–0.9	<0.1–1.3	<0.1–6.1
PBDE 99	<0.1–1.1	<0.1–0.6	<0.1–0.5	<0.1–4.6
PBDE 100	<0.1–0.2	<0.1	<0.1	<0.1–0.8
PBDE 153	<0.1–0.2	<0.1	<0.1	<0.1–0.2
PBDE 154	<0.1	<0.1	<0.1	<0.1–0.2
PBDE 183	<0.1–0.2	<0.1	<0.1	<0.1

n = number of nestlings.

fluorooctanesulfonamide that can be deethylated to perfluorooctanesulfonamide (PFOSA). PFOSA can undergo metabolisation to PFOS in rat liver slices. In rainbow trout (*Oncorynchus mykiss*) microsomes it has been shown that *N*-ethylperfluorooctanesulfonamide can be converted to PFOS (Tomy et al., 2004). These precursors could partly be responsible for the presence of PFOS in liver tissue of Fort IV nestlings because they are more volatile than PFOS. Considering the prevailing wind direction (west, southwest) and the geographical location of Fort IV (10 km southeast from the fluorochemical plant), the plant might be a direct PFOS pollution source for the Fort IV nestlings if airborne pollution is considered.

An indication that the liver PFOS concentration is decreasing rather steeply with increasing distance from the plant, is supported by the observations that nestlings from nestboxes at the eastern side of the Blokkersdijk pond (boxes M4 and C2 for great and blue tit, respectively) had lower hepatic PFOS concentrations than the boxes at the western side although the differences were not significant.

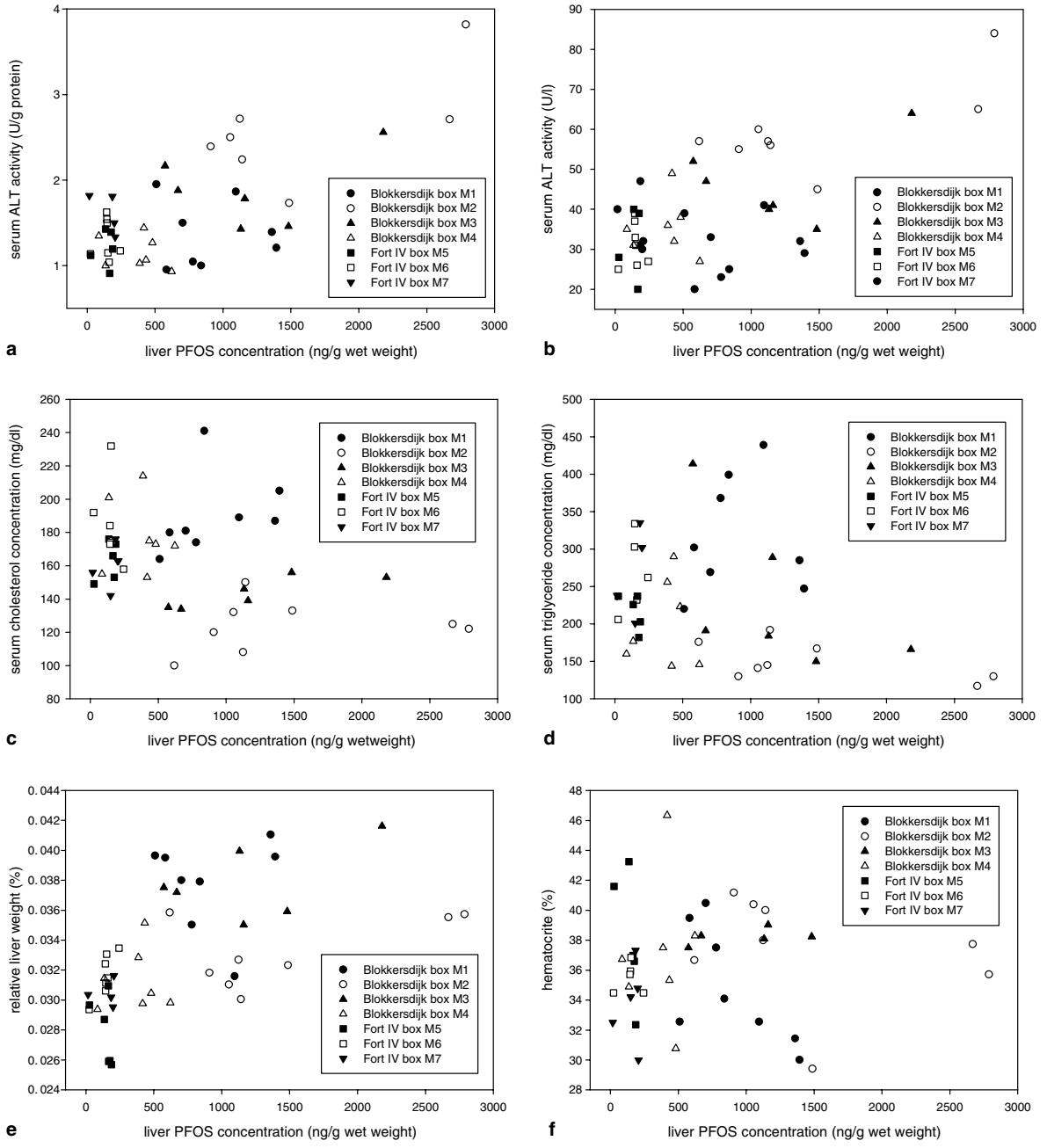

Fig. 3. The relationships between the great tit nestling liver PFOS concentration and the serum ALT activity [(a) $r = 0.45$, $p = 0.002$**, $n = 45$; (b) $r = 0.37$, $p = 0.01$*, $n = 45$], serum cholesterol [(c) $r = -0.34$, $p = 0.02$*, $n = 46$], serum triglyceride concentration [(d) $r = -0.30$, $p = 0.04$*, $n = 46$], relative liver weight [(e) $r = 0.44$, $p = 0.003$**, $n = 46$] and hematocrite [(f) $r = 0.10$, $p = 0.5$, $n = 43$]. PFOS;= perfluorooctane sulfonic acid, ALT = alanine aminotransferase, r = correlation coefficient, $p = p$ value, n = number of nestlings, Spearman rank correlation, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

In Europe, PFOS has been measured in liver tissue from only two avian species: the common cormorant (*Phalacrocorax carbo*) from the Italian coast and the white-tailed sea eagle (*Haliaeetus albicilla*) from the Baltic coast. The measured PFOS concentrations in these species ranged from <3.9 to 150 ng/g ww (Kannan et al., 2002b). In water birds from the USA, Canada and the Northern Pacific, the liver PFOS concentrations ranged between <30 and 1780 ng/g ww (Giesy and Kannan, 2001; Kannan et al., 2001a). In Japan and

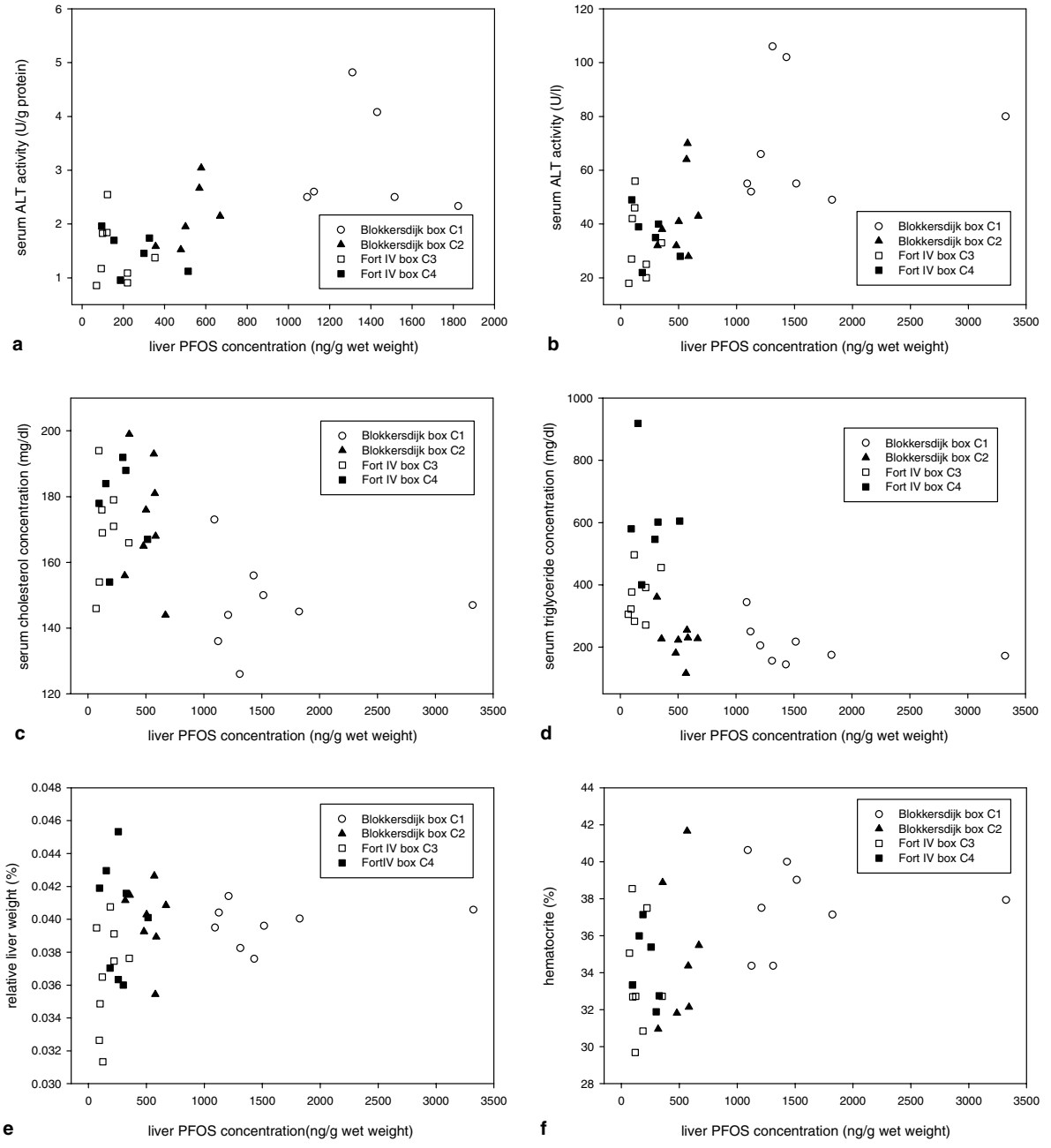

Fig. 4. The relationships between the blue tit nestling liver PFOS concentration and the serum ALT activity [(a) $r = 0.62$, $p = 0.0007$***, $n = 26$; (b) $r = 0.61$, $p = 0.0004$***, $n = 30$], serum cholesterol [(c) $r = -0.41$, $p = 0.03$*, $n = 30$], serum triglyceride concentration [(d) $r = -0.69$, $p < 0.0001$, $n = 30$], relative liver weight [(e) $r = 0.22$, $p = 0.2$, $n = 33$] and hematocrite [(f) $r = 0.39$, $p = 0.03$*, $n = 29$]. PFOS = perfluorooctane sulfonic acid, ALT = alanine aminotransferase, r = correlation coefficient, $p = p$ value, n = number of nestlings, Spearman rank correlation, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Korea, hepatic PFOS levels in fish-eating birds have been reported between <19 and 650 ng/g ww (Kannan et al., 2002a). In comparison with these hepatic PFOS concentrations, it can be concluded that the PFOS concentrations in Blokkersdijk are among the highest values reported in birds. The PFOS concentrations at Blokkersdijk are comparable to the highest ones measured in top predators worldwide such as the bald eagle (2570 ng/ml in plasma), mink (3680 ng/g ww in liver), bottlenose dolphin (1520 ng/g ww in liver) or polar bear

(>4000 ng/g ww in liver, Giesy and Kannan, 2001; Kannan et al., 2001b; Martin et al., 2004). Also in bald eagle nestlings, similar PFOS concentrations as those found in the tit nestlings have been measured (>1000 ng/ml plasma, Kannan et al., 2001a). In the latter study, no sex differences were observed in PFOS contamination levels of the nestlings. That observation is concurrent with the present study because no significant differences in PFOS concentrations were observed between male and female nestlings in Blokkersdijk or Fort IV ($p > 0.05$).

A likely source for PFOS contamination of nestlings is the food. Great and blue tit nestlings have similar nutritional habits. Their diet consists mainly of butterflies and moths (mainly the larval stage) and to a lesser extent of spiders which are generally captured in the vicinity of the nest (Cramp and Perrins, 1993). Therefore, it is very likely that food items for each species have similar PFOS (precursor) contamination levels and contribute at a similar extent to PFOS contamination of the nestlings if uptake/depuration characteristics of PFOS and metabolisation characteristics of its precursors are similar. This seems to be supported by the observation that the hepatic PFOS concentrations were not significantly different between both tit species, as well for Blokkersdijk as for Fort IV ($p > 0.05$).

In addition to PFOS contamination via food items, PFOS (precursor) contamination of nestlings might also occurs via the egg. This possibility is supported by studies in the mallard duck (*Anas platyrhynchos*) and northern bobwhite quail (*Colinus virginianus*) for which PFOS transfer to the egg has been reported (United States Environmental Protection Agency, 2004a,b).

The observed significant relationships between the PFOS liver concentration and the serum alanine aminotransferase (ALT) activity, the relative liver weight and the serum cholesterol and triglyceride concentrations in the tit nestlings suggest that PFOS might affect hepatic integrity and lipid metabolism. This is in agreement with previous reports on PFOS-induced effects where PFOS has been reported to increase the relative liver weight in rodents (Ikeda et al., 1987; Sohlenius et al., 1993; Seacat et al., 2002; Lau et al., 2003; Thibodeaux et al., 2003) and cynomolgus monkeys (*Macaca fascicularis*, Seacat et al., 2003). Also the serum ALT activity has been demonstrated to increase in PFOS-exposed rodents (Seacat et al., 2003) and carps (Hoff et al., 2003). Decreases in serum triglyceride and cholesterol concentrations after PFOS exposure have been shown in rodents (Haughom and Spydevold, 1992; Seacat et al., 2003; Thibodeaux et al., 2003) and cynomolgus monkeys (Seacat et al., 2002).

Although the increase in serum ALT activity and the decreases in serum cholesterol and triglyceride concentration were observed in both tit species, the increases in relative liver weight and the hematocrite were only significantly related to the liver PFOS concentration in the great and blue tit nestlings, respectively. No significant differences were observed in liver PFOS concentration ranges in Blokkersdijk or Fort IV great and blue nestlings, however, suggesting that differences in PFOS liver concentration might not account for the observed species differences in effect profiles. Alternatively, the observed differences could be ascribed to differences in species sensitivity.

Seacat et al. (2003) showed that the lowest observed effect level (LOEL) for a significant increase in relative liver weight in PFOS-exposed male rats after 4 weeks of PFOS exposure corresponded with a liver PFOS concentration of 282 000 ng/g ww. For serum ALT activity increase and cholesterol decrease, the LOEL in males was 568 000 ng PFOS/g liver after 14 weeks of exposure. In females, the relative liver weight was significantly increased at a mean liver PFOS concentration of 635 000 ng/g ww after 14 weeks. In rats exposed to PFOS during gestation, the relative liver weight was shown to be significantly increased on day 2 and 9 after birth corresponding with a mean liver concentration of about 50 000 ng PFOS/g ww at birth (Lau et al., 2003). In cynomolgus monkeys, increased relative liver weights were observed at mean liver PFOS concentrations of 395 000 and 273 000 ng/g ww for males and females after 183 days of PFOS exposure and decreased serum cholesterol concentrations at hepatic PFOS concentrations >100 000 ng/g ww (Seacat et al., 2002). The nestling PFOS concentration ranges for which significant correlations were found with the relative liver weight, serum ALT activity, cholesterol and triglyceride concentrations are well below these values (17–2788 and 69–3323 ng/g ww for great and blue tit, respectively). The reason for this discrepancy is not clear at present but might be due to differences in species sensitivity and differences in exposure conditions between the laboratory and the field. In order to account for laboratory-to-field extrapolation, for within- and between-species variability, and accounting for PFOS′ bioaccumulative capacities and persistence and extrapolation to chronic PFOS exposure conditions a chronic PFOS exposure a correction factor of 1000 has been proposed for extrapolation of PFOS-induced effects in birds and mammals (Canadian Environmental Protection Act, 1999). If this correction factor is applied to the PFOS effect concentrations in rats and cynomolgus monkeys reported above, they come close to the PFOS concentrations measured in the Blokkersdijk tit nestlings (86–2788 and 317–3322 ng/g ww for great and blue tit, respectively) supporting the possibility that PFOS contamination might have affected some biochemical endpoints in the nestlings.

The total serum protein concentration was not significantly related to the presence of PFOS in tit nestling liver tissue. The hematocrite was significantly and positively related to the hepatic PFOS concentration in blue

tit nestlings only suggesting PFOS-mediated erythrocyte enlargement or cell number or dehydration. This finding is concurrent with an observation in feral PFOS-polluted eels with similar hepatic PFOS concentrations (17–9031 ng/g ww, Hoff et al., 2005) but has not been investigated under laboratory conditions. Because the condition index was not shown to be significantly affected in our study, it was not suggested that the observed biological alterations could be indicative of effects on the condition of nestlings.

Because no significant differences were shown in the values of the biological endpoints investigated in males and females, neither for the Blokkersdijk, nor for the Fort IV nestlings, it is suggested that the values of these endpoints are sex-independent. Consequently, sex probably did not confound the studies relationships between the hepatic PFOS concentrations and the biological endpoints.

The present study does not suggest that the measured hepatic organochlorine or organobromine pollutants would have contributed to the modulation of the serum ALT activity, serum cholesterol, triglyceride concentration or hematocrite, endpoints that were significantly correlated with the liver PFOS concentration in both or one of both tit species under investigation. Overall, these results suggest that PFOS might be a relatively important determinant in the alteration of these endpoints.

In conclusion, this study shows for the first time that nestlings from two passerine bird species from Blokkersdijk, an area protected by a European Council directive on the conservation of wild birds, were severely PFOS contaminated. No differences in PFOS contamination were shown between sexes or species. The relative liver weight, serum ALT activity, cholesterol and triglyceride concentrations and hematocrite were suggested to be affected by PFOS contamination but not by any of the measured organochlorine and organobromine pollutants.

It is not clear at present whether these observed alterations could cause deleterious effects on a higher level of biologic organization. Chronic PFOS exposure reproductive studies in which adult mallard duck and northern bobwhite quails were exposed showed that the mean liver PFOS NOAEL values for 14-day survivability was 3.17 and 3.61 μg/g ww in male and female mallard chicks, respectively (United States Environmental Protection Agency, 2004a).

In quail chicks, the mean liver PFOS LOAEL values for 14-day survivability was 5.76 and 5.49 μg/g ww in male and female quail chicks, respectively. (United States Environmental Protection Agency, 2004b). These latter values are in the same order of magnitude than the maximal hepatic PFOS concentrations measured in the Blokkersdijk tits (2788 and 3322 ng/g ww for great and blue tit, respectively) warranting further study on potential effects of PFOS exposure on survival in tit nestlings from Blokkersdijk.

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