

Supplementary Material

Per- and polyfluoroalkyl substances in livers of wild boar (*Sus scrofa*) in Germany: analysis of official monitoring data in relation to local land use characteristics

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Instrumental analysis of the state laboratories

Federal state I (FS I):

Whole liver samples were homogenized using a Grindomix 200 (Retsch GmbH, Haan, Germany). An Aliquot of 0.1 g – 0.3 g homogenized wild boar liver was transferred into a 15 mL PP centrifuge tube (VWR International GmbH, Darmstadt, Germany) and suspended in 100 µL internal standard solution with a concentration of 0.1 µg/mL. The internal standard solution purchased from Wellington Laboratories (Ontario, Canada) consisted of MPFBA Perfluoro-n-(13C4)butanoic acid, MPFHxA Perfluoro-n-(1,2-13C2)hexanoic acid, MPFOA Perfluoro-n-(1,2,3,4-13C4)octanoic acid, MPFNA Perfluoro-n-(1,2,3,4,5-13C5)nonanoic acid, MPFDA Perfluoro-n-(1,2-13C2)decanoic acid, MPFDoA Perfluoro-n-(1,2-13C2)dodecanoic acid, MPFHxS Sodium perfluoro-1-hexane(18O2)sulfonate and MPFOS Sodium perfluoro-1-(1,2,3,4-13C4)octanesulfonate. 2 ml acetonitrile and 2 ml demineralized

water were added before closing the tube and mixing vigorously by shaking for 30 seconds by hand and on a mechanical shaker (Heidolph, Schwabach, Germany) for 20 minutes. After addition of 1.5 g QuEChERS (quick, easy, cheap, effective, rugged and safe) mix containing of 62% magnesium sulphate, 15% sodium chloride, 8% disodium hydrogen citrate sesquihydrate, and 15% tri-sodium citrate dihydrate (Anastassiades et al., 2003) samples were shaken again for 30 seconds by hand and centrifuged (centrifuge sigma 3k15, Osterode am Harz, Germany) for 4 minutes at 4000 rpm. Addition of the salt mixture to the sample resulted in a well-defined phase separation. The organic supernatant was mixed with 2 ml of demineralized water, and the target analytes were enriched by solid-phase extraction using a weak anion exchange mixed-mode sorbent (Oasis WAX 60 mg sorbent, 3 ml cartridge; Waters, Eschborn, Germany). The cartridge which had been conditioned first with 2 ml of 0.1% formic acid in water and then with 2 ml of methanol, served as the solid phase. Finally, the organic supernatant mixed with water was applied and the cartridge was then washed first with 1 ml of 2% formic acid in water and then with 1 ml of methanol. Elution of the target components was performed with 2 ml of 0.3% ammonia in methanol (v/v). The eluate was concentrated under a gentle stream of nitrogen by drying (biostep, sample concentrator, Jahnsdorf, Germany) at 39 °C and the residue was dissolved in 250 µL of methanol and water (50:50 v/v) by means of a vortex mixer (VWR, Darmstadt, Germany). The sample was then strained through a filter (pore size 0.2 µm; Macherey & Nagel, Düren, Germany) attached to a 2 ml disposable syringe and then transferred to a sample vial (Waters, Eschborn, Germany). Analysis was performed by high performance liquid chromatography–tandem mass spectrometry on Alliance e2695 separation module coupled to a Xevo-TQD tandem-MS using an UniSpray ionization source (Waters, Eschborn, Germany). For PFOS quantification, the possible interference from the endogenous Taurodeoxycholic acid (TDCA) was considered, as it shares the same mass transition and results in the same retention time. Thus, PFOS concentrations were evaluated using mass transition 499/80 in all liver samples. In cases of deviations, the results were reported using the second mass transition (499-99). The MRM settings for PFAS analysis are provided in Table S1.

Federal state II (FS II):

Sample preparation was performed using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique. The liver samples were homogenized using a R3-3000 blender (Robot Coupe SNC, Montceau-en-Bourgogne, France). An aliquot of 0.5 g of the homogenized material was transferred into a 30 ml PP tube. 20 µl internal standard (I.S.) solution mix (1 µg/ml) containing Perfluoro-*n*-(1,2,3,4-

$^{13}\text{C}_4$)-butanoic acid, Perfluoro-*n*-(3,4,5- $^{13}\text{C}_3$)-pentanoic acid, Perfluoro-*n*-(1,2- $^{13}\text{C}_2$)-hexanoic acid, Perfluoro-*n*-(1,2,3,4- $^{13}\text{C}_4$)-heptanoic acid, Perfluoro-*n*-(1,2,3,4- $^{13}\text{C}_4$)-octanoic acid, Perfluoro-*n*-(1,2,3,4,5- $^{13}\text{C}_5$)-nonanoic acid, Perfluoro-*n*-(1,2- $^{13}\text{C}_2$)-decanoic acid, Perfluoro-*n*-(1,2- $^{13}\text{C}_2$)-undecanoic acid, Perfluoro-*n*-(1,2- $^{13}\text{C}_2$)-dodecanoic acid, Sodium perfluoro-1-(2,3,4- $^{13}\text{C}_3$)-butanesulfonate, Sodium perfluoro-1-hexane($^{18}\text{O}_2$)-sulfonate, Sodium perfluoro-1-(1,2,3,4- $^{13}\text{C}_4$)-octanesulfonate (purchased as solution from Wellington Laboratories, Ontario, Canada) as well as $^{13}\text{C}_2$ -ADONA (Dünnschichttechnologie u. Mikrosensorik e.V., Teltow, Germany) and 20 µl internal standard (I.S.) solution mix (1 µg/ml) containing Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-(1,2- $^{13}\text{C}_2$)-octane sulfonate and Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-(1,2- $^{13}\text{C}_2$)-decane sulfonate (Wellington Laboratories, Ontario, Canada) were added. Then 10 ml water were given into the tube and the contents were mixed vigorously using a test tube shaker (Vortex-T Genie 2, Scientific Industries, INC, Bohemia, NJ). 10.0 ml of acetonitrile were then added and mixed vigorously again using a test tube shaker (see above). The tube closed by a cap was shaken by a mechanical shaker (Unimax 2010, Heidolph, Schwabach, Germany) for 5 minutes and then extracted in an ultrasonic bath (Sonorex Super RK 102P, Bandelin, Berlin, Germany) at 20 °C starting temperature for 15 minutes. The content of the Citrate Extraction Tube (CET-Tube from the Supel™ QuE Kit) was added. The sample was shaken with a test tube shaker (see above) for 1 min and centrifuged at 3000 rpm for 10 min at 5 °C (Centrifuge 5810 R, Eppendorf SE, Hamburg, Germany). Two or four milliliters of the supernatant solution (depending on PFAS amount in liver sample) were transferred into a 15 ml PP tube, 200 µl of water were added and the solution was evaporated in a current of nitrogen at 40 °C to less than 200 µl. The remaining solution was supplemented to 200 µl with water and 200 µl of acetonitrile-methanol (10+90) were added. After mixing on a test tube shaker the tube was put in an ultrasonic bath (see above) for 1 minute and centrifuged at 3000 rpm for 10 min. The clear supernatant was transferred into an autosampler vial. Analysis was performed by LC–MS/MS using an AB Sciex API3000 (AB Sciex Canada, 2017-2020) or an AB Sciex QTRAP 5500+ (AB Sciex, Canada, 2020-2021, B) LC-MS/MS-system with an ESI source operating in the negative ion mode.

2017-2020: 5 µl of the extract were injected into the HPLC-system (Agilent 1100 LC series with binary pump, 3-line degasser, autosampler and column oven compartment, Agilent, Palo Alto, CA) equipped with a Gemini C18 column (3 µm, 110 Å, 100 × 2 mm, Phenomenex, Aschaffenburg, Germany) with corresponding guard column Gemini C18 (4 × 2 mm). The following gradient program with a flow rate of 270 µl/min was applied: Starting with 70% of 2 mM ammonium acetate in water (95%) mixed with 5%

of acetonitrile (eluent A) and 30% of acetonitrile (60%) mixed with methanol (40%) (eluent B). The proportion of eluent B was increased to 70% within 6 min and consecutively was increased to 98% within another 6 min. The condition was held at 98% for 5 min and then reversed back down to 70% of eluent A in 0.1 min and subsequently equilibrated for 3.9 min. 2020-2021: 3 µl of the extract were injected into the Exion LC-system (ExionLC series with binary pump, 3-line degasser, autosampler and column oven compartment, AB Sciex, Canada) equipped with a Gemini C18 column (see above) with corresponding guard column (see above). The following gradient program with a flow rate of 270 µl/min was applied: 70% of 5 mM ammonium acetate in water (95%) mixed with 5% of acetonitrile (eluent A) and 30% of acetonitrile (60%) mixed with methanol (40%) (eluent B) was held for 0.1 min. The proportion of eluent B was increased to 93% within 14.9 min and then was increased to 98% within another 0.1 min. The condition was held at 98% for 5.9 min and then reversed back down to 70% of eluent A in 0.2 min and subsequently equilibrated for 4.8 min. For PFOS analysis (only non-branches PFOS was quantified), the concentrations in liver samples were calculated using the 499 - 80 transition. In accordance with Ehlers (2012), potential interference from TDCA was considered, and the selectivity of the chromatography was verified during method validation. Additionally, for positive results, the deviation in the ion ratio between the 499 - 80 transition (used for quantification) and the 499 - 99 transition had to be within 30% of the ratio observed in a reference solution of non-branched PFOS in order to rule out false-positive results. The laboratory successfully participated in several round robin tests using the described method e, g. for pike perch, egg yolk and whole egg. The MRM settings for analysis in FS II are provided in Table S1.

Federal state III (FS III):

For sample homogenization, the whole liver was homogenized using Grindomix 300 (Retsch Hann, Germany). Two different sample preparation methods were used in 2014 and 2020. For all samples measured in 2014, a homogenate of 1 g of wild boar liver was transferred into a 50-ml PP tube and 75 µl of 0.5 µg/ml I.S. solution (Perfluoro-[1,2,3,4-¹³C₄] octanoic acid, 1,2,3,4-¹³C₄-Perfluoro-n-octane sulfonate and ¹³C₈-Perfluoro-n-octane sulfonate in MeOH), 5 ml of demineralized water, 1 ml of 0.5 M tetrabutylammonium hydrogensulfate solution (adjusted to pH 10), and 2.5 ml of 0.25 M sodium carbonate buffer were added and mixed thoroughly for 30 min. Subsequently, 15 ml of methyl-tert-butyl ether were added to the sample extract and shaken for 30 min. The extract was then centrifuged at 3000 rpm for 5 min to separate the organic layer from the aqueous layer. The aqueous layer was

transferred in a 15-ml PP tube and evaporated to near-dryness under a gentle stream of nitrogen. The extraction step was repeated twice. The sample was reconstituted with a mixture of 700 µl of 10 mM ammonium acetate solution and methanol (30 + 70; V + V) using the Vibromix and ultrasound treatment and centrifuged at 4000 rpm for 2 min before being transferred into a PP vial via a dosing syringe and an attached syringe filter unit (Pall Life Sciences GHP Acrodisc 0.2 µm/13 mm). The samples were measured using liquid chromatography tandem mass-spectrometry (LC–MS/MS Agilent 1290/6490 Triple Quadrupole) operating in the negative ion mode. Information about the MRM settings for analysis can be found in Table S1.

Samples measured in 2020 were prepared according to Anastassiades et al. (2003). A homogenate of 1 g of wild boar liver was transferred into a 50-ml PP tube and 40 µl of 0.1 µg/ml I.S. solution (Perfluoro-[1,2,3,4-¹³C₄] octanoic acid, 1,2,3,4-¹³C₄-Perfluoro-n-octane sulfonate and ¹³C₈-Perfluoro-n-octane sulfonate in MeOH), 10 ml of demineralized water, 10 ml of acetonitril were added. The samples were then subjected to shaking in an overhead shaker for 5 minutes. Following this, the sample suspensions underwent treatment in an ultrasonic bath for 15 minutes at room temperature (20°C). A Citrate Extraction Kit (BEKOLut® Citrate-Kit-01) was added to each sample, followed by manual shaking for 1 minute to ensure homogeneous distribution. The samples were then centrifuged at 4000 rpm for 10 minutes to separate the phases. An aliquot of 4 mL from the upper phase (acetonitrile) was subsequently pipetted into a 15 mL polypropylene tube, followed by the addition of 200 µL of water and 5 µL of glycerol. The samples were evaporated to 200 µL using nitrogen in a heating block set to 40°C, utilizing graduations or markings on the tubes for accurate volume control. If necessary, the samples were reconstituted with water to reach a final volume of 200 µL. The samples were then adjusted to a final volume of 400 µL using an acetonitrile-methanol mixture (10+90, v+v). After thorough homogenization using a vortex mixer and a subsequent 1-minute treatment in an ultrasonic bath, the samples underwent centrifugation at 4000 rpm for 10 minutes. The clear supernatant was then carefully pipetted into a vial. In cases of cloudy extracts, filtration was performed using syringe filters. The samples were measured using liquid chromatography tandem mass-spectrometry (LC–MS/MS Agilent 1290/6490 Triple Quadrupole) as described above.

Table S1. MRM settings and retention time for PFAS by the federal states (FSs)

Federal state (FS)	Substance	Abbreviation	Precursor Ion m/z	Production Ion m/z	Retention Time
FS I	Perfluorooctanoic acid	PFOA	413	369	8.70
	Perfluorooctanesulfonic acid	PFOS	499	80	9.12
	Perfluorohexanesulfonic acid	PFHxS	399	80	8.05
	Perfluoronanoic acid	PFNA	463	419	9.16
	Perfluorobutanesulfonic acid	PFBS	299	80	4.41
	Perfluoropentanoic acid	PFPeA	263	219	4.29
	Perfluorohexanoic acid	PFHxA	313	269	6.73
	Perfluoroheptanoic acid	PFHpA	363	319	8.05
	Perfluorodecanoic acid	PFDA	513	469	9.54
	Perfluorododecanoic acid	PFDoA	613	569	10.26
FS II	Perfluorooctanoic acid	PFOA	413	169	8.9
	Perfluorooctanesulfonic acid	PFOS	499	80/99	11.0
	Perfluorohexanesulfonic acid	PFHxS	399	80	8.7
	Perfluoronanoic acid	PFNA	463	419	10.2
	4,8-dioxo-3H-Perfluorononanoic Acid	DONA	377	250	7.9
	Perfluorobutanesulfonic acid	PFBS	299	80	5.2
	Perfluoropentanoic acid	PFPeA	263	219	2.9
	Perfluorohexanoic acid	PFHxA	313	269	5.4
	Perfluoroheptanoic acid	PFHpA	363	319	7.4
	Perfluorodecanoic acid	PFDA	513	469	11.2
	Perfluoro-n-undecanoic acid	PFUnA	563	519	12.1
	Perfluoro-1,10-decanedicarboxylic acid	PFDDA	613	569	12.8
	6:2-Fluorotelomersulfonic acid	6:2-FTS	427	81	8.4
	8:2-Fluorotelomersulfonic acid	8:2-FTS	527	81	10.9
FS III	Perfluorooctanoic acid	PFOA	413	369/169	15,7
	Perfluorooctanesulfonic acid	PFOS	499	80/99	17
	Perfluorohexanesulfonic acid	PFHxS	399	80/99	14,5
	Perfluoronanoic acid	PFNA	463	419/219	16,9
	Perfluorobutanesulfonic acid	PFBS	299	80/99	9,9
	Perfluoropentanoic acid	PFPeA	263	219	8,4
	Perfluorohexanoic acid	PFHxA	313	269/119	12,1
	Perfluoroheptanoic acid	PFHpA	363	169/319	14,3
	Perfluorodecanoic acid	PFDA	513	469/219	17,8
	Perfluoro-n-undecanoic acid	PFUnA	563	519/319	18,7

Table S2. Number of samples per county and year reported by the federal states (FSs) regarding the concentrations of diverse PFAS in livers of wild boars (*Sus scrofa*)

Federal state	County	Samples per year (n)						Total
		2014	2017	2018	2019	2020	2021	
FS I	A	.	.	.	.	.	2	2
	B	.	.	.	.	.	2	2
	C	.	.	.	.	.	4	4
	D	.	.	.	.	.	2	2
	E	.	.	.	.	.	4	4
	F	.	.	.	.	.	1	1
	G	.	.	.	.	.	2	2
	H	.	.	.	.	.	4	4
	I	.	.	.	.	.	1	1
	J	.	.	.	.	.	1	1
	K	.	.	.	.	.	17	17
	L	.	.	.	.	.	3	3
	M	.	.	.	.	.	1	1
	Total	.	.	.	.	.	44	44
FS II	A	.	2	5	2	1	1	11
	B	.	.	1	4	.	.	5
	C	.	.	.	1	.	.	1
	D	.	.	.	.	3	.	3
	E	.	.	.	1	5	5	11
	F	.	.	1	.	.	.	1
	G	.	.	.	.	4	.	4
	H	.	.	.	.	3	.	3
	I	.	.	.	1	.	.	1
	J	.	.	.	1	.	.	1
	K	.	.	.	2	.	.	2
	L	.	.	1	.	.	.	1
	M	.	.	.	1	.	.	1
	N	.	.	.	1	.	.	1
	O	.	1	1	1	.	.	3
	P	.	.	.	2	.	.	2
	Q	.	1	.	2	.	.	3
	R	.	.	.	3	.	.	3
	S	.	.	.	2	.	.	2
	T	.	.	.	1	1	.	2
	U	.	.	1	.	.	.	1
	V	.	.	.	1	.	.	1
	W	.	.	.	1	.	.	1
	X	.	1	5	1	.	.	7
	Y	.	.	.	1	.	.	1
	Z	.	1	.	3	.	.	4
	AA	.	.	.	4	2	.	6
	AB	.	.	.	3	.	.	3
	AC	.	.	.	2	1	.	3

	AD	.	.	.	3	.	.	3
	AF	.	.	.	1	.	.	1
	AG	.	.	.	1	1	.	2
	AH	.	.	.	1	.	.	1
	AI	.	.	.	1	.	.	1
	AJ	.	.	.	1	1	.	2
	AK	.	.	.	2	.	.	2
	AL	.	.	.	2	.	.	2
	AM	.	.	.	.	2	.	2
	AN	.	.	.	1	2	.	3
	AO	.	.	.	1	3	1	5
	AP	.	1	.	3	4	.	8
	AQ	.	.	.	1	2	.	3
	AR	.	.	.	1	.	.	1
	AS	.	.	.	3	.	.	3
	AT	.	.	.	1	.	.	1
	Total	0	7	15	64	35	7	128
FS III	A	9	.	.	.	1	.	10
	B	7	.	.	.	5	.	12
	C	1	.	.	.	5	.	6
	D	4	.	.	.	1	.	5
	E	4	.	.	.	.	.	4
	F	5	.	.	.	3	.	8
	Total	30	0	0	0	15	0	45

Table S3. Difference in average sizes and corresponding land coverage of counties included in this study by FS. Q1 and Q3 are the lower and upper quartiles, respectively.

Area	Average size in km ² (Q1 : Q3)		
	FS I	FS II	FS III
Total	1,380.4 (738.4 : 1539.0)	802.1 (695.2 : 1144.6)	3,431.2 (3341.2 : 4766.8)
Forest	497.1 (287.7 : 544.3)	236.5 (176.4 : 397.4)	566.3 (539.2 : 1299.9)
Urban	102.5 (81.8 : 104.4)	61.2 (54.4 : 68.7)	200.3 (200.3 : 226.0)
Industry	15.7 (13.9 : 16.7)	11.6 (9.9 : 12.9)	36.5 (29.0 : 38.0)
Agriculture	663.4 (335.6 : 740.7)	401.9 (316.3 : 513.9)	2,335.7 (2335.7 : 2812.0)

References

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