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Perfluoroalkylated substances in human urine: results of a biomonitoring pilot study

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Abstract: Perfluoroalkylated substances (PFASs) are a class of synthetic chemicals used in a wide range of processes and products due to their unique physical-chemical properties. Through intake of PFASs via food or several consumer products, humans can be exposed. Long-chain PFASs have been associated with adverse effects in laboratory animals, and there is also evidence for adverse health effects in humans. Although investigations of human exposure are mainly conducted in blood samples, some studies have shown that especially short-chain PFASs can be detected in human urine. In the present study, a sensitive analytical method was adapted for the measurement of 12 PFASs in human urine samples by HPLC-MS/MS. For verifying this method, concentrations in 11 male and female participants aged 25-46 years were analysed. In the study population, ranges of urinary PFASs concentrations were n.d.-8.5 ng/l for perfluoropentanoic acid, <LOQ-3.0 ng/l for perfluorohexanoic acid, n.d.-1.8 ng/l for perfluorohexane sulphonate, n.d.-0.99 ng/l for perfluoroheptanoic acid, 0.79-5.1 ng/l for perfluorooctanoic acid, <LOQ-4.9 ng/l for perfluorooctane suphonate, and <LOQ-0.57 ng/l for perfluorononanoic acid. For the other investigated PFASs, no urinary exposure could be identified in any of the samples. The present study shows that several short-chain PFASs are detectable in human urine.

Keywords: human biomonitoring, urinary PFAS, LC-MS/MS

1 Introduction

Perfluoroalkylated substances (PFASs) are a large group of synthetic chemicals, which have been produced since the late 1940s. They are used in a variety of processes and products including water and oil repellents for leather, paper and textiles, inks, hydraulic oils, firefighting foams [1], kitchenware, packaging in food industry, floor polish, insecticides and cosmetics [2]. PFASs consist of fully fluorinated alkyl chain composed of 4-18 carbon atoms and a terminal functional group, which determines their classification into ionic and non-ionic (neutral) PFASs [3]. Additionally, PFASs are classified in relation to their chain length in short-chain and long-chain PFASs, with long-chain PFASs including perfluorocarboxylic acids with carbon chain lengths of ≥ 8 and perfluoroalkyl sulphonates with carbon chain lengths of ≥ 6, as well as their precursors [4].

PFASs have a high chemical, thermal and biological inertness and show unique physical-chemical properties such as being both hydrophobic and oleophobic as well as extremely stable, possessing a great potential to lower surface tension, and being capable of creating stable foams and coatings [1,5]. During their production, application and use, PFASs can be released into the environment. Because of their stability, ionic PFASs are highly resistant to degradation and metabolism. Contrarily, non-ionic PFASs are not usually persistent, but can be transformed into persistent ionic PFASs. Generally, PFAS are ubiquitous and can be found globally in the environment, wildlife and humans. For several PFASs, accumulation in organisms and biomagnification in food chains have been shown [3]. The most abundant PFAS is perfluorooctane sulphonate (PFOS), which is highly persistent, bioaccumulative and toxic. It fulfils the criteria of the Stockholm-Convention of persistent organic pollutants (POPs) and has been included in the list of restricted chemicals since 2009 [6-7]. For perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds, a risk profile has been established under the Stockholm-Convention. As the next step, risk management options will be drafted in the framework of

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the Convention [8]. PFOA is also the subject of a restriction proposal according to Annex XVII of the European REACH regulation [9]. Other long-chain PFASs are also the subject of risk management option analysis within the context of REACH [10].

Humans can be exposed to PFASs via oral (food, drinking water, dust or soil ingestion), dermal (contact with consumer products, e.g. all-weather clothing or textiles) or inhalative (contaminated air) routes of exposure. However, the main source of human exposure is food, with fish and seafood having been identified as major contributors. Additionally, frying and cooking in non-stick cookware and food packaged in repellent-coated material might reflect additional sources for PFASs in foodstuffs [11]. Other contributors to human PFASs exposure are some types of paper or cardboard containing surface coatings with large amounts of PFASs. Thus, food like microwave popcorn can be contaminated due to the migration of PFASs after heating [12].

PFASs have been shown to be easily absorbed but only poorly eliminated by experimental animals. The longer chain compounds are not well metabolised in the organism and undergo enterohepatic circulation [13]. PFASs primarily bind to proteins and can thus accumulate in different body compartments. High concentrations can be found in the blood, liver, kidneys and spleen, but also in the testes and brain [14]. For example, more than 90% of perfluorooctanoic acid (PFOA) binds to serum albumin [15]. After absorption and distribution via blood, PFOA is eliminated as free carboxyl acid primarily via urine and secondarily via faeces, with urinary excretion decreasing with increasing chain-length. In rodents, 100% of the short-chain perfluorohexanoic acid (PFHxA) is eliminated within 24 hours via urine [14].

Studies conducted on experimental animals have determined the half-lives of different PFASs to be short, while the results of human studies, in contrast, indicate considerably longer half-lives. The estimated mean elimination half-lives in human serum are 5.4 years for PFOS, 3.8 years for PFOA and 8.5 years for perfluorohexane sulphonate (PFHxS) based on studies of a highly exposed occupational cohort [16].

PFASs have been identified to lead to adverse health effects. Especially for PFOS and PFOA, extensive toxicity data are available from studies conducted on experimental animals, with effects having been identified on hepatotoxicity and the immune and developmental systems, as well as concerning increased mortality, hormonal effects and a carcinogenic potency [2,5,13,17-18,20-22]. In humans, possible post-exposure effects could be related to different cancer types and the reproductive, developmental and hormone systems [5,23-25].

In order to investigate human exposure to chemical substances, human biomonitoring (HBM) is used as a tool for exposure assessment, with assessments of PFASs exposure being mainly conducted on blood samples [1,5,13-14,20,26-28,30-31]. PFASs were also investigated in other human media such as breast milk [14,20,29]. However, investigations of the occurrence of specific PFASs in human urine are comparably rare [30-37].

For the present study we adapted a sensitive analytical method for the measurement of several PFASs in human urine with a primary focus on short-chain PFASs. Furthermore, we also conducted a pilot study with 11 participants in order to verify the analytical method.

2 Materials and methods

2.1 Chemical analysis

The chemical analysis of 12 PFASs compounds in human urine was performed by high-performance liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) for simultaneous determination based on an analytical method published in Zhang et al. [35]. PFASs compounds included in the analysis and the internal and external standards which were used are listed in Table 1.

To avoid potential contamination of the samples during sample preparation, all glass equipment was pre-treated with methanol and water and heated at 300°C for 24 hours. Solvents were distilled, and all water used was filtered by an Oasis HLB-column (6cc Cartridge 500 mg) *(Waters Corporation, Milford, MA, USA)* and cartridges (Oasis Wax 6cc Vac Cartridge, 500 mg sorbent, 60 µm particle size and Oasis Wax 6cc Vac Cartridge, 150 mg sorbent, 30 µm particle size) *(Waters Corporation, Milford, MA, USA)*. Additionally, for the minimisation of matrix effects during HPLC analysis, an additional clean-up of the solid-phase extraction (SPE) eluates was performed with charcoal.

For sample preparation, 50 ml of urine sample were weighted in polypropylene tubes *(Sarstedt, Nümbrecht, Germany)* and 1 ng of the internal standard solution was added. 50 g of filtered tap water were used for the preparation of blanks. For additional quality assurance, sample container blanks were prepared. For recovery control, the native standard solution was added to samples at two concentration levels (10 and 25 ng/l) and to blanks at a single concentration level (10 ng/l). After centrifugation for 20 minutes at 4,700 rpm, samples were applied to cartridges (Oasis Wax 6cc Vac Cartridge, 150 mg sorbent,

Table 1: Analytes, limits of detection (LOD), limits of quantification (LOQ) and internal standards.

Analyte (chain-length)	Abbreviation	CAS Number	LOD (ng/l)	LOQ (ng/l)	Mass transition(s) (m/z)	Internal Standard
Perfluoropentanoic acid (C5)	PFPeA	2706-90-3	0.50	1.25	263>219	perfluoro-n-[1,2-13C2]hexanoic acid
Perfluorohexanoic acid (C6)	PFHxA	307-24-4	0.20	0.50	313>269	perfluoro-n-[1,2-13C2]hexanoic acid
Perfluorohexane sulphonate (C6)	PFHxS	355-46-4	0.30	0.75	399>99	sodium perfluoro-1-hexane[18O2]sulfonate
Perfluoroheptanoic acid (C7)	PFHpA	375-85-9	0.20	0.50	363>319	perfluoro-n-[1,2,3,4-13C4]octanoic acid
Perfluoroheptane sulfonate (C7)	PFHpS	375-92-8	0.30	0.75	449>80	sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate
Perfluorooctanoic acid (C8)	PFOA	335-67-1	0.20	0.50	413>369	perfluoro-n-[1,2,3,4-13C4]octanoic acid
Perfluorooctane sulphonate (C8)	PFOS	1763-23-1	0.50	1.25	499>99	perfluoro-n-[1,2,3,4-13C4]octanoic acid
Perfluorononanoic acid (C9)	PFNA	375-95-1	0.20	0.50	463>419	perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid
Perfluorodecanoic acid (C10)	PFDA	335-76-2	0.20	0.50	513>469	perfluoro-n-[1,2,-13C2]decanoic acid
Perfluorodecane sulphonate (C10)	PFDS	335-77-3	0.30	0.75	599>80	perfluoro-n-[1,2,3,4-13C4]octanoic acid
Perfluoroundecanoic acid (C11)	PFUnDA	2058-94-8	0.20	0.50	563>519	perfluoro-n-[1,2,-13C2]dodecanoic acid
Perfluorododecanoic acid (C12)	PFDoDA	307-55-1	0.50	1.25	613>569	perfluoro-n-[1,2,-13C2]dodecanoic acid

30 µm particle size; *Waters Corporation, Milford, MA, USA*) which had been pre-treated with methanol (MeOH) *(Pestinorm, VWR International, Leuven, Belgium)* and 0.1 M formic acid (HFA) *(Merck, Darmstadt, Germany)*. PFAS were adsorbed with a flow rate of ca. 3 ml per minute at low vacuum. Cartridges were washed with 5 ml 0.1 M HFA/MeOH (1:1) and 1 ml aqueous ammonia solution (NH_4OH)/HPLC water (1:100) *(Merck, Darmstadt, Germany)*, and were dried under nitrogen for 30 minutes. Afterward, samples were eluted with 5 ml NH_4OH/acetonitrile (ACN) (1:100) *(Optigrade, Promochem, LGC Standards, Wesel, Germany)* in polypropylene tubes and restricted under nitrogen to a volume of 200 µl. After the addition of 30 µl acetic acid (HAc) (100%) *(Merck, Darmstadt, Germany)*, samples were filled up with ACN to a volume of 500 µl. Samples were transferred to 2 ml centrifuge tubes with 25 mg Supelclean ENVI-Carb 120/400 *(Sigma-Aldrich Co. LLC., USA)*. After mixing, samples were centrifuged for 20 minutes at 12,000 rpm. 300 µl of the supernatant were transferred into polypropylene autosampler vials and 300 µl HPLC water *(Optigrade, Promochem, LGC Standards, Wesel, Germany)* were added.

The analysis of PFASs was conducted by HPLC-MS/MS. The HPLC system used was an Agilent Technologies 1290 Infinity Series *(Agilent Technologies, Santa Clara, CA, USA)*, and the MS detector system used was an AB Applied Biosystem MDS SCIEX 4000 QTRAP LC/MS/MS System *(AB Sciex Technologies, Framingham, MA, USA)* which allowed detection through specific mass transitions in electrospray ionization (ESI) negative mode and quantification in multiple reaction monitoring (MRM) mode. A Luna 5 µm C18(2), 100 x 2 mm *(Phenomenex, California, USA)* was used as an analytical column. Injected sample volume was 10 µl. Separation of the investigated PFASs was performed by a gradient elution method using MeOH *(LiChrosolv hypergrade for LC-MS, Merck, Darmstadt, Germany)* and 10 mM NH_4OH/H_2O *(LC/MS Optigrade, Promochem, LGC Standards, Wesel, Germany)*. The external solvent-based calibration included 10 concentrations ranging between 0.01 and 5.0 ng/ml. One run required 13 minutes at a flow rate of 250 µl per minute. Each sample was measured three times. Results of the analysis are expressed as their means corrected by blanks and recoveries of the corresponding internal standard.

The limits of quantification (LOQ) and limits of detection (LOD) of the investigated PFASs are listed in Table 1. The LODs were set as 40% of the respective LOQs. The mean relative recoveries for the different PFASs were calculated based on spiked samples (n=32) and ranged between 56±12% and 74±9%.

2.2 Creatinine analysis

Due to the possibility of urine concentrations or urine dilution affecting concentrations of analytes and due to variable creatinine production during different life stages [19], creatinine adjustment was performed for the correction of these variations. Creatinine concentrations of the urine samples provided were analysed by an external medical laboratory via a photometric method by Roche/Hitachi cobas c 701 analyser *(Roche Diagnostics, USA)*.

2.3 Study design and study population

For verification of the analytical method adapted for urinary PFASs analysis, a pilot study was conducted. Participants were recruited in August 2016 and included 11 staff members of the Environment Agency Austria (EAA). Before inclusion in the pilot study, participants had to sign an informed consent. Urine samples (first morning void) were collected from all participants and were stored at -20°C at the EAA laboratory until analysis. Questionnaires were provided to survey personal information (e.g. age, sex), health, lifestyle (e.g. use of functional sportswear), working place, living place and nutrition including a food frequency questionnaire for the previous two months as well as for the last three days before sampling to potentially find possible indications of statistical associations and/or differences between the surveyed parameters and the PFASs exposure.

In total, 11 healthy staff members comprising 6 males and 5 females aged 25-46 years were included in the pilot study. Details on the study population are given in Table 2.

2.4 Statistical analysis

Statistical analysis was conducted using IBM® SPSS® Statistics Version 21. Descriptive statistics including ranges, medians, means and corresponding standard deviations were determined for unadjusted urinary PFASs concentrations (expressed in ng/l urine) and for creatinine-adjusted urinary PFASs concentrations (expressed in ng/g creatinine). For data treatment, PFASs levels below the LOQ were set to LOQ/2, and levels <LOD were set to zero.

Urinary PFASs levels were tested for their normal distribution with a Kolmogorov-Smirnov test. For the investigation of correlations between the different PFASs concentrations, Pearson tests were used for normal distributed data and Spearman's rank tests for data which were not normally distributed. For the statistical analysis of differences between groups (e.g. sex), Mann-Whitney U and Kruskal-Wallis tests were used depending on the type of data. Values of $p < 0.05$ were considered for the indication of statistical significance.

3 Results and discussion

The concentrations of a total of 12 PFASs in 11 first morning void urine samples of male and female adults are shown in Table 3 – in ng/l for unadjusted levels and in ng/g for creatinine-adjusted levels. The compounds PFHxA, PFOA, PFOS and PFNA were found in all urine samples investigated in concentrations (unadjusted) ranging between <LOQ-3.0 ng/l (median: 1.5 ng/l), 0.79-5.1 ng/l (1.9 ng/l), <LOQ-4.9 ng/l (1.6 ng/l) and <LOQ-0.57 ng/l (<LOQ), respectively. In 72.7% of the urine samples, PFPeA and PFHxS were detected at levels of n.d.-8.5 ng/l (1.8 ng/l) and n.d.-1.8 ng/l (0.80 ng/l), respectively. PFHpA was found in concentrations of n.d.-0.99 ng/l (<LOQ) in 90.9% of the samples. PFHpS and PFASs with high chain-lengths (> 10 carbon atoms) including PFDA, PFDS, PFUnDA and PFDoDA were not detected in any urine sample investigated. Regarding individual creatinine excretion, creatinine-adjusted PFASs concentrations ranged between <LOQ-2.8 ng/g (median: 1.6 ng/g), 1.1-7.8 ng/g (1.6 ng/g), <LOQ-4.2 ng/g (1.5 ng/g) and <LOQ-0.63 ng/g (<LOQ) for PFHxA, PFOA, PFOS and PFNA, respectively. PFPeA, PFHxS and PFHpA were found in adjusted concentrations between n.d. and 6.3 ng/g (2.0 ng/g), between n.d. and 2.2 ng/g (0.82 ng/g) and between n.d. and 0.82 ng/g (<LOQ), respectively. The median PFASs concentrations were similar between substances with chain-lengths of between 5 (PFPeA) and 8 (PFOS, PFOA), ranging between 0.80 ng/l for PFHxS and 1.9 ng/l for PFOA, except in the case of substances with chain-lengths of 7, as PFHpA exhibited a median level of <LOQ and PFHpS of n.d. Figure 1 shows the graphical distribution of investigated PFASs detectable in the urine samples for unadjusted and creatinine-adjusted concentrations, respectively.

No statistically significant differences and/or associations were found between urinary PFASs exposure

Table 2: Description of the pilot study population group.

	All	males	females
n	**11**	**6**	**5**
Age [years] (mean±SD)	25-46 (35±7)	31-46 (37±6)	25-44 (32±7)
Body weight [kg] (mean±SD)	50-96 (69±14)	67-96 (80±10)	50-63 (57±6)
Body height [cm] (mean±SD)	163-180 (173±5)	170-180 (175±5)	163-173 (170±4)

Abbreviations: n: sample size; SD: standard deviation.

Table 3: Urinary unadjusted (in ng/l) and creatinine-adjusted (in ng/g) PFAS concentrations in first morning void urine samples of 11 pilot study participants (ranges, medians, means ± standard deviations; detection rate in % of positive detected samples).

Substance		Unadjusted concentrations (ng/l)			Creatinine-adjusted concentrations (ng/g creatinine)		
	DR (%)	**Range**	**Median**	**Mean±SD**	**Range**	**Median**	**Mean±SD**
PFPeA	*72.7*	n.d.-8.5	1.8	2.6±3.0	n.d.-6.3	2.0	2.3±2.2
PFHxA	*100*	<LOQ-3.0	1.5	1.6±0.84	<LOQ-2.8	1.6	1.6±0.64
PFHxS	*72.7*	n.d.-1.8	0.80	0.79±0.62	n.d.-2.2	0.82	0.82±0.74
PFHpA	*90.9*	n.d.-0.99	<LOQ	0.41±0.29	n.d.-0.82	<LOQ	0.41±0.25
PFHpS	*0*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFOA	*100*	0.79-5.1	1.9	2.1±1.2	1.1-7.8	1.6	2.4±1.9
PFOS	*100*	<LOQ-4.9	1.6	1.8±1.2	<LOQ-4.2	1.5	1.8±1.0
PFNA	*100*	<LOQ-0.57	<LOQ	0.28±0.1	<LOQ-0.63	<LOQ	0.33±0.16
PFDA	*0*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDS	*0*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFUnDA	*0*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFDoDA	*0*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Abbreviations: DR, detection rate; n, number of analysed samples; n.d., not detectable; LOQ, limit of quantification; SD, standard deviation.

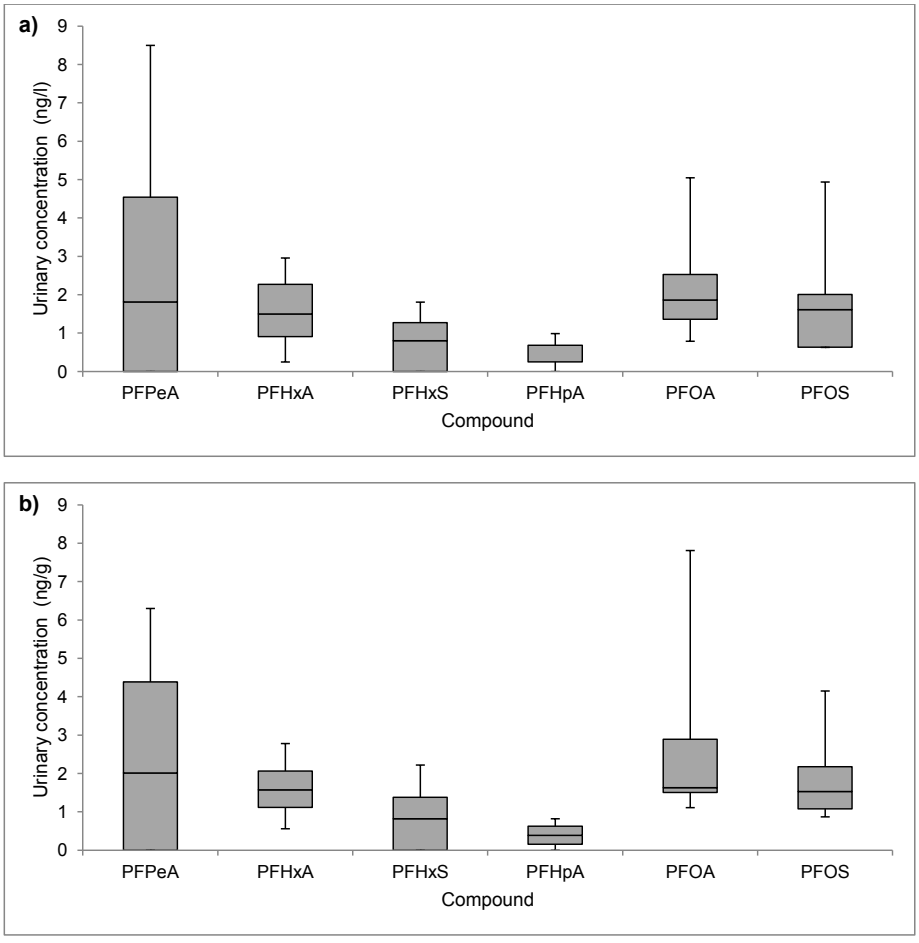

Figure 1: Graphic distribution of selected a) unadjusted PFAS concentrations (in ng/l) and b) creatinine-adjusted PFAS concentrations (in ng/g) in the human urine samples analysed (n=11).

and age, sex, the use of potentially PFASs-containing products such as functional sportswear, baking parchment or non-stick cookware, weight loss, or the consumption of microwave popcorn or fish. Statistically significant correlations could be identified between unadjusted PF6C and PF8C levels (r=0.764) as well as between creatinine-adjusted PF8C and PF9C levels (r=0.782) at a significance level of 0.01. However, it is important to note that the low sample size is very likely to be insufficient for the determination of statistically significant differences and/or associations.

Only a few studies have investigated PFASs exposure in human urine (see Table 4). Harada et al. estimated urinary PFOA and PFOS concentrations in 48 adults from Japan based on 24-hour urine samples, which were found in all samples in concentrations ranging between 8.7 and 39 ng/d (median: 8.6 ng/d) and between 1.4 and 36 ng/d (9.9 ng/d), respectively [32]. A study conducted in a medical researcher from Canada in 2007 analysed seven different PFASs in urine, finding that only PFOA was detectable at a concentration of 3,720 ng/l [33]. Zhang et al. analysed several PFASs in urine samples of 86 Chinese adults. PFOS and PFOA were found in all investigated samples with the highest concentration of 1,040 ng/l found for PFOA. The detection rates for the other compounds analysed ranged between 67% (PFHxA) and 98% (PFHxS and PFUnDA) [34]. An investigation of PFOS and PFOA in urine samples from Chinese adults aged 22-62 years in 2010 showed exposure levels of n.d.-296 ng/l (median: < 4 ng/l) for PFOS and of n.d.-41 ng/l (9.0 ng/l) for PFOA with detection rates of 48% and 76%, respectively. In the same study, the investigation of pregnant women aged 21-39 years showed urinary exposure to PFOS of n.d.-132 ng/l (median: < 4 ng/l) and of PFOA of n.d.-30 ng/l (< 4 ng/l) with detection rates of 11% for PFOS and of 30% for PFOA [35]. In a study conducted in 2012 of children aged 5-13 years and adults aged 20-29 years from South Korea, urinary exposure to a total of 15 PFASs was investigated. Most compounds were not detectable or only detectable in frequencies of 1%. PFPeA, PFHxA and PFHpA were found in 11% (PFHxA) to 70% (PFPeA) of urine samples of children, with the highest concentration being found for PFPeA at 11,600 ng/l. In adults, the named substances were analysed in 5% (PFHxA) to 25% (PFPeA) of the investigated samples, with the highest maximum concentration within this population group reaching 17,600 ng/l for PFPeA [36]. Li et al. analysed urinary PFOS and PFOA exposure levels in 63 Chinese adults aged 19-43 years. They reported PFOS concentrations in urine of up to 160 ng/l (median: 37 ng/l) in 95% of the investigated samples, and PFOA concentrations of up to 57 ng/l (8.0 ng/l) in 56% of the samples [37]. As shown in Table 4, results of PFASs levels measured in human urine are highly variable in different investigations. For example, the median concentrations of PFOS and PFOA ranged in different studies from n.d. [33,36] to 37 ng/l [37] and n.d. [36] to 3,720 ng/l [33], respectively. A possible explanation for these variations could be different lifestyles and nutrition, such as different consumption frequencies of contaminated fish and seafood in the different populations.

For the majority of the available studies, long-chain PFASs were not detectable or were found only in low concentrations. This is in concordance with findings from the present study, where PFASs with chain-lengths of > 10 could not be detected in any of the investigated samples. These findings support the assumption that compounds with longer chain length are not well metabolised [13], bind to serum proteins and accumulate in the body [14,15]. Additionally, studies have shown that urinary excretion decreases with increasing chain length [15].

Compared with the results of previous studies, PFASs concentrations in the present pilot study were notably lower. However, because of the small sample size investigated in the present study, comparisons should be conducted with care.

4 Conclusions

A sensitive analytical method was adapted for the analysis of PFASs in human urine by HPLC-MS/MS. The method was employed to analyse urine samples of 11 EAA employees to test the analytical method.

PFASs with chain lengths of 5 to 9 carbon atoms were detectable in the investigated urine samples with detection frequencies between 72.7% and 100%. The highest concentration was found for PFPeA (C5), reaching 8.5 ng/l in one sample. Compounds of chain lengths from 10 to 12 carbon atoms were not found in any of the samples. No differences in PFASs exposure with respect to age or sex were identified. However, the sample size was too small to allow for statistically significant conclusions.

The adaption of a sensitive analytical method for PFASs analysis in human urine and its application within a small pilot study shows that especially short-chain PFASs can be found in urine, which is also in accordance with findings of previous studies.

The measurement of PFASs in urine enables the study of exposure within populations thus so far only infrequently investigated, such as infants and children. It further facilitates the investigation of dietary habits and allows for the identification of sources of PFASs.

Table 4: Urinary PFAS concentrations (in ng/l) from selected studies of different populations.

Study	Japanese adults aged 45.3±23.9 years (n=48)[a] [32]	Canadian male researcher aged 51 years (n=1) [33]	Healthy Chinese adult volunteers (n=86) [34]	Chinese adults aged 22-62 years (n=54) [35]	Chinese pregnant women aged 21-39 years (n=27) [35]	South Korean children aged 5-13 years (n=120) [36]	South Korean adults aged 20-29 years [36]	Chinese adults aged 19-53 years (n=63) [37]	*this study*
Sampling period	2004	2007	2010	2010	2010	2012	n.r.	n.r.	2016
Analytical method	LC-MS/MS	LC-MS/MS	HPLC-MS/MS	UPLC-MS/MS	UPLC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Substance (ng/l) *range (mean±SD; median)* *LOQ / LOD (DR [%])*									
PFBA	–	–	–	–	–	n.d. n.r. / 175 (0)	n.d.-1,720 (1,720; n.r.) n.r. / 175 (1)	–	–
PFBS	–	–	–	–	–	n.d.-492 (492; n.r.) n.r. / 113 (1)	n.d. n.r. / 113 (0)	–	–
PFPeA	–	–	–	–	–	n.d.-11,600 (2,340; n.r.) n.r. / 225 (70)	n.d.-17,600 (2,390; n.r.) n.r. / 225 (25)	–	n.d.-8.5 (2.6±3.0; 1.8) 1.25 / 0.5 (72.7)
PFHxA	–	–	–	–	–	n.d.-2,340 (731; n.r.) n.r. / 163 (11)	n.d.-5,630 (1,380; n.r.) n.r. / 163 (5)	–	<LOQ-3.0 (1.6±0.84; 1.5) 0.5 / 0.2 (100)
PFHxS	–	n.d. 0.5 ng/g / n.r. (0)	n.d.-35 (2.4; 1.1) 0.045 / n.r. (98)	–	–	n.d. n.r. / 88 (0)	n.d. n.r. / 88 (0)	–	n.d.-1.8 (0.79±0.62) 0.75 / 0.3 (72.7)
PFHpA	–	–	n.d.-19 (1.9; 0.82) 0.173 / n.r. (67)	–	–	n.d.-4,440 (1,350; n.r.) n.r. / 125 (36)	n.d.-1,080 (495; n.r.) n.r. / 125 (7)	–	n.d.-0.99 (0.41±0.29; <LOQ) 0.5 / 0.2 (90.9)
PFHpS	–	–		–	–	n.d. n.r. / 113 (0)	n.d. n.r. / 113 (0)	–	n.d. 0.75 / 0.3 (0)
PFOA	8.7-39 (17.6±8.6; 15) ng/d 1,000 / n.r. (100)	3,720 0.5 ng/g / n.r. (100)	2.6-1,040 (81; 19) n.r. (100)	n.d.-41 (11; 9.0) 4.0 / n.r. (76)	n.d.-30 (6.0; <LOQ) 4.0 / n.r. (30)	n.d. n.r. / 163 (0)	n.d. n.r. / 163 (0)	n.d.-57 (13; 8.0)[b] 3.5 / 1.1 (56)	0.79-5.1 (2.1±1.2; 1.9) 0.5 / 0.2 (100)
PFOS	1.4-36 (13±9.9; 9.9) ng/d 1,000 / n.r. (100)	n.d. 0.5 ng/g / n.r. (0)	2.0-184 (37; 25) n.r. (100)	n.d.-296 (23; <LOQ) 4.0 / n.r. (48)	n.d.-132 (15; <LOQ) 4.0 / n.r. (11)	n.d. n.r. / 138 (0)	n.d. n.r. / 138 (0)	<LOQ-160 (50; 37)[b] 7.0 / 2.0 (95)	<LOQ-4.9 (1.8±1.2; 1.6) 1.25 / 0.5 (100)

continued **Table 4:** Urinary PFAS concentrations (in ng/l) from selected studies of different populations.

Study	Japanese adults aged 45.3±23.9 years (n=48)[a] [32]	Canadian male researcher aged 51 years (n=1) [33]	Healthy Chinese adult volunteers (n=86) [34]	Chinese adults aged 22-62 years (n=54) [35]	Chinese pregnant women aged 21-39 years (n=27) [35]	South Korean children aged 5-13 years (n=120) [36]	South Korean adults aged 20-29 years [36]	Chinese adults aged 19-53 years (n=63) [37]	*this study*
PFNA	–	n.d. 0.5 ng/g / n.r. (0)	n.d.-22.1 (2.2; 1.7)– 0.133 / n.r. (85)	–	–	n.d. n.r. / 200 (0)	n.d. n.r. / 200 (0)	–	<LOQ-0.57 (0.28±0.1; <LOQ) 0.5 / 0.2 (100)
PFDA	–	n.d. 0.5 ng/g / n.r. (0)	n.d.-11 (0.46; 0.22) 0.077 / n.r. (92)	–	–	n.d. n.r. / 113 (0)	n.d.-495 (495; n.r.)– n.r. / 113 (1)		n.d. 0.5 / 0.2 (0). <LOQ) 0.28tudies have shown that the urinary excretion decrease with increasing chain length [15].rum proteins and accumula
PFDS	–	n.d. 0.5 ng/g / n.r. (0)		–	–	n.d. n.r. / 138 (0)	n.d. n.r. / 138 (0)	–	n.d. 0.75 / 0.3 (0)
PFUnDA	–		n.d.-2.43 (0.42; 0.3) 0.032 / n.r. (98)	–	–	n.d. n.r. / 138 (0)	n.d. n.r. / 138 (0)	–	n.d. 0.5 / 0.2 (0)
PFDoA	–	n.d. 0.5 ng/g / n.r. (0)	–	–	–	n.d. n.r. / 181 (0)	n.d.-442 (442; n.r.)– n.r. / 181 (1)		n.d. 1.25 / 0.5 (0)
PFTrA	–	–	–	–	–	n.d. n.r. / 207 (0)	n.d. n.r. / 207 (0)	–	–
PFTeA	–	–	–	–	–	n.d. n.r. / 194 (0)	n.d. n.r. / 194 (0)	–	–

[a] 24-h pooled urine samples.

[b] The authors reported that samples below LOD were removed. Concentrations of detectable samples of <LOQ were set to LOQ.

Abbreviations: DR, detection rate; HPLC-MS/MS, high performance liquid chromatography and tandem mass spectrometry; LC-MS/MS, liquid chromatography and tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; n, sample size; n.d., not detected; n.r., not reported; SD, standard deviation; TFC-HPLC-MS/MS, turbulent flow chromatography and tandem mass spectrometry; UPLC-MS/MS, ultra-performance liquid chromatography and tandem mass spectrometry.

Substances: PFBA, perfluorobutanoic acid; PFBS, perfluorobutane sulphonate; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFDS, perfluorodecane sulphonate; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulphonate; PFHxA, perfluorohexanoic acid; PFHxDA, perfluorohexadecanoic acid; PFHxS, perfluorohexane sulphonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanic acid; PFODA, perfluorooctadecanoic acid; PFOS, perfluorooctane sulphonate; PFOSA, perfluorooctane suphoneamide; PFPeA, perfluoropentanoic acid; PFTeA, perfluorotetradecanoic acid; PFTrA, perfluorotridecanoic acid; PFUnDA, perfluoroundecanoic acid.

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Abbreviations

DR, detection rate; EAA, Environment Agency Austria; HBM, human biomonitoring; HPLC-MS/MS, high-performance liquid chromatography tandem-mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; n.d., not detected; PFASs, perfluoroalkylated substances; PFDoDA, perfluorododecanoic acid; PFDA, perfluorodecanoic acid; PFDS, perfluorodecane sulphonate; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptane sulphonate; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulphonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulphonate; PFPeA, perfluoropentanoic acid; PFUnDA, perfluoroundecanoic acid

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