Perfluoroalkyl acids (PFAAs)

concentrations and oxidative status in two generations of great tits inhabiting a contamination hotspot

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Supplementary Information

Number of pages: 16

Number of figures: 4

Number of tables: 12

DETAILED DESCRIPTION OF THE METHODS

We provide below a more detailed description of some of the methods used in this study.

PFAAs analysis in plasma.

A mixture of isotopically mass-labelled internal standards (ISTDs), comprising $^{13}C_4$-PFBA, [1,2-$^{13}C_2$]PFHxA, [1,2,3,4-$^{13}C_4$]PFOA, [1,2,3,4,5-$^{13}C_5$]PFNA, [1,2-$^{13}C_2$]PFDA, [1,2-$^{13}C_2$]PFUnDA, [1,2-$^{13}C_2$]PFDoDA, $^{18}O_2$-PFHxS and [1,2,3,4-$^{13}C_4$]PFOS, were purchased by Wellington Laboratories (Guelph, Canada). HPLC grade acetonitrile (ACN; Merck Chemicals, N.V./S.A. (Millipore), Overijse, Belgium) and water (VWR International, Leuven, Belgium) were used.

Sample extraction

Egg content was transferred into a polypropylene (PP) tube and homogenized by repeatedly sonicating and vortex-mixing. Blood plasma (10 µL), or homogenized egg (~0.4g) samples were transferred into PP tubes. Hereafter, 80 µL of the previously described ISTD mixture, containing 125 pg/µL of each ISTD (in 50:50 ACN:HPLC grade water), and 10 mL ACN was added to each sample. After sonication (3x10 min), samples were left overnight on a shaking plate at room temperature. After centrifugation (4°C, 10 min, 2400 rpm, Eppendorf centrifuge 5804R) the supernatant was transferred into a 14 mL tube and loaded on HR-XAW columns that were preconditioned and equilibrated with 5 mL ACN and 5 mL Milli-Q (MQ) water, respectively. Samples were washed with 5 mL 25 mM ammonium acetate and 2 mL ACN. Finally, samples were eluted from the SPE columns using 2 x 1 mL 2% ammonium hydroxide in ACN. The eluent was completely dried using a rotational-vacuum-concentrator at 30°C (Eppendorf concentrator 5301, Hamburg, Germany), reconstituted with 200 µL 2% ammonium hydroxide in ACN and vortex-mixed for at least 1 minute. Prior to the analysis, samples were filtrated through an Ion Chromatography Acrodisc 13 mm Syringe Filter with 0.2 µm Supor (PES) Membrane (VWR International, Leuven, Belgium) attached to a PP auto-injector vial.

UPLC-TQD analysis

To separate PFAAs, an ACQUITY BEH C18 column (2.1 x 50 mm; 1.7 µm, Waters, USA) was used. Mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in ACN (B). Solvent gradients were 65% A to 0% A in 3.4 min and 65% A at 4.7 min. The injection volume was 10 µL at a flow rate of 450 µL/min, with a total run time of 6.7 min.An ACQUITY BEH C18

pre-column (2.1 x 30 mm; 1.7 µm, Waters, USA) was inserted between the solvent mixer and injector, to retain any PFAAs contamination originating from the system. Identification and quantification of individual PFAAs was based on multiple reaction monitoring (MRM) of two diagnostic transitions per analyte or ISTD.

Calibration

A constant amount of ISTD was added to varying amounts of non-labelled standards, ACN and water, to construct calibration curves. A linear regression function with a highly significant linear fit for all target analytes (all $p < 0.001$; $R^2 > 0.98$) described the relationship between the ratio of unlabelled and labelled PFAA concentrations and the ratio of the area of the unlabelled and labelled PFAAs. With exception of PFPeA, PFHpA, PFTrDA, PFTeDA, PFBS and PFDS, which were all quantified using the ISTD of the compound closest in terms of functional group and size (individual PFAAs were quantified using their corresponding ISTD.

Quality assurance

The quality of the method was assured by regular analysis of procedural blanks (one per batch of 20 samples) and contained no contamination. The limit of quantification (LOQ) was determined, based on a signal-to-noise ratio of 10 and ranged from 1.1 to 8.2 pg/µL for all compounds with exception of PFHxS (129.2 pg/µL), PFOS (LOQ = 46.6 pg/µL) and PFPeA (52.4 pg/µL) and which had considerably higher LOQs due to high noise.

Antioxidant and oxidative stress parameters measurement in red blood cells

For the detection of molecular antioxidants in red blood cells: reduced glutathione (GSH) and oxidised glutathione (GSSG), high-performance liquid chromatography with electro-chemical detection by a reversed-phase HPLC of Shimadzu (Shimadzu, ‘s Hertogenbosch, The Netherlands) was used, following the protocol as described by Sinha et al. (2014). Approximately, 10 mg of RBC per sample were used. The ratio between GSH/ GSSG was used as an index of redox state with lower values indicating higher oxidative stress (Jones 2006).

Activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were determined from haemolysates of red blood cells. Approximately, 10 mg of RBC were homogenized by MagNALyser (Roche, Vilvoorde, Belgium) in 250 µl of extracting

buffer (pH 7.4; 1.15% KCl and 0.02 M EDTA in 0.01 M PBS). All measurements were scaled down for semi-high throughput using a micro-plate reader (Multiskan RC plate reader type 351; Synergy Mx, Biotek Instruments Inc., Vermont, USA). SOD activity was determined by measuring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm ($\varepsilon 530 = 12.8\ mM^{-1}\ cm^{-1}$), following Dhindsa et al. (1981). CAT activity was measured following Aebi (1984), by monitoring the rate of decomposition of H_2O_2 ($\varepsilon 240 = 39.4\ M^{-1}cm^{-1}$). Activity of GPX was determined following Drotar et al. (1985) by measuring the decrease in NADPH absorbance measured at 340 nm and calculated from the 6.22 $mM^{-1}\ cm^{-1}$ extinction coefficient. A modified ferric ion reducing antioxidant power (FRAP) assay was used to estimate the total antioxidant capacity (TAC) (Benzie and Strain 1996). Homogenised red blood cells were mixed with the FRAP reagent, and the absorption was measured at 600 nm after 30 min. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as the standard.

Finally, we measured protein carbonyls (marker of protein damage) in red blood cells as oxidative damage markers. We followed the procedure explained in the "Protein Carbonyl Colorimetric Assay Kit" by Cayman Chemical's (Ann Arbor, MI, USA; see also Levine et al. 1990) to measure protein carbonyl content after samples had been diluted with buffer extract to 2 mg protein ml^{-1}.

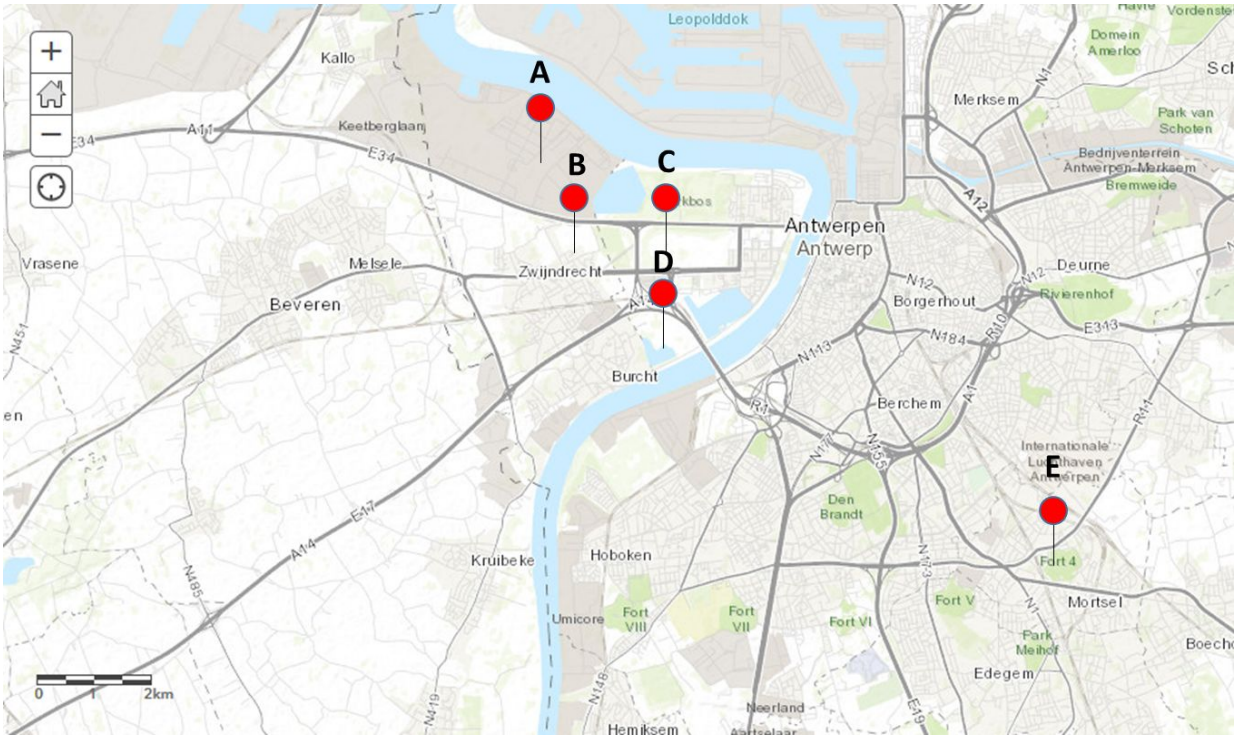

Figure S1. Overview of the different study sites located along a distance gradient of 11 km from an active fluorochemical plant in Antwerp (Belgium). A: Fluorochemical plant; B: Vlietbos; C: Rot; D: Burchtse Weel; E: Fort 4. (Map created using ArcGIS® software by Esri).

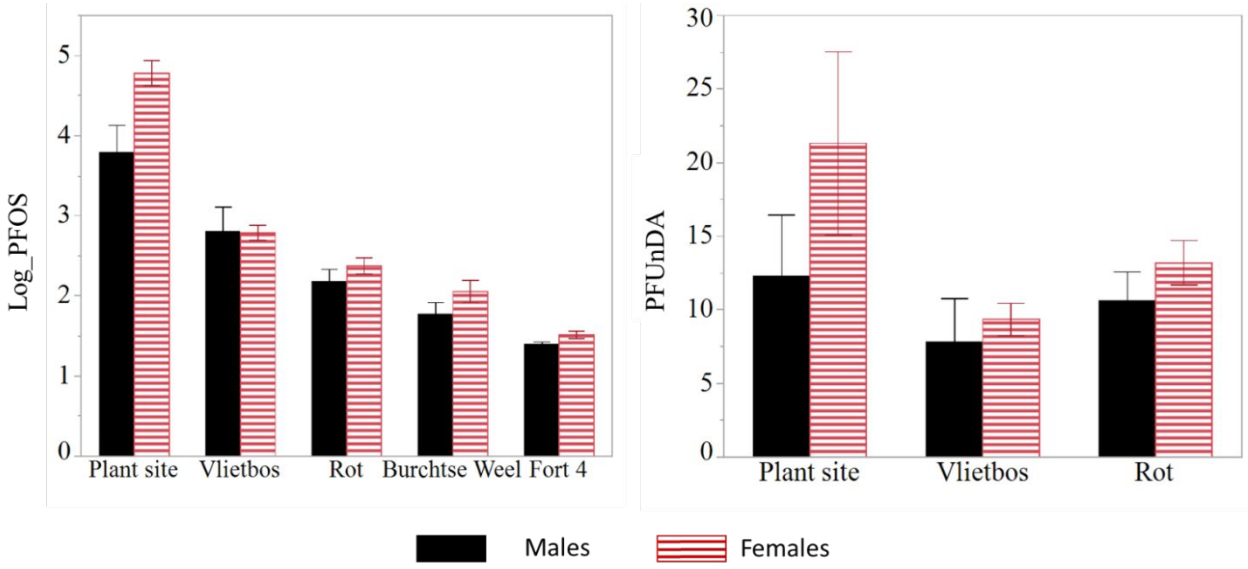

Figure S2. Mean concentrations (pg/µL) of PFUnDA and logPFOS (±SE) found in adult birds' plasma (temporal data were pooled together (adults from both the late winter and the spring)), at the five sampling sites, separated by sex. Sample sizes are (F/M): Plant site=16/8; Vlietbos=7/24; Rot=14/8; Burchtse Weel=12/11; Fort 4=12/19.

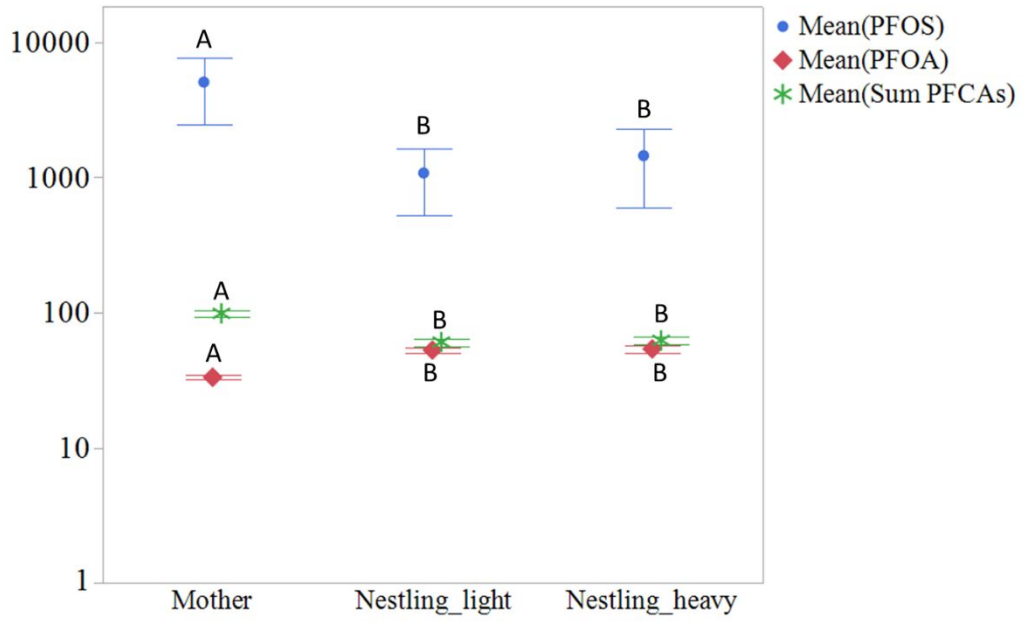

Figure S3. Mean concentrations (pg/µL ±SE) of PFOA, PFOS and ∑PFCAs found in the blood of the mother and the offspring (the lightest and the heaviest nestlings in the nest, n=40 nests). Different letters indicate significantly different concentrations between different sample types.

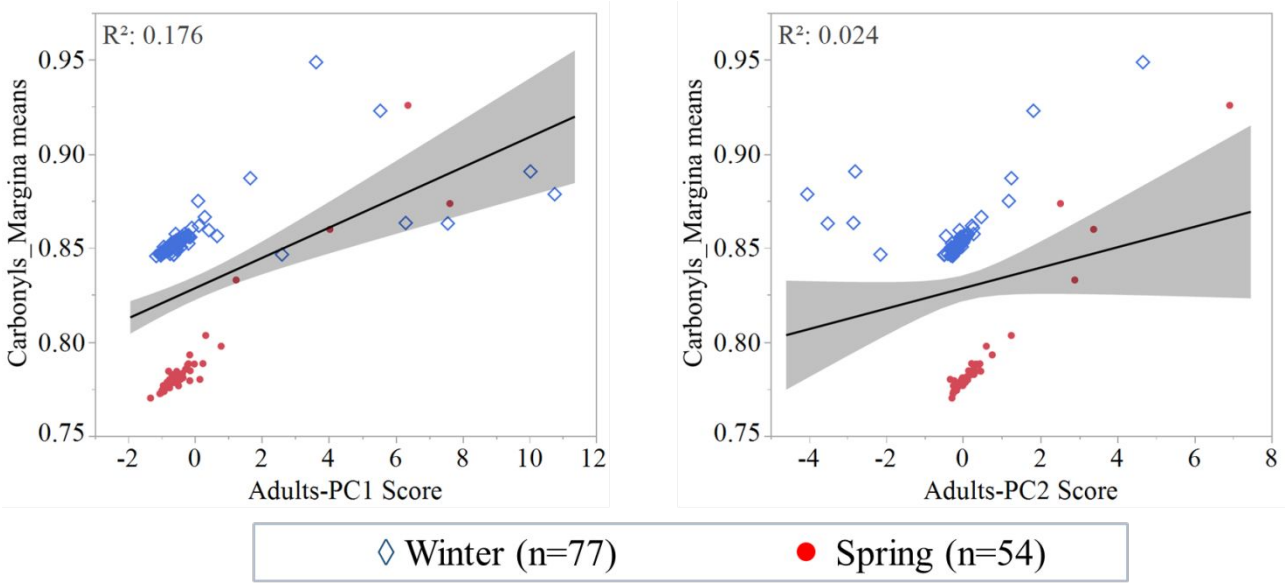

Figure S4. Relationship between Adults-PC1 and Adults-PC2 and protein carbonyl (marginal means as obtained in the mix model when considering season as a factor and ring number as random effect) content in blood of adult birds sampled in winter and spring. Regression lines are shown with 95% confidence bands shaded.

Table S1. Target PFAA compounds (11 perfluoroalkyl carboxylic acids and 4 perfluoralkyl sulfonic acids), chemical formula and their acronyms (the used abbreviations for PFAA compounds are according to Buck et al. 2011)

Family	Compound	Formula	Acronym
Perfluoroalkyl carboxylic acids $C_nF_{2n+1}COOH$ (PFCAs)	Perfluorobutanoic acid	C_3F_7COOH	PFBA
	Perfluoropentanoic acid	C_4F_9COOH	PFPeA
	Perfluorohexanoic acid	$C_5F_{11}COOH$	PFHxA
	Perfluoroheptanoic acid	$C_6F_{13}COOH$	PFHpA
	Perfluorooctanoic acid	$C_7F_{15}COOH$	PFOA
	Perfluorononanoic acid	$C_8F_{17}COOH$	PFNA
	Perfluorodecanoic acid	$C_9F_{19}COOH$	PFDA
	Perfluoroundecanoic acid	$C_{10}F_{21}COOH$	PFUnDA
	Perfluorododecanoic acid	$C_{11}F_{23}COOH$	PFDoDA
	Perfluorotridecanoic acid	$C_{12}F_{25}COOH$	PFTrDA
	Perfluorotetradecanoic acid	$C_{13}F_{27}COOH$	PTeDA
Perfluoroalkyl sulfonic acids $C_nF_{2n}+SO_3H$ (PFSAs)	Perfluorobutane sulfonic acid	$C_4F_9SO_3H$	PFBS
	Perfluorohexane sulfonic acid	$C_6F_{13}SO_3H$	PFHxS
	Perfluorooctane sulfonic acid	$C_8F_{17}SO_3H$	PFOS
	Perfluorodecane sulfonic acid	$C_{10}F_{21}SO_3H$	PFDS

Table S2. Limits of quantification (LOQ: pg/µL), mean and median concentrations (pg/µL), range and detection frequencies (%) of most frequently found PFAA compounds (PFCAs; perfluoroalkyl carboxylic acids. PFSAs; perfluoroalkyl sulfonic acids) in plasma of adult great tits sampled at the five sampling sites. Different upper case letters indicate significantly different mean concentrations among locations. Different lower case letters indicate significantly different detection frequencies among locations. Temporal data were pooled together (adults from the late winter and the spring). *For PFOA, concentration in the plant site was only significantly higher in winter (no differences in spring).

		PFCAs						**PFSAs**
		PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFOS
LOQ		2.6	4.1	5.5	6.4	1.8	1.4	46.6
Detection frequency		99	26.7	24.1	47.8	62.9	32.7	71.7
Plant site (n =24)	Median	75.1	12.8	5.57	9.99	15.7	5.60	20168
	Mean	94.9^{A*}	21.8	89.2	17.7	23.1^{A}	8.54	43428^{A}
	Range	<LOQ -244	<LOQ – 81.0	<LOQ - 477	<LOQ – 57.2	<LOQ – 122	<LOQ – 40.0	<LOQ - 161333
	Freq	95	70 ^{a}	50	50 ^{ab}	75	55	100 ^{a}
Vlietbos (n =31)	Median	40.0	<LOQ	<LOQ	8.15	3.38	<LOQ	488
	Mean	44.8^{B}	<LOQ	<LOQ	8.92	4.16^{B}	<LOQ	1780^{B}
	Range	25.5 – 94.7	<LOQ - 23.9	<LOQ - 19.1	<LOQ - 24.5	<LOQ – 17.4	<LOQ - 3.17	65.4 - 21139
	Freq	100	17 ^{b}	28	62 ^{a}	76	38	100 ^{a}
Rot (n=22)	Median	41.1	<LOQ	<LOQ	10.3	3.44	<LOQ	178
	Mean	41.5	<LOQ	<LOQ	11.2	3.46^{B}	<LOQ	260^{BC}
	Range	28.8 - 69.1	<LOQ - 11.7	<LOQ - 11.0	<LOQ – 22.8	<LOQ – 8.25	<LOQ - 4.18	<LOQ - 1182
	Freq	100	20 ^{b}	20	70 ^{a}	70	35	84 ^{ab}
Burchtse Weel (n =23)	Median	40.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	59.6
	Mean	47.1^{B}	<LOQ	<LOQ	<LOQ	<LOQ	1.56	118^{D}
	Range	18.4 - 104	<LOQ - 8.36	<LOQ - 20.4	<LOQ - 22.1	<LOQ - 9.15	<LOQ - 9.95	<LOQ - 657
	Freq	100	15 ^{b}	15	32 ^{b}	45	25	60 ^{b}
Fort 4 (n=31)	Median	41.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Mean	44.0^{B}	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Range	7.63 - 75.4	<LOQ - 12.2	<LOQ - 10.5	<LOQ - 27.4	<LOQ - 9.87	<LOQ - 6.20	<LOQ - 91.22
	Freq	100	18 ^{b}	11	26 ^{b}	48	15	25 c

Table S3. Limits of quantification (LOQ: pg/µL) and detection frequencies (%) of PFAA compounds rarely found (less than 20% of the samples above the LOQ) in plasma of adult great tits sampled at the five sampling sites.

		PFCAs			
		PFBA	PFPeA	PFHxA	PFTeDA
LOQ		6.5	52.4	8.2	1.3
Plant site	Freq	12.5	0	0	8.3
Vlietbos	Freq	0	22.6	9.7	3.2
Rot	Freq	0	22.7	4.5	4.5
Burchtse Weel	Freq	0	4.3	0	4.3
Fort 4	Freq	6.4	6.4	0	6.4

Table S4. Results of the Principal Component Analysis conducted on the PFAA compounds measured in great tits plasma; Adults (n=131), nestlings (n= 170). Variable loadings greater than 0.6 or lower than -0.6 are highlighted in bold.

	Adults		Nestlings
	PC1	PC2	PC1
PFBA			**0.738**
PFOA	**0.758**	-0.136	**0.856**
PFNA	**0.841**	-0.419	
PFDA	**0.858**	-0.355	
PFUnDA	**0.756**	0.185	
PFDoDA	**0.751**	0.586	**0.855**
PFTrDA	0.555	**0.763**	
PFOS	**0.933**	-0.260	**0.950**
Variance explained			
Proportion	61.91	19.26	72.76
Cumulative		81.17	

Table S5. Coefficient (r) and probability (p) of the correlations found between different PFAA compounds (those with detection frequency ≥ 50% in each site) at the five sampling sites in the plasma samples of the adults.

		Plant site (n = 24)		**Vlietbos (n = 31)**		**Rot (n = 22)**		**Burchtse Weel (n = 23)**		**Fort 4 (n = 31)**	
		r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
PFOA	**PFNA**	0.83	**<.0001**								
	PFDA	0.55	**0.005**								
	PFUnDA	0.27	0.197	0.03	0.851	0.32	0.144				
	PFDoDA	0.46	**0.027**	-0.19	0.322	0.33	0.130				
	PFTrDA	0.16	0.451								
	PFOS	0.58	**0.003**	-0.13	0.488	0.20	0.373	-0.50	**0.015**		
PFNA	**PFDA**	0.65	**0.001**								
	PFUnDA	0.49	**0.018**								
	PFDoDA	0.44	**0.041**								
	PFTrDA	0.16	0.475								
	PFOS	0.84	**<.0001**								
PFDA	**PFUnDA**	0.49	**0.014**								
	PFDoDA	0.53	**0.009**								
	PFTrDA	0.25	0.252								
	PFOS	0.74	**<.0001**								
PFUnDA	**PFDoDA**	0.60	**0.002**	-0.21	0.266	0.66	**<.001**				
	PFTrDA	0.47	**0.022**								
	PFOS	0.55	**0.005**	0.05	0.806	0.37	0.0880				
PFDoDA	**PFTrDA**	0.81	**<.0001**								
	PFOS	0.70	**<.001**	-0.05	0.786	0.56	**0.006**				
PFTrDA	**PFOS**	0.50	**0.014**								

Table S6. Limits of quantification (LOQ: pg/µL), mean and median concentrations (pg/µL), range (pg/µL) and detection frequencies (%) of most frequently found PFAA compounds (PFCAs; perfluoroalkyl carboxylic acids. PFSAs; perfluoroalkyl sulfonic acids) in plasma of great tits nestlings (14 days old) at the five sampling sites. Different upper case letters indicate significantly different mean concentrations among locations. Different lower case letters indicate significantly different detection frequencies between locations.

		PFCAs			**PFSAs**
		PFBA	PFOA	PFDoDA	PFOS
LOQ		6.5	2.6	1.8	46.6
Plant site	Median	16.4	93.3	10.2	17137
(n =38nestlings /14 nests)	Mean	24.1	139^{A}	12.2	14514^{A}
	Range	<LOQ - 112	32.1 - 438.7	<LOQ - 49.8	612 -35624
	Freq	60.5^{a}	100	80.5^{a}	100^{a}
Vlietbos	Median	<LOQ	48.4	<LOQ	123
(n =47nestlings /18 nests)	Mean	<LOQ	50.1^{B}	1.9	464^{B}
	Range	<LOQ -10.8	20.2 - 81.0	<LOQ -9.39	<LOQ -3292
	Freq	10.4^{b}	100	19.1^{b}	91.3^{a}
Rot	Median		54.1	<LOQ	47.3
(n=22nestlings / 10 nests)	Mean		52.9^{B}	<LOQ	68.3^{C}
	Range	All <LOQ	27.8 - 83.2	<LOQ - 7.74	<LOQ - 198
	Freq	0^{c}	100	30.4^{b}	52.2^{b}
Burchtse Weel	Median	<LOQ	46.5	<LOQ	<LOQ
(n =35nestlings / 14 nests)	Mean	<LOQ	49.1^{B}	<LOQ	<LOQ
	Range	<LOQ - 7.16	23.9 - 83.4	<LOQ - 7.58	<LOD - 247
	Freq	5.7^{bc}	100	14.3^{b}	28.6^{b}
Fort 4	Median	<LOQ	51.6	<LOQ	<LOQ
(n=28nestlings /14 nests)	Mean	<LOQ	50.3^{B}	<LOQ	<LOQ
	Range	<LOQ - 8.36	26.2 - 72.9	<LOQ - 10.9	<LOQ - 138
	Freq	17.9^{b}	100	25^{b}	7.14^{c}

Table S7. Coefficient (r) and probability (p) of the correlations found between different PFAA compounds (those with detection frequency ≥ 50% in each site) at the five sampling sites in the plasma samples of the nestlings.

		Plant site (n = 38)		**Vlietbos (n = 47)**		**Rot (n = 22)**		**Burchtse Weel (n = 35)**		**Fort 4 (n = 28)**	
		r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
PFBA	**PFOA**	0.38	**0.020**								
	PFDoDA	0.37	**0.030**								
	PFOS	0.62	**<.0001**								
PFOA	**PFDoDA**	0.71	**<.0001**								
	PFOS	0.67	**<.0001**	0.47	**<.001**	0.0984	0.6630				
PFDoDA	**PFOS**	0.73	**<.0001**								

Table S8. Limits of quantification (LOQ: pg/µL) and detection frequencies (%) of PFAA compounds rarely found (detection frequency < 20%) in plasma of great tit nestlings at the five sampling sites.

		PFCAs				
		PFNA	PFDA	PFUnDA	PFTrDA	PFTeDA
LOQ		4.1	5.5	6.4	1.4	1.1
Plant site (n =38nestlings /14 nests)	Freq	39	10	0	21	5
Vlietbos (n =47nestlings /18 nests)	Freq	0	4	4	2	2
Rot (n=22nestlings / 10 nests)	Freq	0	0	4	0	0
Burchtse Weel (n =35nestlings / 14 nests)	Freq	0	6	3	3	0
Fort 4 (n=28nestlings /14 nests)	Freq	4	7	11	4	0

Table S9. ∑PFAAs, ∑PFCAs, PFOA, PFDoDA and PFOS mean (± SE) concentrations in mothers, eggs and both nestlings (the lightest and the heaviest in the nest; mean ± SE) at the five sampling sites (n=40 nests)

		Location				
		Plant site	**Vlietbos**	**Rot**	**Burchtse Weel**	**Fort 4**
Mother (pg/µL)	∑PFAAs	43902 ± 16891	1391 ± 323	530 ± 153	354 ± 60	130 ± 12
	∑PFCAs	145 ± 22	102 ± 7.3	90.0 ± 3.5	77.4 ± 4.1	87.2 ±8.0
	PFOA	44.8 ± 3.4	33.1 ± 1.6	36.3 ± 3.0	27.5 ± 2.3	30.2 ± 3.0
	PFDoDA	23.5 ± 10	3.6 ±0.6	3.5 ±0.4	5.0 ±1.3	4.6 ±1.0
	PFOS	43757 ± 16870	1289 ± 324	410 ± 156	276 ±62	43 ± 8.0
Egg (ng/g)	∑PFAAs	81032 ± 38056	908 ± 253	363 ± 104	89.8 ± 9.0	41.7 ± 8.1
	∑PFCAs	171 ± 67	14.2 ± 3.1	9.7 ± 1.8	6.8 ± 1.1	9.5 ± 2.2
	PFOA	18.2 ± 2.1	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.3	1.0 ± 0.1
	PFDoDA	57.5 ± 26	<LOD	1.7 ± 0.3	<LOD	2.0 ± 0.7
	PFOS	80231 ± 37684	890 ± 251	351 ± 104	80.0 ± 9.1	29.2 ± 6.0
Chick light (pg/µL)	∑PFAAs	8517 ± 3980	464 ± 208	128 ± 37	94 ± 8.7	90 ± 15
	∑PFCAs	115 ± 12	55.6 ± 4.0	55.2 ± 8.2	50.7 ± 3.2	52.5 ± 4.8
	PFOA	88.0 ± 4.1	50.2 ± 3.8	49.2 ± 7.2	44.5 ± 3.0	48.0 ± 5.1
	PFDoDA	7.3 ± 3.7	<LOD	2.8 ± 1.1	<LOD	<LOD
	PFOS	11203 ± 3997	409 ± 206	73 ± 42	<LOD	<LOD
Chick heavy (pg/µL)	∑PFAAs	12419 ± 6509	372 ± 146	84 ± 15	123 ± 22	82 ± 7.2
	∑PFCAs	115 ± 27	54.7 ± 3.5	52.3 ± 10.4	62.3 ± 4.9	55.3 ± 6.4
	PFOA	85.2 ± 19	48.3 ± 3.2	45.9 ± 9.1	57.4 ± 4.8	49.2 ± 5.8
	PFDoDA	6.8 ± 3.0	<LOD	<LOD	<LOD	<LOD
	PFOS	16406 ± 7113	336 ± 151	<LOD	60 ± 18	<LOD

Table S10. Mean (± SE) values of body condition and oxidative stress biomarkers (in red blood cells) in adult great tits at the five sampling sites. Different letters indicate significant differences between locations at the $p < 0.05$ level according to Tukey test results.

	Location				
	Plant site	**Vlietbos**	**Rot**	**Burchtse Weel**	**Fort 4**
Body condition	17.6 ± 0.3	17.0 ±0.2	17.2 ± 0.2	17.7 ± 0.2	17.4 ± 0.2
TAC	10.5 ± 0.5A	10.9 ± 0.5A	10.7 ± 0.33A	10.9 ± 0.5A	8.8 ± 0.5B
GPX	0.29 ± 0.03BC	0.38 ± 0.02AB	0.30 ± 0.02BC	0.46 ± 0.04A	0.27 ± 0.03C
SOD	0.82 ± 0.09	0.81 ± 0.12	0.98 ± 0.29	0.83 ± 0.11	1.14 ± 0.12
Protein carbonyls	7.71 ± 0.21A	5.90 ± 0.22C	7.87 ± 0.16A	6.11 ± 0.28BC	7.05 ± 0.29AB
CAT	13.2 ± 1.1	15.6 ± 1.2	14.6 ± 1.40	19.9 ± 2.2	12.7 ± 1.30
GSH	1.25 ± 0.18AB	0.80 ± 0.16B	1.36 ± 0.17A	1.37 ± 0.23AB	0.76 ± 0.11AB
GSSG	0.57 ±0.07A	0.99 ± 0.12B	0.74 ± 0.09AB	1.19 ± 0.33AB	0.55 ± 0.08A
GSH/GSSG ratio	3.36 ±1.08A	0.73 ± 0.13B	3.28 ± 0.87A	1.70 ± 0.52AB	2.43 ± 0.55A

Body condition (Scaled mass index);TAC (Total antioxidant capacity (µmol trolox/g)); GPX (glutathione peroxidase (µmol NADPH/mg protein)); SOD(Superoxide dismutase (U/mg protein)); Protein carbonyls (nmol/mg protein); CAT (catalase (µmol H_2O_2/mg protein)); GSH (Reduced and total glutathione (µmol/g)); GSSG (oxidized glutathione (µmol/g))..

Table S11. Mean (± SE) values of body condition and oxidative stress biomarkers (in red blood cells) in nestlings at the five sampling sites. Different letters indicate significant differences between locations at the $p < 0.05$ level according to Tukey test results.

	Location				
	Plant site	**Vlietbos**	**Rot**	**Burchtse Weel**	**Fort 4**
Body condition	15.6 ± 0.3	16.6 ± 0.2	15.63 ± 0.4	15.9 ± 0.3	15.0 ± 0.3
TAC	5.33 ± 0.60AB	8.18 ± 0.65A	3.87 ± 0.54B	6.06 ± 0.47AB	7.74 ± 0.60A
GPX	0.18 ± 0.01AB	0.21 ± 0.01A	0.14 ± 0.01B	0.17 ± 0.01AB	0.19 ± 0.01AB
SOD	0.66 ± 0.05A	0.45 ± 0.03B	0.54 ± 0.03AB	0.53 ± 0.07AB	0.68 ± 0.07A
Protein carbonyls	6.60 ± 0.29AB	7.91 ± 0.28A	5.84 ± 0.37B	7.39 ± 0.36A	6.81 ± 0.38AB
CAT	7.65 ± 0.64	7.64 ± 0.43	6.33 ± 0.54	6.59 ± 0.53	6.05 ± 0.65
GSH	1.15 ± 0.11	1.23 ± 0.09	1.02 ± 0.15	1.37 ± 01.7	1.39 ± 0.11
GSSG	1.05 ± 0.09	1.15 ± 0.09	0.84 ± 0.08	1.02 ± 0.13	0.98 ± 0.07
GSH/GSSG ratio	1.37 ± 0.22	1.45 ± 0.18	1.75 ± 0.54	2.64 ± 0.80	1.57 ± 0.15

Body condition (Scaled mass index);TAC (Total antioxidant capacity (µmol trolox/g)); GPX (glutathione peroxidase (µmol NADPH/mg protein)); SOD(Superoxide dismutase (U/mg protein)); Protein carbonyls (nmol/mg protein); CAT (catalase (µmol H_2O_2/mg protein)); GSH (Reduced and total glutathione (µmol/g)); GSSG (oxidized glutathione (µmol/g))..

Table S12. PFAA concentration (range; pg/µL) measured in plasma of different bird species around the world.

Species	Place	Year	PFCAs								PFSA		REF
			PFBA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	PFOS	
Great tit Adults	Belgium	2015	<-133	<-232	<–81	<-477	<–57	<–61	<-25	<-2.4			<-161333	Current
Great tit nestlings			<-112	20-438	<–19	<–15	<–25	<–50	<–12	<–6.7			<-35624	
Bald eagle	USA	90s											1-2570	1
Albatrosses	North Pacific Ocean	1992-1996		0.1-0.30									0.310-13.4	2
†Carrion crow	Japan	2002									<45		68-1200	3
Glaucous gull	Norwegian Arctic	2004		0.70-0.74	2.3-6.3	3.1-15		2.9-24	3.6-30			0.3-2.7	48-349	4
*†Black-backed gull	Norway	2005						1.2±0.08	2.4±0.17			1.0±0.06	37±2.7	5
†Griffon vulture	Israel	2007		1.4–3.5									2.2–7.4	6
Tree swallow	Minnesota	2008-2009		2.1–3.5	1.8–7.6	3.4–13		0.7-4.3				4.5–19.2	75-190	7
Bald eagle nestlings	Midwestern USA	2006-2011		<–15	0.3-160	0.1–85	1.3-110	0.04–33	0.13–63	<-310	<-4100	<–47	6.6-4200	8
Kittiwakes	Svalbard	2012	<-78	0.03–0.12	0.8–3.0	1.3–2.8		1.5-4.0	4.5-29.7			0.01-0.22	6.8–14	9
*Kittiwakes	Svalbard	2012-2014			1.2±0.1	2.2 ± 0.1	12± 0.6	2.5± 0.1	11 ± 1.4				11±0.6	10
†Calonectris shearwaters	Mediterranean and Atlantic	2014					0.9–9.3	0.2-3.8	0.1-2.5				3.2 - 53	11
*Kittiwakes	Svalbard	2016			2.0±0.9	2.9±1.2	10.3±3.7	1.7±0.8	8.6±3.1	1.0±0.8			13±6.2	12

*Mean concentrations ± standard error. † Measured in whole blood (concentrations expressed in plasma would be 2 to 5-fold higher (Kannan et al. 2001))

< Concentrations below the LOQ.

References (Table S12):

1. Giesy, J. P.; Kannan, K.. Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol*. **2001**, *35*: 1339-1342.
2. Tao, L.; Kannan, K.; Kajiwara, N.; Costa, M. M.; Fillmann, G.; Takahashi, S.; Tanabe, S. Perfluorooctanesulfonate and related fluorochemicals in albatrosses, elephant seals, penguins, and polar skuas from the southern ocean. *Environ. Sci. Technol*. **2006**, *40*, 7642-7648.
3. Taniyasu, S.; Kannan, K.; Horii, Y.; Hanari, N.; Yamashita, N. A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan. *Environ. Sci. Technol*. **2003**, *37*, 2634-2639.
4. Verreault, J.; Houde, M.; Gabrielsen, G. W.; Berger, U.; Haukas, M.; Letcher, R. J.; Muir, D. C. G. Perfluorinated alkyl substances in plasma, liver, brain, and eggs of glaucous gulls (Larus hyperboreus) from the Norwegian Arctic. *Environ. Sci. Technol*. **2005***, 39*, 7439-7445.
5. Bustnes, J. O.; Erikstad, K. E.; Lorentsen, S.-H.; Herzke, D. Perfluorinated and chlorinated pollutants as predictors of demographic parameters in an endangered seabird. *Environ. Pollut.* **2008***, 156*, 417-424.
6. Shlosberg, A.; Rumbeiha, W. K.; Lublin, A.; Kannan, K. A database of avian blood spot examinations for exposure of wild birds to environmental toxicants: The DABSE biomonitoring project. *J. Environ. Monitor*. **2011**, *13*, 1547-1558.
7. Custer, C. M.; Custer, T. W.; Schoenfuss, H. L.; Poganski, B. H.; Solem, L. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reprod. Toxicol.* **2012,** *33*, 556-562.
8. Route, W. T.; Key, R. L.; Russell, R. E.; Lindstrom, A. B.; Strynar, M. J. Spatial and temporal patterns in concentrations of perfluorinated compounds in bald eagle nestlings in the upper Midwestern United States. *Environ. Sci. Technol* .**2014***, 48*, 6653-6660.
9. Tartu, S.; Gabrielsen, G. W.; Blévin, P.; Ellis, H.; Bustnes, J. O.; Herzke, D.; Chastel, O. . Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in arctic breeding black-legged kittiwakes. *Environ. Sci. Technol*. **2014**, *48*, 13504-13510.
10. Blevin, P.; Angelier, F.; Tartu, S.; Bustamante, P.; Herzke, D.; Moe, B.; Bech, C.; Gabrielsen, G. W.; Bustnes, J.O.; Chastel, O. Perfluorinated substances and telomeres in an Arctic seabird: Cross-sectional and longitudinal approaches. *Environ. Pollut.* **2017***, 230*, 360-367.

11. Escoruela, J.; Garreta, E.; Ramos, R.; González-Solís, J.; Lacorte, S. Occurrence of Per- and Polyfluoroalkyl substances in Calonectris shearwaters breeding along the Mediterranean and Atlantic colonies. *Mar. Pollut. Bull.* **2018***, 131*, 335-340.
12. Costantini, D.; Blévin, P.; Herzke, D.; Moe, B.; Gabrielsen, G. W.; Bustnes, J. O.; Chastel, O. Higher plasma oxidative damage and lower plasma antioxidant defences in an Arctic seabird exposed to longer perfluoroalkyl acids. *Environ. Res.,* **2019***, 168*, 278-285. doi:https://doi.org/10.1016/j.envres.2018.10.003