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Screening for per- and polyfluoroalkyl substances in drinking water samples collected at schools and public fountains in Veneto (Italy)

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Introduction

Per- and polyfluoroalkyl substances

It is estimated that per- and polyfluoroalkyl substances (PFASs) were found for the first time in the decade of 1950, as a consequence of electrochemical fluorination and telomerization processes [1, 2]. Since then, they have been used in a variety of industrial processes and commodities, as emulsifiers, surfactants, lubricants and water and oil repellents, in adhesives, cements, coatings, gasoline, fire extinguishing foams, foodstuff containers, textile and clothing goods, personal care products and household items [3-7].

The organisation for economic co-operation and development (OECD) estimated the historic production volume of PFOS and related substances in 4,500 tons per year [8]. This quantity was severely reduced once the production of these substances was regulated.

PFASs present two different ways of introduction in the environment. The first one is direct, from industrial and manufacture processes, and the second one is through chemical reactions and degradation processes. PFOA and PFOS are the final result of the transformation of perfluorinated carboxylic acids, perfluoroalkyl sulfonamides and fluorotelomer alcohols [9-12].

PFASs show an elevated persistence in the environment [13, 14] and many have high levels of absorption and bioaccumulation. Many of these substances are absorbed rapidly by oral exposure [15, 16] and present long half-life times in the body ($t_{1/2}$), with periods for PFOS and PFOA of 5.4 and 3.8 years for humans, respectively [17-19]. PFASs have been detected in human blood [20-22].

PFOA is considered a powerful immune suppressor, increasing the number and the severity of infections, together with a higher tumor rate [23]. PFOS also affects the production of antibodies [24], reduces the fetuses' weight and generates bone problems, cardiac abnormalities and death of newborns [25]. At the same time, it is considered an endocrine disruptor [26].

Chronic and acute toxicity studies conducted on fish revealed harmful effects on fish exposed to these chemicals. PFASs can affect the proper development of the colony [27], PFOS produces a decrease on the cardiac frequency and movement complications and PFOA modifies the estrogenic activity of male fishes [28].

For the general population, mineral water can be a major ingestion source for PFCs. A recent study determined the presence of these substances in tap water in The Netherlands and Greece, finding PFOA and PFOS at maximum concentrations of 11.1 ng/L and 5 ng/L, respectively, while longer chain PFASs (C₉-C₁₁) were not detected [29]. Similar results were found in an analysis undertaken in Australia, where PFOS and PFOA were detected in 49% and 40% of the tap water samples, respectively, while the longer chain analytes were not found [30]. Regarding short chain PFASs (C₄-C₇), some years before, PFBA was found at a maximum concentration of 11 ng/L in Germany [31], 10 ng/L in China and 25 ng/L in India [32], PFPeA at 77 ng/L in Germany, PFHxA at 22 ng/L in Germany and 20 ng/L in China, PFHpA at 23 ng/L in Germany, while PFBS appeared at a maximum concentration of 26 ng/L in Germany and 15 ng/L in China and PFHxS at 81 ng/L in India.

Concern about the persistence, bioaccumulation potential and toxicity of PFOA and PFOS has led to production restrictions of PFAAs in developed countries. The United States Environmental Protection Agency (US EPA) limited their production by the main manufacturer, 3M, in 2000 [33]. PFOA and PFOS production is also internationally restricted since they were included in the list of POPs of the Stockholm Convention) [34].

The European Union registration, evaluation, authorization and restriction of chemicals (REACH) [35] addressed the production of chemical substances within the EU, especially the substances of very high concern (SVHC) because of their potential negative impacts on human health and environment. The most recent update occurred on January 2017 to include 173 substances in total [36]. PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA and PFTeDA are part of this candidate list. On the other hand, the marketing and use of PFOS within the EU was prohibited in 2008 by the Directive 2006/122/EC [37], which amends the 76/769/EEC one, setting a maximum concentration limit of 1 µg/m² in textiles.

Afterwards, the European Union also released the Directive 2013/39/EU [38], which amends the Directive 2000/60/EC, establishing an action framework in the field of water policy. This Directive includes PFOS in its list of priority substances, setting up the maximum concentration level for this compound in surface water at 36 µg/L.

In the United States more than 200 PFASs are included in the significant new use rule (SNUR) [39], which means that EPA regulates whether a new product containing one of the listed compounds can be manufactured or imported in the USA, while in Canada PFOS, PFOA and long chain perfluorinated carboxylic acids are part of the list of toxic substances [40].

Due to these restrictions, other polyfluorinated materials have been developed, but little is known so far about many of them. For some PFC compounds either being released to, or present in, the environment, the identity of individual PFCs remains unknown. Some recent studies have provided information on some such PFCs, for example, Newton *et al.* recently investigated surface water near a manufacturing facility and identified a series of 9 polyfluorinated carboxylic acids, each differing by CF₂CH₂, together with a

polyfluoroalkyl sulfate with differing mixes of hydrogen and fluorine substitution, which had not previously been characterised [41].

Samples

25 drinking water samples were taken at the region of Veneto (Italy). 18 of them from schools and 7 from public fountains in urban parks. **Table 1** summarises the sampling dates and locations.

Table 1. Sampling locations and dates of the samples included in this report

Sample	Type	Municipality	Date
1	Drinking Fountain	Verona	4-Apr-17
2	School	San Giovanni Lupatoto	4-Apr-17
3	School	San Bonifacio	4-Apr-17
4	School	Albaredo D'Adige	4-Apr-17
5	School	Legnago	4-Apr-17
6	Drinking Fountain	Santa Maria Maddalena	4-Apr-17
7	Drinking Fountain	Polesella	4-Apr-17
8	School	Padova	4-Apr-17
9	School	Vicenza	5-Apr-17
10	School	Brendola	5-Apr-17
11	School	Montecchio Maggiore	5-Apr-17
12	School	Arzignano	5-Apr-17
13	School	Montebello Vicentino	5-Apr-17
14	School	Montorso Vicentino	5-Apr-17
15	School	Sarego	5-Apr-17
16	Drinking Fountain	Lonigo	5-Apr-17
17	Drinking Fountain	Pojana Maggiore	5-Apr-17
18	Drinking Fountain	Noventa Vicentina	5-Apr-17
19	School	Veronella	6-Apr-17
20	Drinking Fountain	Cologna Veneta	6-Apr-17
21	School	Pressana	6-Apr-17
22	School	Roveredo di Gu	6-Apr-17
23	School	Zimella	6-Apr-17
24	School	Montagnana	6-Apr-17
25	School	Lozzo Atestino	6-Apr-17

Materials and methods

Sample preparation

The 25 samples were prepared following a solid-phase extraction (SPE) procedure by an accredited Italian lab. The extracts corresponding to the samples were analyzed in duplicates as received, without any further treatment.

Sample analysis

A semi-target screening analysis for fluorinated compounds was conducted for all the extracts.

1 μ L of extract was injected in a Dionex UltiMate 3000 LC system, Thermo Scientific (Hemel Hempstead, UK), furnished with a quaternary pump, a C18 Accucore aQ (100 x 2.1 mm; 2.6 μ m) LC column protected by a C18 Accucore aQ (10 x 2.1 mm; 2.6 μ m) column-guard, placed inside a column oven set at 25° C, and a temperature controlled autosampler set at 15° C. The mobile phase flowed at 0.3 mL/min with a gradient composition, result of the mixture of A: water (2% MeOH, 0.1% formic acid and 5 mM ammonium formate) and B: MeOH (2% water, 0.1% formic acid and 5 mM ammonium formate) as follows: 0 min 2% B, 0.5 min 2% B, 7 min 70% B, 9-12 min 100% B. Column was re-conditioned between injections for 5 min.

The LC column flow was directed to an Orbitrap Q Exactive Focus, Thermo Scientific, equipped with a HESI-II source, a quadrupole, an HCD collision cell, the C-trap and the high-resolution Orbitrap mass analyser. The ionisation parameters were set at: sheath flow rate 30, auxiliary gas flow rate 5, sweep gas flow rate 0, spray voltage 4 kV, capillary temperature 325° C, S-lens RF level 50 and auxiliary gas heater temperature 350° C.

Extracts were analysed in the full scan range of 100-1000 Da in the negative ionisation mode. The resolution of this analysis was 70,000 and the maximum injection time was set to 200 ms or the minimum for 1.00E6 AGC target. Simultaneously, data dependant-MS² analysis were conducted at a resolution of 17,500, with a maximum injection time of 100 ms or the minimum for 1.00E3 AGC target, for those ions included in the suspect list, with the HCD collision energy stepped at 15, 30 and 45 eV, aiming the generation of fragment ions that were further used, in combination with the mzCloud spectral database (<https://www.mzcloud.org/>), to confirm the identity of each substance.

The suspect list (**Table 2**) was built with the data provided by the Italian lab plus other PFASs found at online databases.

TraceFinder 4.1 software, Thermo Scientific, was used to control the LC-ESI-QOrbitrap-MS system, while the FreeStyle 1.1 application, Thermo Scientific, was applied for data visualization.

Table 2. Suspect list with the compounds included in the screening performed in the present work, showing CAS number, formula, retention time (minutes) and molecular ion ([M-H]⁻)

Acronym	Compound	CAS	Formula	Ret. time (min)	[M-H] ⁻
<i>Carboxylic acids</i>					
TFAA	trifluoroacetic acid	76-05-01	C2HF3O2		112.9856
PFBA	Perfluoro butanoic acid	375-22-4	C4HF7O2		212.9792
PFPeA	Perfluoro pentanoic acid	2706-90-3	C5HF9O2		262.9760
PFHxA	Perfluoro hexanoic acid	307-24-4	C6HF11O2	8.35	312.9728
PFHpA	Perfluoro heptanoic acid	375-85-9	C7HF13O2	9.08	362.9696
PFOA	Perfluoro octanoic acid	335-67-1	C8HF15O2	9.59	412.9664
PFNA	Perfluoro nonanoic acid	375-95-1	C9HF17O2	9.98	462.9632
PFDA	Perfluoro decanoic acid	335-76-2	C10HF19O2	10.26	512.9600
PFUnDA	Perfluoro undecanoic acid	2058-94-8	C11HF21O2		562.9714
PFDoDA	Perfluoro dodecanoic acid	307-55-1	C12HF23O2		612.9536
PFTTrDA	Perfluoro tridecanoic acid	72629-94-8	C13HF25O2		662.9504
PFTeDA	Perfluoro tetradecanoic acid	376-06-7	C14HF27O2		712.9472
PFHxDA	Perfluoro hexadecanoic acid	67905-19-5	C16HF31O2		812.9409
PFODA	Perfluoro octadecanoic acid	16517-11-6	C18HF35O2		912.9345
<i>Sulfonic acids</i>					
TFMS	Trifluoro methane sulfonic acid	1493-13-6	CHF3O3S		148.9526
PFBS	Perfluoro butane sulfonic acid	375-73-5	C4HF9O3S	7.56	298.9430
PFHxS	Perfluoro hexane sulfonic acid	355-46-4	C6HF13O3S	9.11	398.9366
PFHpS	Perfluoro heptane sulfonic acid	375-92-8	C7HF15O3S		448.9334
PFOS	Perfluoro octane sulfonic acid	1763-23-1	C8HF17O3S	10.01	498.9302
PFDS	Perfluoro decane sulfonic acid	335-77-3	C10HF21O3S		598.9238
<i>Carboxylic acid derivatives</i>					
HPFHpA	7H-Perfluoro heptanoic acid	1546-95-8	C7H2F12O2		344.9790
H2PFDA	2H,2H-Perfluoro decane acid	-	C10H3F17O2		476.9789
H4PFUnDA	2H,2H,3H,3H-Perfluoro undecanoic acid	34598-33-9	C11H5F17O2		490.9945
DONA	Perfluoro-3H-4,8-dioxa nonanoic acid	-	C9H2F16O4		476.9625
DMPFOA	Perfluoro-3,7-dimethyl octanoic acid	172155-07-6	C10HF19O2		512.9600
<i>Sulfonic acid derivatives</i>					
H4PFOS	1H,1H,2H,2H Perfluoro octane sulfonic acid	27619-97-2	C8H5F13O3S		426.9679
H4PFDS	1H,1H,2H,2H-Perfluoro decane sulfonic acid	39108-34-4	C10H5F17O3S		526.9615
PFOSA	Perfluoro octane sulfonamide	754-91-6	C8H2F17NO2S	10.69	497.9462
MePFOSA	N-methyl perfluoro octane sulfonamide	31506-32-8	C9H4F17NO2S		511.9618
EtPFOSA	N-ethyl perfluoro octane sulfonamide	4151-50-2	C10H6F17NO2S		525.9775
EtPFOSAA	N-Ethylperfluoro 1-octane sulfonamido acetic acid	2991-50-6	C12H8F17NO4S		583.9830
MePFOSAA	N-Methylperfluoro 1-octane sulfonamidoacetic acid	2355-31-9	C11H6F17NO4S	10.73	569.9673

Quality control

Calibration of the mass axis was performed in a daily basis, before the analysis of each sequence of samples, considering ions with m/z values of 265.1479 (sodium dodecyl sulfate), 514.2844 (sodium taurocholate) and 1379.9972, 1479.9844, 1579.9780 and 1679.9717 and 1779.9653 (Ultramark 1621).

[M-H]⁻ parent ions and M⁺ fragment ions were extracted from MS and MS² spectra with narrow mass error windows of 5 and 20 ppb, respectively, based on the resolution of each acquisition process. This fact ensured the unequivocal detection of the compounds and their elemental composition.

Chromatographic peak areas found in samples were, at least, three times higher than in methanol blanks.

Results and Discussion

Table 3 summarises the presence of PFASs in the analysed samples. Three perfluorinated sulfonic acids, PFOS, PFHxS and PFBS, were present in all the samples. Among the screened perfluorinated carboxylic acids, PFHpA was present in most of the samples, while PFOA and PFHxA were also found in 17 and 11 samples, respectively. On the other hand, PFNA and PFDA appeared in only one of the samples. No carboxylic acid derivatives were found at all, but all the samples contained MePFOSA, a sulfonic acid derivative. Another sulfonic acid derivative, PFOSA, was present in the 36% of the samples.

Table 3. Compounds found present in the analysed samples 1-25 (marked with an x)

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
TFAA																									
PFBA																									
PFPeA																									
PFHxA				x	x										x	x	x	x	x	x	x		x	x	
PFHpA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PFOA			x	x	x		x			x	x	x		x		x	x	x	x	x	x	x	x	x	x
PFNA								x																	
PFDA												x													
PFAUnDA																									
PFAoDA																									
PFATrDA																									
PFATeDA																									
PFAHxDA																									
PFOA																									
TFMS																									
PFBS	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PFHxS	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PFHpS																									
PFOS	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PFDS																									
H2PFDA																									
H4PFAUnDA																									
DONA																									
DMPFOA																									
H4PFOS																									
H4PFDS																									
PFOSA						x	x		x			x		x			x		x	x	x				
MePFOSA																									
EtPFOSA																									
EtPFOSAA																									
MePFOSAA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

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