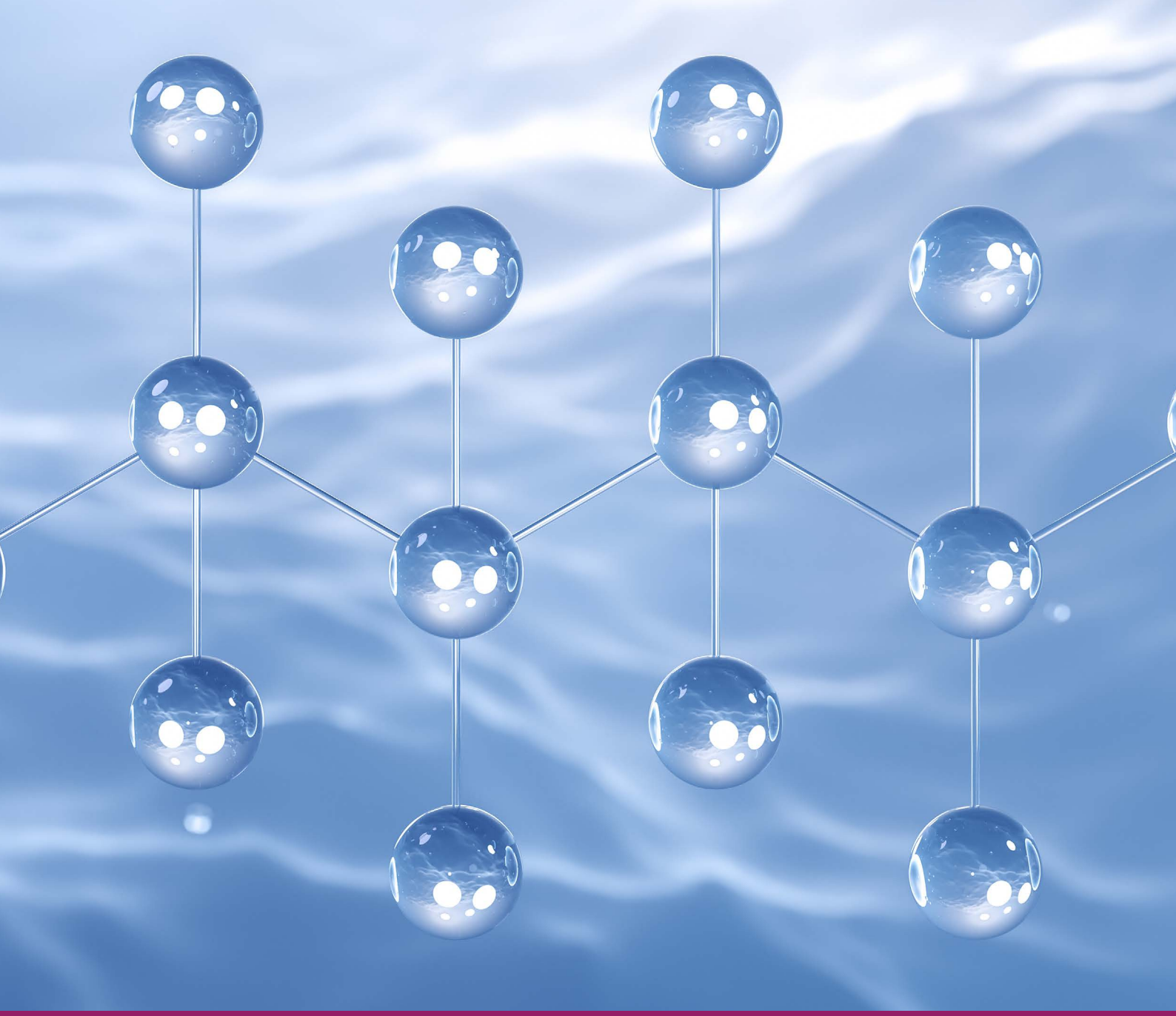

Per- and Polyfluoroalkyl Substances

Contamination and Analysis

Expert Insights

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Contents

Navigating PFAS Challenges: Insights into Contamination and Analysis

Per- and polyfluoroalkyl substances (PFAS) represent a class of synthetic chemicals that have garnered substantial attention due to their widespread presence, persistence, and potential health and environmental implications. PFAS compounds are characterized by their unique carbon-fluorine bond, which imparts remarkable stability and resistance to degradation. This has led to their extensive use in various industrial applications and consumer products, including waterproof textiles, non-stick cookware, firefighting foams, and more.

However, the very same properties that make PFAS valuable in these applications also contribute to their persistence in the environment, earning them the moniker "forever chemicals". Their resistance to breakdown has resulted in their accumulation in soil, water, and even living organisms.

Concerns have arisen due to the potential adverse health effects associated with prolonged exposure to PFAS, including links to certain cancers, developmental issues, and immune system disruption. As a result of growing awareness about their prevalence and possible dangers, regulatory measures and research efforts have been intensified globally to address PFAS contamination, reduce their production, and explore effective remediation strategies.

This eBook offers a glimpse into the multifaceted dimensions of the PFAS issue, from their chemical structure and applications to the intricate challenges posed by their persistence and potential impact on human health and the environment.

The first article within this compilation, by Hassel, K.L. *et al.* [1], examined the uptake and elimination kinetics of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and hexafluoropropylene oxide dimer acid (GenX) in benthic fish using LC-TQ. While PFAS contamination in aquatic environments is known, limited understanding exists about depuration and accumulation mechanisms in fish through different exposure routes.

Next, Piva, E. *et al.* [2] discuss the determination of per- and polyfluoroalkyl substances (PFAS) in shellfish using liquid chromatography coupled with accurate mass spectrometry. The study focuses on the health and environmental effects of different types of PFAS isomers and their accumulation in shellfish, often used as indicators of contamination. The research demonstrates that the LC/Q-TOF method can detect both linear and branched PFAS compounds in shellfish samples at nanogram per gram levels.

We also highlight a recent Agilent Technologies interview with Tarun Anumol, the Global Environmental division's Director, where we delved into strategy development and water treatment research findings and explored emerging technologies for environmental monitoring. The conversation underscores PFAS analysis importance, sheds light on challenges, and Agilent's role in innovative solutions. FluoroMatch 3.0 and software tools for non-targeted PFAS analysis are detailed, as well as the Triple Quad LC/MS system's role in enhancing understanding and regulatory support, addressing contamination concerns.

Finally, we draw attention to two posters cited in the interview: *FluoroMatch 3.0 – Automated PFAS Non-Targeted Analysis and Visualizations Applied to Mammalian Biofluids and Strategies for Ultimate Sensitivity of Per and Polyfluoroalkyl Substances (PFAS) in Water.* The posters showcase groundbreaking advancements in PFAS analysis, revolutionizing the understanding of contaminant presence and potential health impacts.

Together, the articles, the interview, and posters offer transformative insights that could reshape how we analyze and address the challenges posed by PFAS contamination in both biological samples and water sources. In this comprehensive eBook, readers gain a profound understanding of PFAS contamination, its far-reaching implications, and the cutting-edge methodologies that are shaping a more effective and sensitive approach to detection and analysis. For more information, we encourage you to visit agilent.com to learn more and explore options to enhance your research.

Dr. Cecilia Kruszynski
Editor at Wiley Analytical Science

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[1] Hassell, K.L. *et al.* (2020). Dietary Uptake and Depuration Kinetics of Perfluorooctane Sulfonate, Perfluorooctanoic Acid, and Hexafluoropropylene Oxide Dimer Acid (GenX) in a Benthic Fish. *Environmental Toxicology and Chemistry*. DOI: 10.1002/etc.4640.

[2] Piva, E. *et al.* (2022). Per- and polyfluoroalkyl substances (PFAS) determination in shellfish by liquid chromatography coupled to accurate mass spectrometry. *Drug Testing and Analysis*. DOI: 10.1002/dta.3282.

Dietary Uptake and Depuration Kinetics of Perfluorooctane Sulfonate, Perfluorooctanoic Acid, and Hexafluoropropylene Oxide Dimer Acid (GenX) in a Benthic Fish

Adapted from Hassell, K.L. *et al.* 2019

Introduction

While prior studies have characterized the distribution and accumulation of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in aquatic environments [1], the same in-depth knowledge is lacking concerning the mechanisms of depuration, accumulation, and relative contribution to body burden through digestive or aqueous exposure [2,3]. Profiles of PFAS compound in benthic fish are similar to the same profile in the local sediment [1,4], in aquatic species, perfluorooctanesulfonic acid (PFOS) is the most detected PFAS [2], and bioaccumulation is influenced by habitat, food source (prey), metabolism, and route of exposure [5]. General bioaccumulation of PFAS is reduced in fish compared to other non-aquatic animals, most likely because gills have a higher capacity to eliminate PFAS than lungs [6].

PFOS and perfluorooctanoic acid (PFOA) exist in various biological samples and can be detected even at low quantities using triple quadrupole (TQ) or quadrupole time-of-flight mass spectrometry (Q-TOF). They usually occur at a higher frequency than other PFAS and are known to be toxic to the environment and health [7]. PFAS are hepatotoxic, capable of inducing liver tumors [8,9], can cause immunotoxicity [10], and in fish adversely affect reproduction [11].

This emphasizes the generation of a less toxic alternative. One such alternative, hexafluoropropylene oxide dimer acid (HPFO-DA, trade name GenX), is primarily in use as a PFOS replacement [12]. This chemical was found at high concentrations in waters of industrial regions in China [13], the U.S. [12], Germany, and the Netherlands [13], but the overall extent of environmental contamination is as of yet unknown [3]. In algae, invertebrates, and fish, the toxicity of GenX was shown to be either low or undetectable [14], and rodent studies have shown that orally administered GenX was rapidly eliminated through urine, with no associated metabolic activity [15].

As organic content and salinity also affect the absorption characteristics of PFOS and PFOA, and as most studies on PFAS contamination of fish are centered around freshwater fish models [2,16], this study investigated PFAS contaminations in a benthic, sediment-associated estuarine fish species, specifically adult blue spot gobies (*Pseudogobius sp.*) from the Werribee River, Australia.

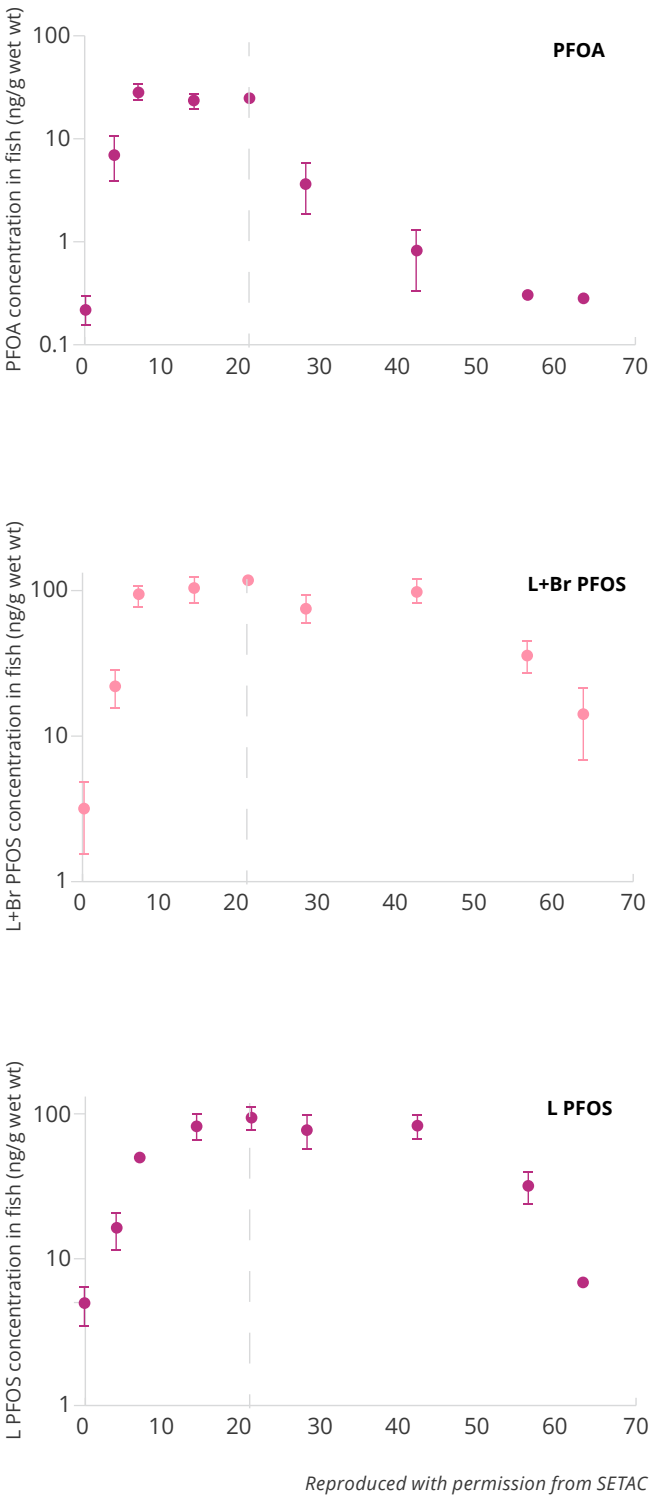
Methods

The fish were housed individually, which allowed adjustment of specific food portions (based on individual fish weight), ensuring the same dose of food was offered to each fish, subsequently enabling a calculation of individual ingestion rates. Variations between the ingestion rates were insignificantly different. The experiment presented 40 male and 8 female fish that were assigned to treatment and control groups; experiments lasted for 11 weeks: 14-day acclimatization, 21-day uptake, and 42-day depuration. Whole body samples were extracted and analyzed on an ultra-high performance liquid chromatography instrument (Agilent Technologies 1290 Infinity II LC device coupled to an Agilent Technologies 6495B tandem MS (MS/MS)), at low limits of reporting (LOR) of PFOA – 0.3 ng g^{-1}, linear PFOS – 0.6 ng g^{-1}, linear+branched PFOS – 0.6 ng g^{-1}, and GenX – 1.0 ng g^{-1}.

Results and Discussion

All fish showed low but robustly detectable PFOA and PFOS concentrations at the beginning of the experiment (pre feeding with laced food), and those values were considered the background. PFOA, linear PFOS, and linear+branched PFOS accumulated in blue spot gobies, and steady-state whole-body concentrations were reached after 14 days (Fig. 1), which is roughly in line with a published observation in juvenile rainbow trout [16].

Figure 1



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Whole-body concentration (ng/g^{-1}) of PFOA, linear PFOS (L PFOS), and linear+branched PFOS (L+Br PFOS) in blue spot gobies fish throughout uptake (21 days) and depuration (42 days) phases. Each data point represents the mean +/- standard error of the mean (SEM) of N = 4, except for day 63, where N = 2. Whole body concentrations under the limit of reporting (LOR) were substituted with a ½ LOR value.

While the whole-body concentration in the gobies was much higher than in rainbow trout [17], the depuration rate and biological half-lives remained similar (Table 1). PFOA showed faster depuration rates than PFOS (regardless of branching state): 7 days of depuration had only 15% of PFOA remaining, while 81% of linear PFOS and 65% of linear+branched PFOS remained. The compound half-lives (PFOA 5.9 days, linear PFOS 15.4 days, and linear+branched PFOS 16.7 days) were roughly in line with previously published reports for pelagic or freshwater fish [16,17].

PFOS isomers are fairly consistently composed at a ratio of 70% (linear) and 30% (branched) [18,19] but this frequency can deviate strongly in aquatic specimens. Indeed, linear PFOS isomers amounted to 77 to 81% of total PFOS in the uptake phase, and increased to 90% in the depuration phase, indicating that the depuration of linear and branched PFOS follows different kinetics. This observation is not just in line with previous results from studies on fish [19], but also other invertebrates [20] and polar bears [21]. While the reasons for these differences are not well known, a multitude of isomer-specific factors are thought to play a role, including pharmacokinetics, biotransformation, elimination capacity, and different sources [18,19]. Interestingly, it appears that in rainbow trout, the gills and kidneys preferentially eliminate branched-PFOS19, and if it is the same in blue gobies, that could be the reason for the enrichment of linear PFOS in the depuration phase.

GenX, the replacement product for PFOS, showed no accumulation in the gobies at any time during sampling. Still, the food was confirmed to contain GenX, which means the lack of GenX contamination is due to a lack of uptake or very rapid elimination. Given that other studies have previously shown the rapid elimination of orally administered GenX [15], this may also be the case for our observation.

Conclusion

Given the presented data, it would be of interest to look into specific PFOS isomers, especially in regards to branched and linear PFOS, as they are associated with different biological properties and toxicities [18,21]. Additionally, performing a similar investigation specifically in different tissues would be important, as that could shed light on the metabolic regulation of these compounds. Finally, the authors believe that additional studies into newer PFAS replacement compounds are essential, especially focused on organ-specific biological half-life data, as these might be of significantly lower burden for the environment and potential implications for human consumers.

Table 1

Whole-body concentrations (ng g^{-1} wet wt) and kinetics information for perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and GenX in blue spot gobies (*Pseudogobius sp.*), following dietary exposure of up to 21 days and depuration of up to 42 days.

[a]Steady state ($_{tss}$) was defined as the time after which there was no statistically significant increase in whole-body concentration during the uptake phase.

[b]Assimilation efficiency (a measure of the absorption of PFAS (across the gut) was calculated at the end of the uptake period (21 days).

GenX = trade name for hexafluoropropylene oxide dimer acid; PFAS = per- and poly-fluoroalkyl substance; BMF = biomagnification factor; L = linear; Br = branched; LOQ = limit of quantification; NA = not available.

		Mean whole-body concentration (ng/g wet wt)							
PFAS compound	Structure	Beginning of uptake period (0 d)	End of uptake period (21 d)	End of depuration period (63 d)	Depuration rate constant (k_d; d^{-1})	$t_{1/2}$ (d)	BMF	t_{ss} (d)[a]	Assimilation efficiency[b] (%)
PFOA	$C_8HF_{15}O_2$	0.11 ± 0.002	38.8 ± 15.1	0.12 ± 0.00	0.1473	5.9	0.021 ± 0.001	14	22.43 ± 1.54
L-PFOS	$C_8HF_{17}SO_3$	2.66 ± 1.54	99.6 ± 17.4	7.00	0.0449	15.4	0.346 ± 0.015	14	60.64 ± 3.84
L+BrPFOS	$C_8HF_{17}SO_3$	3.07 ± 1.80	123.1 ± 8.84	14.6 ± 7.56	0.0416	16.7	0.261 ± 0.011	14	42.17 ± 2.66
GenX	(HPFO-DA: $C_6HF_{11}O_3$)	<LOQ	<LOQ	<LOQ	NA	NA		NA	NA

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Per- and polyfluoroalkyl substances (PFAS) determination in shellfish by liquid chromatography coupled to accurate mass spectrometry

Adapted from Piva, E. *et al*. 2022

Introduction

PFAS substances, either with a carboxylic acid (PFCA) or a sulfonic acid group (PFSA) have been industrially produced for more than 70 years [1] and have been linked to numerous health problems in humans (including kidney and liver disease, altered immune function, and cancer) [2,3]. Data specifically on the health/environmental consequences of branched and linear PFAS isomers are rare. Branched isomers are reported to concentrate more in soil and sediments [4,5]; they differ in their bioaccumulation, metabolism, and toxicity [6,7], and may be excreted more efficiently than linear PFAS, reducing their accumulating power. This, however, is inconsistent between different PFAS substances, and branched isomers are not readily detected in all test organisms. An additional concern is that PFOS and PFOA substances are still the predominantly detected compounds in various biomonitoring studies, regardless of efforts

Table 1

Sensitivity, calibration range, matrix-effect, and intra and interday bias of the developed LC/Q-TOF method for PFAS determination in bivalves. Abbreviations: LOD. limit of detection: LOQ. limit of quantification; LC/Q-TOF. liquid chromatography coupled to accurate mass spectrometry PEAS, per-and polyfluoroalkyl substances.

Analyte	LOD (ng/g)	LOQ (ng/g)	Calibration range (ng/mL)	Matrix effect (%)	Intraday QC @ 0.6 ng/mL (% bias)	Interday QC @ 0.6 ng/mL (% bias)	Intraday QC @ 1.2 ng/mL (% bias)	Interday QC @ 1.2 ng/mL (% bias)
11-Cl-PF3OUd5	0.01	0.03	0.1-10	86	-10	-15	-8	-14
4:2 FTS	0.004	0.01	0.05-10	110	9	15	-3	7
6:2 FTS	0.002	0.007	0.05-10	99	1	2	1	4
8:2 FTS	0.002	0.007	0.05-10	79	4	5	-2	4
9-Cl-PF3ONS	0.01	0.03	0.1-10	86	-9	-10	-5	-9
ADONA	0.01	0.04	0.2-10	97	3	9	2	3
HFPO-DA	0.01	0.05	0.5-10	92	-5	9	1	2
PFBA	0.01	0.04	0.1-10	109	4	10	5	-13
PFBS	0.005	0.02	0.05-10	93	-2	3	-3	-2
PFDA	0.01	0.04	0.2-10	80	4	12	2	10
PFDoA	0.03	0.10	0.5-10	89	-7	9	-3	1
PFEESA	0.01	0.04	0.2-10	92	1	2	1	3
PFHpA	0.03	0.09	0.5-10	97	6	12	3	4
PFHpS	0.006	0.02	0.05-10	86	1	11	2	8
PFHxA	0.02	0.07	0.2-10	99	2.5	3.5	4	5
PFHpS	0.004	0.01	0.05-10	96	3	11	-3	4
PFMBA	0.03	0.09	0.5-10	109	8	13	4	10
PFNA	0.05	0.15	0.5-10	95	9	-15	3	10
PFOA	0.009	0.02	0.1-10	90	3	5	2	-3
PFOS	0.007	0.02	0.1-10	86	-2	5	1	-1
PFPcA	0.02	0.08	0.5-10	96	7	13	4	-9
PFPeS	0.005	0.02	0.05-10	96	4	6	3	5
PFUnA	0.02	0.08	0.5-10	83	-8	12	-2	-4

taken to limit their diffusion, probably due to their resistance to biodegradation [8,9].

Biomonitoring studies extensively focus on oceanic waters, biota, and sediments. PFAS is predominantly accumulated in those areas, mostly due to direct or indirect anthropogenic discharges [10]. Monitoring PFAS levels by using sentinel animals, specifically fish and bivalves, is typical, not only because they are native to the area but also because they represent the primary dietary PFAS source for humans [11,12]. Filter-feeding organisms such as mussels, clams, oysters, and scallops, accumulate xenobiotics in their environment and are therefore optimally suited for biomonitoring studies [13–16].

Methods

This study analyzed mussel, clam, and oyster samples provided by the National Reference Laboratory for Marine Biotoxins in Italy, as well as locally bought Atlantic and Pacific clams. Analysis was carried out on 100 g of homogenized material, using liquid chromatography and mass spectrometry (1290 Infinity II LC coupled to 6546 quadrupole-time-of-flight mass spectrometer), and reference solutions for accurate mass measurements were from Agilent Technologies. An in-house library of 150 PFCA and PFSA compounds was prepared for non-targeted analysis. Details of the parameters of the developed LC/Q-TOF method are given in Table 1.

Results and Discussion

At least one PFAS substance was detected in every sample, and out of the 12 PFAS that were detected overall (above the LOD), seven were quantified, with a sum ranging from 0.03 to 0.57 ng/g. Two out of four Mediterranean mussel samples were characterized with PFAS levels below the limit of quantification (LOQ). Overall, PFAS compounds consistently occurred at various detection frequencies (DFs): PFOS > PFOA > PFBS > PHFpA > PFHxA / PFHxS / PFPeA / 6:2 FTS / PFNA / PFDaA > PFDA / PFUnA (Table 1). Interestingly, Mediterranean mussel samples tested negative for PFOA, with only a low accumulation of PFOS, compared to clams.

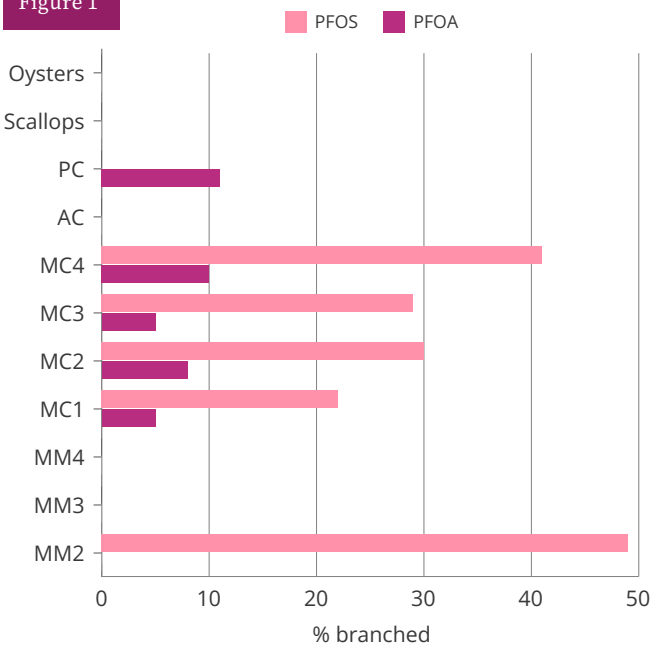
The sum of PFAS contamination in the different organisms aligns with the previously published PFAS contamination pattern of oysters < scallops < mussels < clams (Table 2). Locality mattered in terms of PFAS contamination levels, as clams from the Mediterranean Sea displayed higher levels compared to clams from the Pacific or Atlantic. The methodology developed in this study allowed differential detection of branched and linear PFOS and PFOW isomers. Branched PFOA was exclusively present in Mediterranean and Pacific clams (range 5–11% of total PFOS), while branched PFOS amounted to nearly half of total PFOS (range 22–49%) (Figure 1).

Table 2

Results of PFAS determination in the samples. Note: The detected molecules are reported only in columns. AC: Atlantic Clams, LOQ: limit of quantification, MC: Mediterranean clams, MM: Mediterranean mussels, PC: Pacific clams, PFAS: per-and polyfluoroalkyl substances. [a]Linear + branched isomers, [b]Only linear isomers.

	PFMxA (ng/g)	PFHpA (ng/g)	PFHxS (ng/g)	PFPeA (ng/g)	PFBS (ng/g)	6:2 FTS (ng/g)	PFOA[a] (ng/g)	PFOS[a] (ng/g)	PFNA[a] (ng/g)	PFDA (ng/g)	PFDoA (ng/g)	PFDoA (ng/g)	ΣPFAS (ng/g)
MM1	<LOQ				<LOQ			<LOQ					-
MM2					<LOQ			0.03			<LOQ		0.03
MM3					<LOQ			<LOQ				0.10	0.10
MM4	<LOQ				<LOQ								-
MCI			0.015		0.02		0.23	0.18	<LOQ		<LOQ		0.45
MC2					0.02		0.11	0.05					0.18
MC3		<LOQ	0.016	<LOQ	0.03	0.05	0.33	0.14	<LOQ				0.57
MC4				<LOQ	0.02	0.09	0.12	0.04		0.04			0.31
Scallops							0.08[b]						0.08
Oysters		<LOQ					0.03[b]	<LOQ					0.03
AC		<LOQ					0.04[b]	<LOQ					0.04
PC							0.12	<LOQ					0.12

Figure 1



Distribution of branched PFOS and PFOA in the samples. MM: Mediterranean mussels, MC: Mediterranean clams, PC: Pacific clams, AC: Atlantic clams.

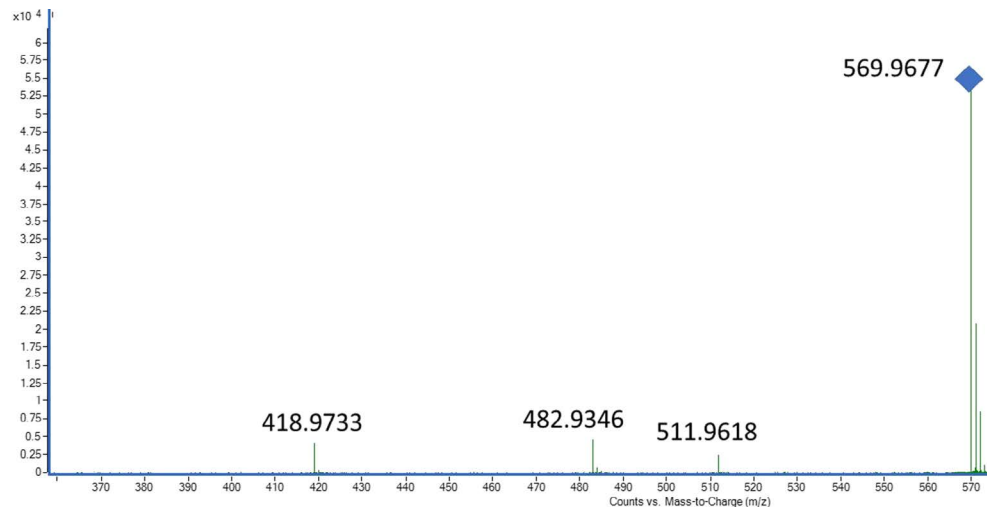
The untargeted detection approach revealed no novel PFAS compounds, but three potential new PFOS precursors (pre-PFOS): N-MeFOSA, N-EtFOSA (perfluorooctane sulfonamides), and N-MeFOSAA (perfluorooctane sulfonamide acetic acid). N-MeFOSAA was validated with a reference standard (Fig. 2), while N-MeFOSA and N-Et-FOSA were only identified by database match.

Detecting PFAS levels is performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) [4,12,17]. This provides adequate sensitivity for most routine/research applications, however, certain short-chain PFAS substances may be undetectable with this method. This limitation can be overcome using high resolution accurate mass mass spectrometers (HRAM-MS) [8], and the LC/Q-TOF method used in this study was chosen based on this.

Comparing the results of this study with published literature is challenging due to changing temporal trends, and various existing and detected compounds. For example, mussels and oysters from the French coast of the Mediterranean Sea had a lower chemical burden of PFOS compared with PFCA [18], while the results of this study reported the opposite, but this might be the consequence of regional contaminations. Still, the sum of PFAS concentration in French samples was consistent with what was observed in this study, highlighting chemical contamination along the international coast of the Mediterranean Sea. A recent study confirmed that South Africa-farmed shellfish contained PFPeA at the highest levels, which was also the highest PFAS contaminant in the water habitat [11]. In clams, mussels, scallops, whelks, and oysters in the semi-closed Bohai Sea, PFOS made up 87.2% of the total PFAS concentration. The different organisms also vary in their uptake efficiency. The samples from the Bohai Sea had the highest levels of PFAS in clams, which agrees with this study [17]. Notably, PFAS contaminations do not appear to be permanent, as maintaining oysters in a depuration system and relocating them to a non-contaminated site reduced the amount of PFOS significantly [19].

Branched isomers exist in soil and sediments at a high concentration, and indeed, clams that thrive in sediments showed variable levels of branched PFOA

Figure 2



MS/MS spectra of accurate mass fragments for N-Me-FOSAA.

and PFOS, and branched PFOA accounted for 100% of total PFOA in Pacific clams. The high degree of branched PFAS observed in this study might be partially a consequence of the sample proximity to the Veneto region in northeastern Italy, where a huge contamination of linear and branched PFAS has occurred [4]. As previously stated, branched PFAS isomers appear to affect human health differently than their linear counterparts, and finding them in edible food may pose a currently under-investigated threat.

Conclusion

This study showed that the LC/Q-TOF method can detect linear and branched PFAS substances in shellfish, at a ng/g resolution. The trend of PFAS contamination among species confirmed the previously published pattern of oysters < scallops < mussels < clams, and while linear isomers are the predominantly detected isomers of PFOA and PFOS, branched isomers were detected, most prominently in clams (range 8–34%). Additionally, the methodology employed in this study could also detect PFAS precursors, specifically 6:2 FTS (in two Mediterranean clams) and N-MeFOSAA (in all Mediterranean clams and mussels).

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Insights into Environmental Analysis and PFAS Detection

Exploring Emerging Contaminants and Advanced Analytical Strategies for Environmental Health

In a recent interview, Tarun Anumol, Ph.D., an expert from Agilent Technologies discusses strategy development for the environmental market focusing on PFAS, their significance, and the challenges of analyzing them. Agilent's role in advancing PFAS analysis and environmental health through innovative technologies over the last decade is also discussed, along with insights into the FluoroMatch 3.0 technology and Agilent's software tools for non-targeted PFAS analysis. Finally, the role of Agilent's Triple Quad LC/MS system in enhancing PFAS understanding and regulatory support is explored, alongside strategies to mitigate background contamination issues.

Can you tell us about your role as the Global Director for the Environmental Market at Agilent Technologies? What are your main responsibilities and areas of focus in this position?

Currently, I'm the Director of Agilent's Global Environmental market. In this role, my primary responsibilities revolve around shaping the strategy for the environmental market. This involves identifying crucial testing areas that require attention within the testing market for environmental customers. Additionally, I'm involved in gathering insights from the environmental testing field and relaying them to the company so we can provide tools for our customers to answer their key environmental questions. This entails staying attuned to emerging opportunities and testing areas within the environmental market and channeling this feedback back to the organization. Crafting and refining the strategy for our environmental testing market is at the core of my responsibilities.

Your Ph.D. research focused on water treatment strategies for water reuse and the identification of emerging contaminants. Could you share some key findings or insights from your research that have practical implications for the environmental field?

During my Ph.D. research, I focused on water reuse, particularly the treatment and analysis of emerging contaminants. The study delved into various aspects, including pharmaceuticals, hormones, and PFAS disinfection byproducts, exploring their presence and removal in water. The outcomes were enlightening. Firstly, it became evident that numerous unregulated contaminants pervade our water sources, often escaping our awareness. This predicament is substantial in scale. Another crucial realization was the need for advanced analytical testing methods for measuring these compounds, especially to detect them at extremely low levels in water. Compounds like PFAS and hormones exemplify this challenge that requires detection at low nanogram per liter ranges. Lastly, the research highlighted the continuous emergence of new components demanding a diverse array of tools for both analysis and treatment. A universal solution is unattainable; tailored strategies are imperative based on the compound classes under consideration.

Are there any emerging technologies or methodologies that are shaping the way we approach environmental monitoring and analysis? What significant trends have you observed?

The positive aspect I'd like to highlight is the increased public focus and interest in environmental safety and sustainability. Our lifestyles have become accustomed to relying on multiple chemicals for their maintenance, whether in outdoor clothing, plastics, home appliances, or water bottles. The ongoing trend indicates a rise in emerging chemicals in the environment that can't be adequately monitored with conventional methods. To address this, we should explore alternative monitoring

techniques. A promising approach involves utilizing higher resolution mass spectrometry (MS) instruments like quadrupole time-of-flight (QTOF) with GC and LC, enabling simultaneous screening of several thousand compounds, coupling this with tools that give us the biological effects of these chemicals is important. Prioritization based on findings could guide targeted measurement. Among environmental trends, attention to PFAS compounds is evident, with a realization of the importance of studying even the volatile and smaller chain variants. Thus, complementary techniques such as Gas Chromatography (GC) and GC-MS are necessary to get a complete picture. These emerging trends call for new testing strategies to tackle contaminants related to water and air quality, given their significant importance in the public eye.

Can you explain briefly what PFAS are and why they have garnered significant attention from the media, public, and government regulators?

PFAS stands for per- and polyfluoroalkyl substances and are synthetic chemicals that vary in definition, potentially ranging from 5,000 to over 1,000,000 compounds. These substances have been utilized since the 1940s, persisting in the environment for more than 80 years. Despite legacy contamination, PFAS usage continues globally due to their robust carbon-fluorine bond, rendering them stable and unique for many daily uses. They were employed in non-stick cookware, fire suppression, and as water and fat repellents, finding applications in carpets and garments. This extensive use has led to their omnipresence in daily life and subsequently, the environment. The concern with PFAS relates to potential health impacts, especially from prolonged exposure, an aspect still under thorough study by regulatory bodies. Although health effects are confirmed for only a subset of PFAS, the broad occurrence of these compounds has sparked considerable public and governmental apprehension about their potential widespread health implications.

The focus of research and regulation has primarily been on two PFAS, namely PFOA and PFOS. Why do you think there's limited attention given to other PFAS, and what can be improved to collect and monitor further?

I think for a long time, we thought those were the most prevalent and hence the two most studied PFAS. What's becoming clear is these are legacy PFAS used in high abundance a while ago. Since the 1990s, those compounds have been widely replaced by other PFAS, what we term 'emerging PFAS'. There are at least over 5,000 PFAS thought to be present today. Research shows many others are present at significant concentrations, not just in water, soil, and air, but also in food, food contact materials, consumer products, and chemical manufacturing. The toxicological profile of these compounds takes a long time to determine, as studies are time-consuming and require suitable rigor. We are still at the tip of the iceberg in terms of information on PFAS occurrence and toxicity studies. Information is available for only a handful of them, but it's prudent to monitor as many as possible now for baseline levels. This way, when we have toxicological information, determinations can be made quickly based on their occurrence.

Can you explain the difference between targeted and non-targeted analysis and how the latter can help in expanding the understanding of PFAS?

Traditionally, we adhere to a routine and regulatory approach known as targeted analysis, where we identify specific compounds for measurement and design an appropriate analytical method for them. The gold standard in targeted analysis often involves utilizing a mass spectrometer, particularly a triple quadrupole mass spectrometer for compounds like PFAS. These substances, being relatively non-volatile and having substantial molecular weight, typically require a liquid chromatograph coupled with a mass spectrometer. However, the limitation of targeted analysis lies in its focus on predetermined PFAS compounds for quantification, excluding others from consideration. This is where non-targeted methods come into play, allowing measurement across a broader spectrum without preconceived biases. These methods are facilitated by high-resolution mass spectrometers that provide accurate mass measurements, offering confidence in compound identification. The true advantage of non-targeted analysis is its capacity to screen and potentially quantify thousands of compounds simultaneously, and its unbiased nature permits retrospective analysis even long after the initial assessment, offering a comprehensive and enduring view of sample composition.

Finally, how do you envision Agilent Technologies contributing to further advancements in the field of PFAS analysis and environmental health?

The focus has been on the PFAS market for a significant period. This is a crucial growth market on

the environmental front, holding significant public and environmental importance. The emphasis has been on promptly providing customers with solutions for routine testing and PFAS discovery. This includes the entire workflow from sample collection to preparation, complementary instrument analysis, data analysis, and reporting. This comprehensive value chain is supported by products, consumables, and supplies. Specific PFAS products are also available to ensure cleanliness and low background for sensitive analysis. Agilent boasts 40+ years of leadership in environmental testing, showcasing expertise in analysis and customer collaboration.
The goal is to facilitate quick implementation of PFAS analysis with robust methods, minimizing time spent on method development and maintenance. The focus has also extended to developing a non-targeted analysis portfolio, particularly in software. The new software suite, MassHunter Explorer, aids the non-targeted identification of PFAS compounds and offers statistical analysis and predictive tools to compare multiple samples while the ChemVista software has several hundred PFA spectra that coupled with our high-resolution MS instruments providing customers a more comprehensive picture of PFAs in their sample. Collaboration with Innovative Omics resulted in the Fluoromatch software, dedicated to PFAS annotation and identification without the need for analytical standards, which is a critical piece in increasing the ease of PFAS discovery in samples.

Based on this publication:
Fluoromatch 3.0 – Automated PFAS Non-Targeted Analysis and Visualizations Applied to Mammalian Biofluids

Could you provide more details about FluoroMatch 3.0 technology and other software tools for the measurement of unknown and emerging PFAS?

The essential aspect of non-targeted analysis lies in the software, particularly in data analysis and interpretation, where users invest the majority of their time. Agilent's MassHunter suite allows users to process and interpret data from the GC/MS and LC/MS with triple quadrupole and quadrupole time-of-flight instruments. A recent addition is the software package MassHunter Explorer, designed for non-targeted identification and statistical profiling of samples. This software streamlines the process with integrated statistical tools, catering even to novice users. Another notable software is Chem Vista, a library manager introducing Agilent's expert-curated Spectra of over 5,000 compounds. In addition to this, customers can also screen and identify compounds against Agilent's libraries, as well as open-source databases like Mass Bank and EPA's CompTox dashboard. Additionally, ChemVista collaborates with Fluoromatch, an open-source software, enhancing PFAS analysis by identifying compounds without needing analytical standards, effectively closing the mass balance.

Based on this publication:
Strategies for Ultimate Sensitivity of Per and Polyfluoroalkyl Substances (PFAS) in Water

As an expert in the environmental testing industry, what role do you see Agilent's Triple Quad LC/MS system playing in advancing our understanding of PFAS and supporting regulatory decisions in the future?

Regarding the LC/MS Triple Quad, it is currently the standard for quantifying PFAS compounds, crucial due to global regulatory interest and ongoing monitoring. The LC/MS Triple Quad is made more accessible to novice users, a focus of Agilent's work. Our latest triple quadrupole instruments incorporate user-friendly tools. One such tool is an intelligent reflex, a new feature that can detect if a sample is outside the range of a calibration curve triggering an automatic reinjection at a lower volume as well as prompting automatic blank runs to prevent carryover that can be determined by the user when setting up the worklist of samples. This saves users time and reduces reruns freeing up instrument time to run more samples as well as spending more time on generating valuable data insights. Agilent also offers guided maintenance suggestions with the Early Maintenance Feedback dashboard, much the same as how newer cars offer preemptive alerts for care and maintenance. This approach prevents both under and over-maintenance, targeting the key areas and affording customers more uptime. Specific data processing and reporting for PFAS EPA methods streamlines data collection for customers. Enhanced electronics in modern instruments enable heightened sensitivity with precision and robustness, allowing PFAS measurements at sub-part-per-trillion levels. These levels are significantly lower than those of other regulated contaminants. Agilent's overarching goal is to simplify instrument use for novices, thereby expanding the user base of LC/MS tools.

What's the difference between MCL and HAL? How does the 4th generation iFunnel technology on the new 6495D LC/TQ compare to the 3rd generation iFunnel technology (G6495C) in terms of achieving HAL values and sensitivity?

Regarding your inquiry about water quality regulations in the US, there are key distinctions between the Maximum Contaminant Level (MCL) and the Health Advisory Level (HAL). The MCL, established by the US EPA, serves as a regulatory standard for drinking water compounds subject to regulation and enforcement. In contrast, the HAL is a health-based guideline without legal enforcement. It signifies the concentration at which the lowest health effect is observed for a particular compound. The MCL considers not only health effects but also factors like accurate analytical measurement and associated costs of treatment. For instance, for PFAS compounds, while MCL concentrations range between 2 and 4 ng/L, HALs are much lower, ranging from 4 to 20 parts per quadrillion (pg/L). Labs often seek to measure up to the MCLs due to their regulatory nature, yet some opt for HAL measurements to anticipate potential future changes in regulations. Agilent's latest technology, the 6495D triple quadrupole LC/MS system, enhances sensitivity and confidence in measurements at these ultra-low concentrations, bolstering reliable and robust analysis.

What are the challenges associated with achieving the HAL values for PFOA due to background contamination, and what strategies can be employed to mitigate this problem with Agilent's PFAS solution and the new 6495D LC/TQ?

The main challenge in achieving desired HAL levels lies not in the analytical sensitivity but rather in the background PFAS contamination stemming from lab or general-use substances, like impurities in widely used fluoropolymers, such as air conditioning filters. This issue is pervasive across lab equipment and analytical solvents, contributing to contamination. To address this, Agilent employs a multi-pronged strategy. They scrutinized manufacturing processes, identifying points where fluoropolymers might have been used, and created a PFC-free kit for engineers to replace such parts reliably. This kit significantly reduces PFAS background in analytical instruments. To tackle solvent-related contamination, a PFAS delay column is integrated into the instrument, drawing from Agilent's decade-long focus on PFAS. Additionally, pretested PFAS sample prep cartridges, vials, and caps ensure customer assurance regarding specific PFAS concentrations. These measures alleviate customer concerns about background contamination, aligning with Agilent's comprehensive workflow approach. This approach, including specialized supplies, consumables, solvents, and analytical standards, ensures reliable PFAS analysis from sample collection to data reporting, with the 6495D LC/TQ playing a crucial role within the broader workflow alongside PFAS-specific resources.

FluoroMatch 3.0 – Automated PFAS Non-Targeted Analysis and Visualizations Applied to Mammalian Biofluids

ASMS 2023
ThP 102

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Yale

Agilent

Funding from: R01ES032196

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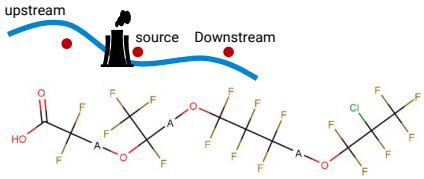
Introduction

Per and poly-fluorinated substances (PFAS) have gained considerable attention from the media, public, and government regulators due to their persistence and toxicity. Most research, media attention, and regulation focus on 2 PFAS (PFOA and PFOS) whereas even broad targeted methods seldom measure over 30 PFAS. Targeted PFAS analysis in serum is incomplete often measuring less than 40% of total PFAS. The portion of uncommonly measured or unknown PFAS is only increasing across time as companies manufacture alternative structures. Therefore, non-targeted PFAS analysis is needed to increase coverage of PFAS measurement to those uncommonly measured or unknown to understand the full implications of PFAS loads on human health. For this purpose, we release FluoroMatch 3.0. Here we present new development in FluoroMatch 3.0 and application to dried blood spots.

Libraries

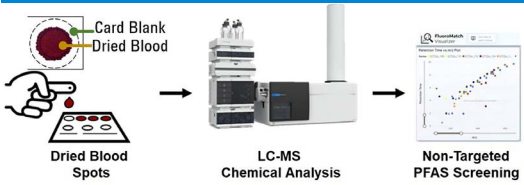
Class based libraries with fragmentation rules were generated for over 10,000+ species across over 80 different types of PFAS classes. These include biotransformation products, emerging PFAS, and legacy PFAS, as well as predicted structures not currently contained in any database



EPA

In collaboration with the EPA, new PFAS discovered at the 5 major manufacturer sites (surface water) will be added to FluoroMatch libraries continuously

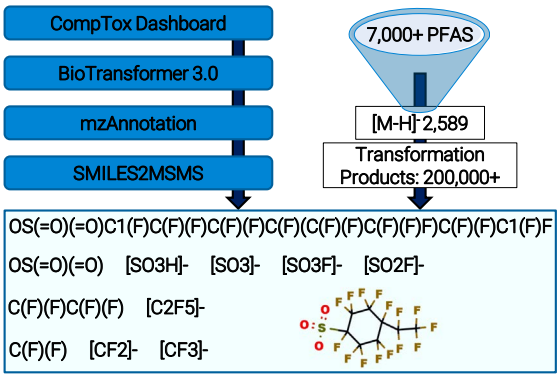
Dried Blood Spot Analysis



Reference whole blood (UTAK; #44600-WB(F)) was dried onto blood spot cards (QIAcard). Cards were spiked with a mixture of 20 native PFAS standards (Accustandard). To account for background contamination a blank portion of the card was analyzed. An Agilent 1290 Infinity II LC with a Poroshell ECC18 (2.1 x 100 mm, 2.7 um) column and PFC Delay Column (4.6 x 30 mm) connected to an Agilent 6546 LC/Q-TOF was used for analysis.

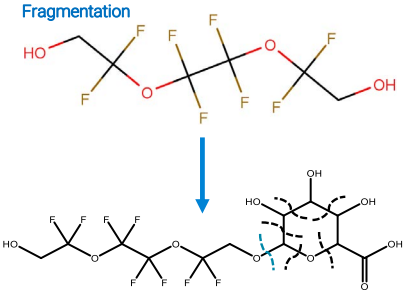
In-Silico Biotransformation Libraries

Predicted Biotransformation Products

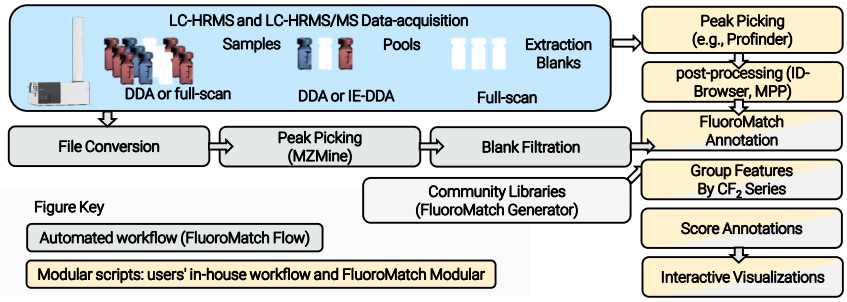


Chemical Standards Based biotransformation Libraries

(Biotransformed with Mouse Enzymes)
21 transformers identified across 13 parent PFAS
75 Transformation Products With Class Based Fragmentation



Entire Acquisition and Software Workflow

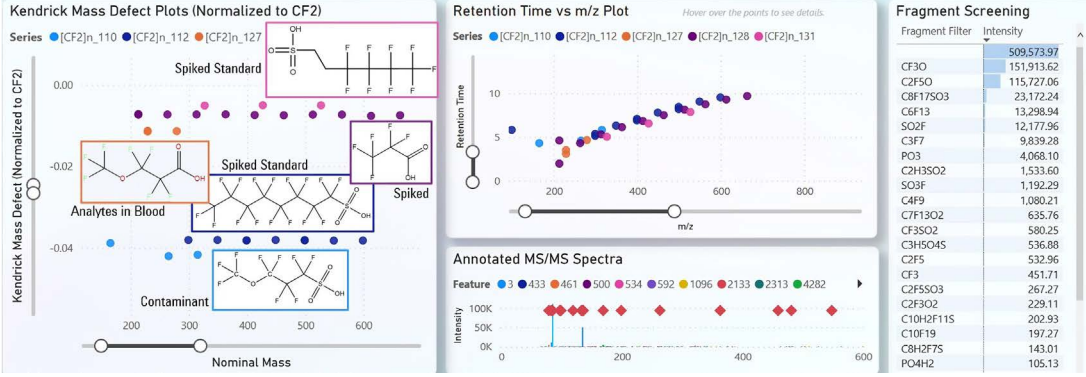


FluoroMatch Flow and FluoroMatch Modular (acquisition and data-processing workflow)

The FluoroMatch software data analysis workflow starts by importing data collected using MS, and MS/MS data dependent (DDA), iterative exclusion MS/MS (IE-DDA), or targeted MS/MS modes from individual, pooled and blank samples. FluoroMatch algorithms cover file conversion, blank filtering, feature annotation, and visualization. FluoroMatch Software also directly imports data processed initially using Agilent's Mass Profiler software or other peak picking software.

In this study, IE-DDA was performed on pooled samples

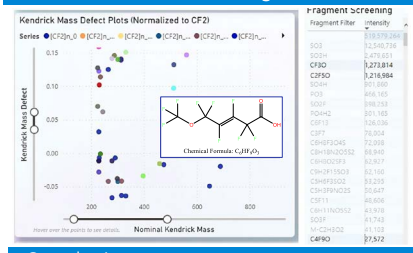
Visualizer Interface: Dried Blood Spot Results – Homologous Series



FluoroMatch Visualizer Outputs (Left are All Homologous Series Automatically and Manually Assigned) (Right are All Features After Filtering by Ether Related Fragments)

Left: A total of 28 PFAS across 5 homologous series were annotated in dried blood spot samples. These series were identified as fluorotelomer perfluoroalkyl sulfonic acids (FTS), perfluoroalkyl carboxylic acids (PFCA), perfluoroalkyl ether carboxylic acids (PFECA), perfluoroalkyl sulfonic acids (PFSA), and perfluoroalkyl ether sulfonic acids (PFESA). These annotations captured 95% of the standards spiked onto samples for validation (19 of 20 spiked standards, 5% false negative rate). Three PFECA species (two C4 isomers and C5) were above levels in the card blank and neat standard solution, and hence were likely from the blood and indicative of human exposure.
Right: Fragment screening for $[C_3F_5O]^-$, $[C_2F_5O]^-$, $[C_3F_7O]^-$, and $[C_4F_9O]^-$, showing many potential ether-linked PFAS in dried blood spots. One additional annotated species is shown.
The interactive dataset can be review for new PFAS at innovativeomics.com/datasets

Visualizer Interface: Fragment Screening



Conclusions

FluoroMatch can be used to classify mixtures, identify compounds, and determine unknowns

- Incorporates MS/MS, MS, EICs, homologous series, and retention time
- Has over 200,000 species with fragmentation in libraries; fragment screening (777) and substructure assignment for unknowns
- Five novel or rarely screened PFAS were found in whole blood using the workflow: PFECA, PFSA branched isomer, and unsaturated PFECA
- < 5% false positive and false negative rate

Innovative Omics

To install the software please visit:
Innovativeomics.com/software
Questions? Trainings? Collaboration?
Contact: jeremykoelmel@gmail.com

For Research Use Only. Not for use in diagnostic procedures.

Poster Reprint

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Strategies for Ultimate Sensitivity of Per- and Polyfluoroalkyl Substances (PFAS) in Water

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Introduction

Introduction

Per and polyfluorinated alkyl substances (PFAS) are a group of man-made compounds that are ubiquitous in environment. Possible adverse effects to humans and animals have made them a public health concern[1]. In June 2022, the USEPA issued interim drinking water health advisory limits (HALs) for PFOA at 0.004 ng/L, PFOS at 0.02 ng/L, GenX at 10 ng/L and PFBS at 2,000 ng/L to reduce the risk to the public from exposure to these PFAS[1].

In March 2023, EPA proposed National Primary Drinking Water Regulation for six PFAS[1], adding PFHxS and PFNA to the list covered in the HALs. The proposed maximum contaminant level (MCL) for both PFOA and PFOS was set to 4 ng/L, higher than the toxicologically based HALs. The remaining compounds are proposed to be covered by a Combined Hazard Index Calculation.

With the 3rd generation iFunnel technology (G6495C) we showed that with a large volume injection utilizing either a focusing guard cartridge or a sandwich injection that achieving the HAL values was possible when extracting per EPA 533[2]. However, background contamination is very problematic and usually exceeds the HAL value for PFOA.

While achieving the HAL level is not necessary, it is ideal to maintain sensitivity with routine implementation of EPA 533. Here we show what can be achieved with the 4th generation iFunnel technology on the new 6495 LC/TQ (G6495D) with a typical injection volume with comparison to the earlier model.

Figure 1. Infinity II 1290 and 6495 LC/TQ and Agilent Bond Elut PFAS WAX SPE Cartridge.

Experimental

Methods

A bottled drinking water, two different tap waters and reagent water blank were collected and extracted following US EPA Method 533[3] using the Agilent Bond Elut PFAS WAX SPE cartridge. Native PFAS standards and isotopically labeled analogues were purchased from Wellington Labs. Standards were diluted to the low pg/mL range to evaluate instrument sensitivity while using a routine injection volume for EPA 533 analysis. The native samples were evaluated to confirm sensitivity and background. The extracts and standards were in 80:20 methanol:water.

An intelligent source optimization algorithm (MassHunter Source Optimizer) was used to define the ideal source temperatures and conditions for EPA 533 target compounds. LC and instrument parameters are shown in Table 1.

LOQ Determination

The LOQ determination required a peak S/N (> 10), reproducibility (< 20%), accuracy within 30% and a calibration fit with R^2= 0.99 and Relative Standard Error (RSE) < 20.

Table 1. LC and 6495 LC/TQ Parameters

Column	• Zorbax Eclipse Plus C18, 2.1 x 100mm, 1.8 um • PFC Delay Column, 4.6 x 30 mm
Flow Rate	0.4 mL/min
Injection volume	3 uL
Column Temperature	40 °C
Mobile Phase	A: 2 mM Ammonium Acetate in Water B: 95:5 Acetonitrile: water
Run time	12.5 minutes
Gas Temperature	150 °C
Gas flow	18 L/min
Nebulizer	25 psi
Sheath Gas Temperature	390 °C
Sheath Gas flow	18 L/min
Capillary Voltage	2500 V (ESI-)
Funnel voltages	Standard

Results and Discussion

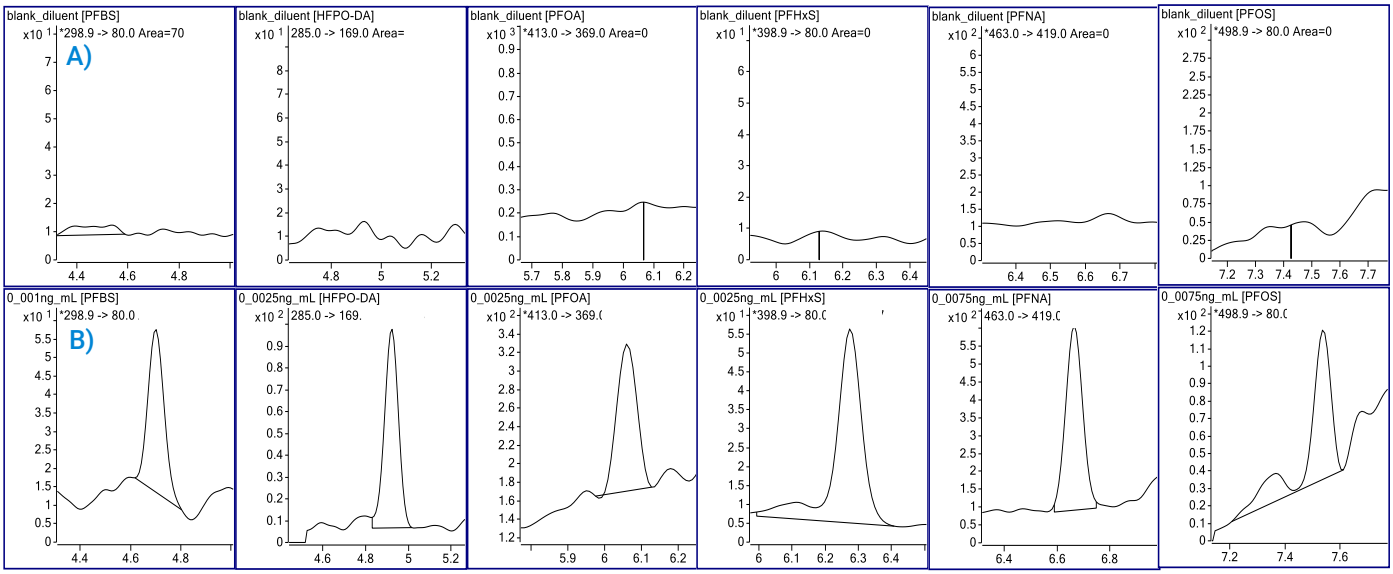

Figure 2. Chromatograms-A) Blank B) Compound Chromatograms at LOQ level (New 4th Generation 6495 LC/TQ (G6495D). From Left, PFBS, HFPO-DA, PFOA, PFHxS, PFNA, and PFOS

Table 2. Concentration corrected Limit of Quantitation (LOQ) on Each Instrument Models

	3rd Generation iFunnel LOQ (ng/L)	4th Generation iFunnel LOQ (ng/L)
PFBS	0.01	0.004
HFPO-DA	0.01	0.01
PFOA	0.02	0.01
PFHxS	0.02	0.01
PFNA	0.03	0.03
PFOS	0.2	0.03

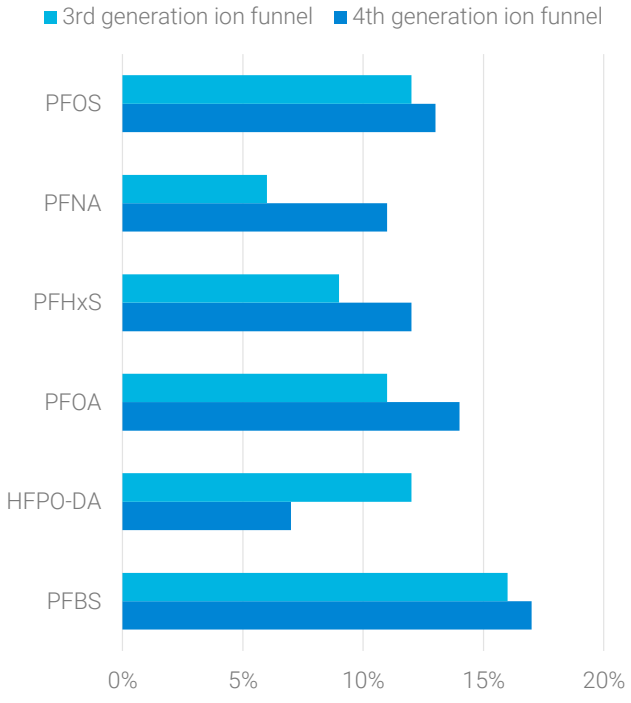

Figure 3. RSD (n=8) at LOQ Level for Both Instrument Models

Results and Discussion

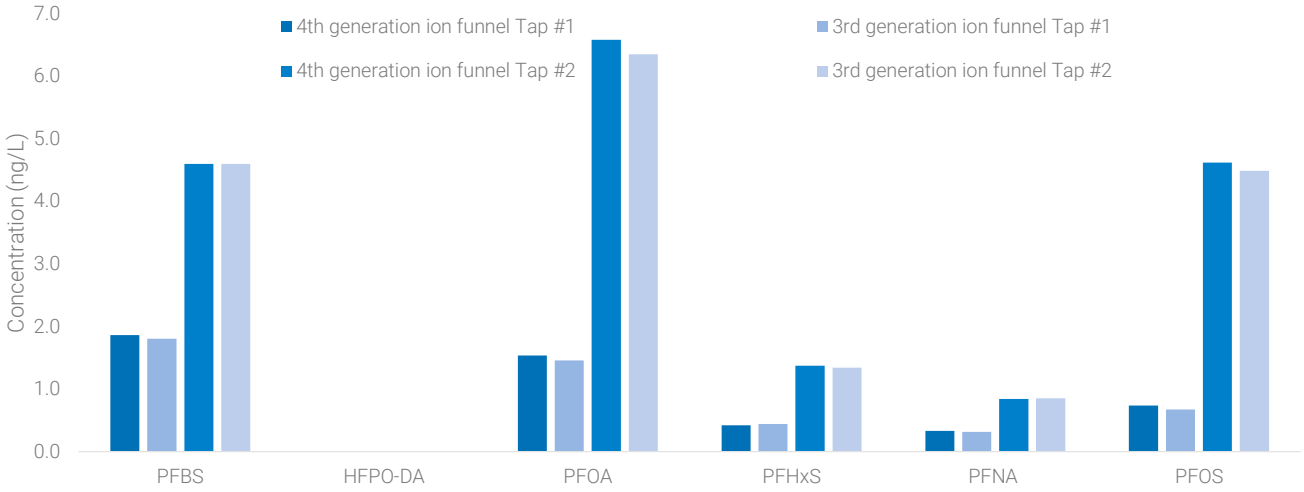

Figure 4. Concentration in Water Samples. No MCL compounds were detected in method blank or bottled water sample

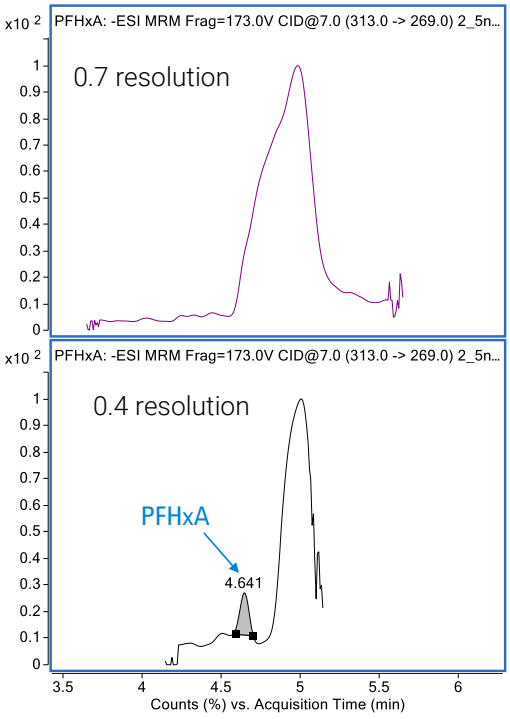

Figure 5. Narrow MS Resolution Width Removed PFHxA Interference at Low Concentrations

MS Resolution Width

The 6495 LC/TQ offers a "narrow" (0.4) isolation width as a compound setting. During method development a large interfering peak was noticed for PFHxA at low concentration levels. Updating the isolation width to narrow removed the interference (Figure 5). While still allowing sensitive performance within the calibration range.

Results

The 4th generation iFunnel showed improved performance with a 2.5 – 7x increase in sensitivity. The increase was compound dependent with PFOS showing the greatest increase.

Extracted water samples concentrations analyzed separately on both the 6495 LC/TQ and its predecessor were comparable. The bottled water sample and method blank sample did not contain any significant level of the MCL compounds. While the both tap water samples showed detectable levels of 5/6 compounds.

Conclusions

- The 4th generation iFunnel showed improved performance with a 2.5 – 7x increase in sensitivity for the 6 MCL PFAS compounds.
- Narrow MS Resolution removed an interference at low concentrations.

References

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