Italian Mass Spectrometry Society
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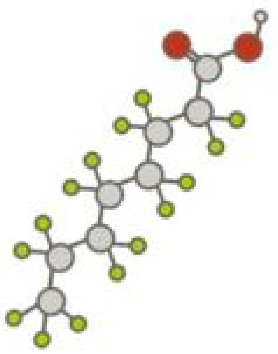
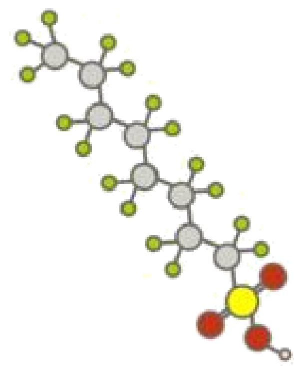
IMaSS Emerging Contaminants Days

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues





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Bologna, September 15, 2022

Aula Magna U.E.1 - Polo Didattico Navile
Via della Beverara 123/1

Conference Proceedings

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Italian Mass Spectrometry Society

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Traditional and “Emerging” PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022
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Via della Beverara 123/1

P R O G R A M

Morning Session, Chairman: Andrea Raffaelli

10:00 Andrea Raffaelli: Introduction

10.10 Marinella Vitulli (Food Contact Center s.r.l., Pistoia)
PFAS in raw materials, food contact items, packaging and related industrial waste: sample preparation and targeted&untargeted testing

10:50 Andrea Perissi (Waters)
Sensitive PFAS analysis from Edible Produce on Xevo TQ-XS after QuEChERS extraction

11:10 Debora d'Addona (Thermo Fisher Scientific)
Utilizzo della Spettrometria di Massa ad Altissima Risoluzione per la determinazione di PFAS in matrici diverse: evidenti vantaggi e possibilità analitiche

11:30 Stefano Fiorina (Sciex)
PFAS nelle acque potabili e superficiali: un viaggio fra legislazione, semplicità e sensibilità

11:50 Marco Fontana (ARPA, Piemonte e Veneto)
Sviluppo e validazione di metodi per il monitoraggio PFAS su campioni di aeriformi

12:10 Lorenzo Zingaro (Agilent)
Analysis of Perfluoroalkyl Substances in Human Hair by Agilent LC/MS Accurate Mass Spectrometry Q-TOF: A rapid biomonitoring screening method using the Agilent 1290 Infinity II LC and the Agilent 6546 Q-TOF

12.30 Lunch Break

Afternoon Session Chairman: Laura Mercolini

14:30 Michele Mazzetti (ARPAT, Livorno)
First investigation of per- and poly-fluoroalkylsubstances (PFAS) in striped dolphin Stenella coeruleoalba stranded along Tuscany coast (North Western Mediterranean Sea)

15:00 Damiano Bracchitta (APPA, Trento)
L'analisi in routine dei PFAS, strategie analitiche per il miglioramento dei limiti di quantificazione

15:20 Sara Valsecchi (CNR-IRSA, Brugherio)
Analytical strategies in LC-MS for the analysis of next generation PFAS

15:40 Claudio Carrer – Loretta Gallocchio (InterCinD—LSA)
L'importanza dei PTP dinamici per la valutazione delle prestazioni dei test valutativi di laboratorio utilizzando matrici naturali - PFAS

16.00 Alessandro Pellizzaro - Massimo Fant (Acque del Chiampo S.p.A.)
Evaluation of perfluoroalkyl acids uptake by hydroponic crops and emerged macrophytes: from extraction to LC-MS/MS determination

16:20 General Discussion and Conclusions

Italian Mass Spectrometry Society

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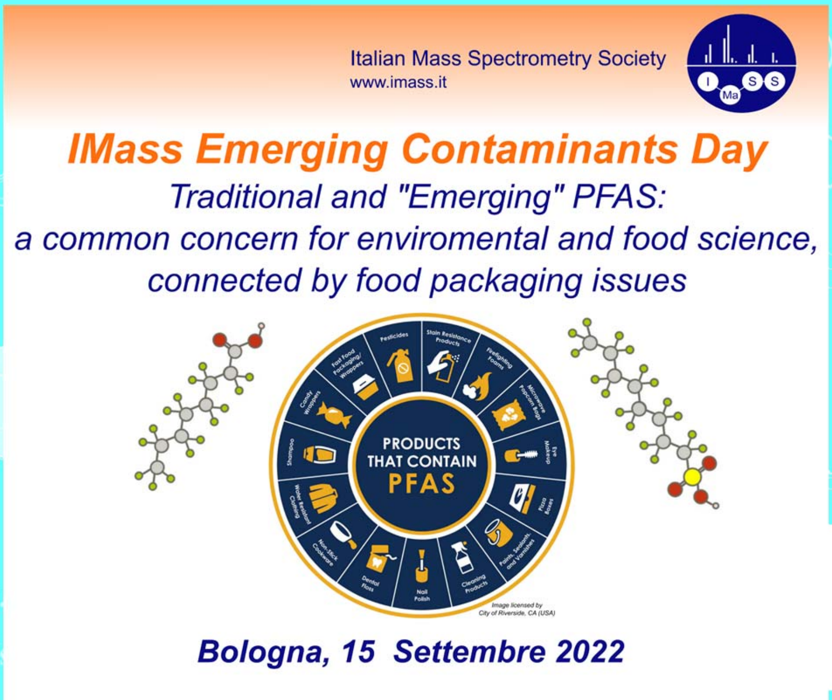
INTRODUZIONE
(Andrea Raffaelli)



New Classes of "Emerging" Pollutants

- During the meeting held on-line last September 22, 2021, we focused our attention on some classes of "emerging pollutants", such as PPCPs
- Going on about taking care of environment, as well as caring about our food, in this occasion we will try to get a sort of second order emergence.
- Indeed, if PFAS are included in the list of emerging contaminants, recently there is an increasing interest for "emerging PFAS", that become "emerging-emerging".
- Beyond the commonly monitored "traditional PFAS", we need new, untargeted approaches for their identification.

I.F.C.

Istituto di Fisiologia Clinica

Traditional and Emerging PFAS: Who Concern

- PFAS are widely employed in several fields, such as domestic use items, medical devices, paper and general packaging materials, fabrics manufacturing, automotive, and so on…
- They are a critical issue for environment.
- They are as well a very critical issue for their presence in food.
- Indeed, a lot of PFAS-containing materials bump into food: food packaging, cookware (for instance, non-stick pans), single use plates, glasses and silverware (materials later released in the environment…)
- Food packaging, hence, become a sort of link between environment and food safety

Speakers List

- Damiano BRACCHITTA (ARPA Trento)
- Claudio CARRER (InterCInd Labservice)
- Debora D'ADDONA (Thermo Fisher Scientific)
- Massimo FANT (Acque del Chiampo)
- Stefano FIORINA (Sciex)
- Marco FONTANA (ARPA Piemonte)
- Loretta GALLOCCHIO (InterCInd Labservice)
- Michele MAZZETTI (ARPA Toscana)
- Alessandro PELLIZZARO (Acque del Chiampo)
- Andrea PERISSI (Waters)
- Sara VALSECCHI (CNR-IRSA)
- Marinella VITULLI (Food Contact Center s.r.l.)
- Lorenzo ZINGARO (Agilent)


I.F.C.

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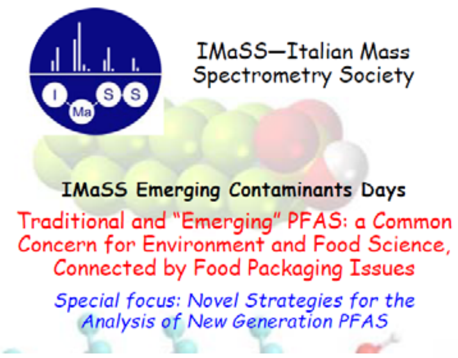
Marinella VITULLI

PFAS in raw materials, food contact items, packaging and related industrial waste: sample preparation and targeted&untargeted testing



Marinella Vitulli

PFAS in raw materials, food contact items, packaging and related industrial waste: sample preparation and targeted&untargeted testing

Food Contact Center - Pistoia- Tuscany

Food Contact Center is a laboratory; our headquarters are based in Tuscany, Via E.Pestalozzi 63, Pistoia, where documentary evaluations and laboratory tests are performed, according to good laboratory practices and ISO/IEC 17025 requirements; some tests are performed in partner laboratories, under direct supervision.

We are Accredited ISO 17025 by Accredia, laboratory 1786L


ACCREDIA

CERTIFICATO DI ACCREDITAMENTO

Accreditation Certificate

1786L

DIPARTIMENTO LABORATORI DI PROVA

Food Contact Center Srl

New Location–transfer in 2023

Food Contact Center - Pistoia- Tuscany

FOOD CONTACT CENTER

Analytical techniques

(some techniques are available thanks to synergies with partner laboratories)

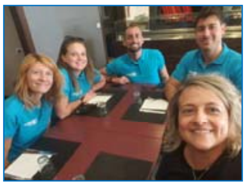
- Gravimetric analysis, GC FID-MS and LC-MS. Metal analysis ICP OES, ICP MS
  - UV VIS spectrometry, infrared spectrophotometry with ATR and microscope
- Mechanical analysis of bending, traction and compression
- DSC-TGA thermal analysis
- GPC for molecular weight analysis of polymers
- Optical microscopy, X-ray diffraction
- SEM with EDXS microanalysis
- Permeability to various gases
- Sensory analysis
- Devices for the evaluation of technological compliance

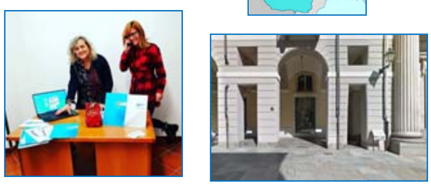
All operations and office processes in the Food Contact Center are managed through **LIMS** **(Visual Lims- Infoteam)**

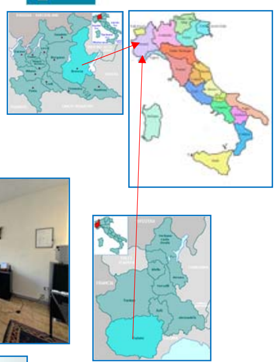
Food Contact Center – North & Center Italy

FOOD CONTACT CENTER

Food Contact Center opened in 2020 a **materials laboratory** (accredited) in Via Aldo Moro, 16, **Brescia**







We also have an office in **Cuneo**, Via Roma 64

www.foodcontactcenter.com

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LAUREA TRIENNALE IN TECNOLOGIE ALIMENTARI

DETERMINAZIONE DEI METALLI PESANTI SU CARTA A CONTATTO CON ALIMENTI:

IL CASO DEL PIOMBO

ISTITUTO ITALIANO IMBALLAGGIO The ITALIAN INSTITUTE of PACKAGING The packaging community

AMI

PACKAGING EDUCATION

SMITHERS INNOVATE WITH CONFIDENCE

ILSI Europe Packaging Materials

aibo-fce associazione italiana business operator food contact expert

ILSI Europe Packaging Materials

FOOD CONTACT CENTER participates to the TECHNICAL GROUP Best Practices in identifying and quantifying unknown migrants from food contact materials' from ILSI Europe's Packaging Materials Task Force.

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FCC Regulatory MATRIX

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PFAS in FCM: sample preparation and targeted&untargeted testing

Agenda

- ❑ PFAS: definition and uses in FCM

- ❑ Testing approach, sample preparation for different Items and PFAS

- ❑ Hidden PFAS in paper food contact materials: untargeted screening of perfluoroalkyl substances and Total Oxidizable Precursor Assay

FCM: Food Contact Materials

The main purpose of packaging:

- Protect
- Preserve
- Promote the product on the market.

The final packaging is very important…

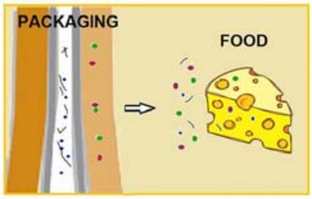
But we must not underestimate the risk related to **industrial packaging and surfaces, cooking/kitchen materials**!

FCM: Food Contact Materials

The European Legislation on Food Contact Materials is based on the framework Regulation (EC) No 1935:2004.

This Regulation establishes the **general requirements** for food contact materials and the authorization of new substances.

Regulation (EC) No. 1935:20041 includes the following requirements:



Food contact materials:

Must be safe.

Must not **transfer their components into food in quantities that could endanger human health**, change food composition in an unacceptable way or deteriorate its taste and odour.

FCM: Food Contact Materials

in quantities that…. ?

Risk evaluation

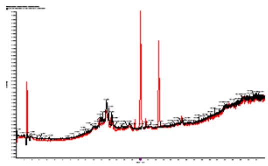
Risk assessment

Risk management

Risk evaluation:

Hazard = any source of potential adverse health effect, harm or damage (in our case it refers to the toxicity of substances)

Exposure = the extent to which someone is subjected to a hazard



MIGRATION TESTING

Risk = the likelihood that a person exposed to a hazard will be armed

FCM: Food Contact Materials – Migration testing

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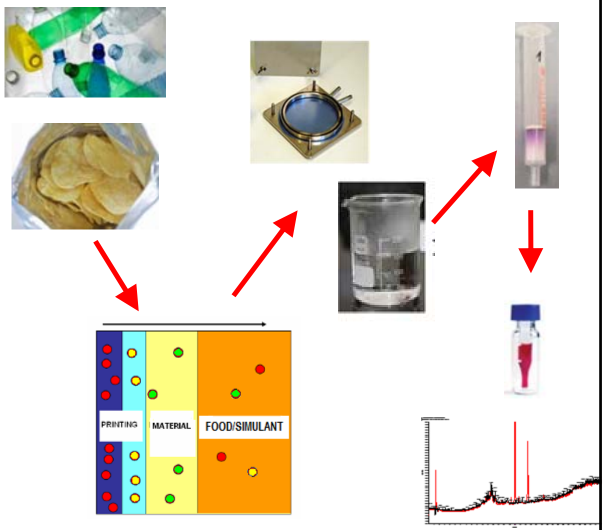
+

Hazard (toxicity) No exposure = No Risk

Hazard + exposure = RISK



FCM: Food Contact Materials – Inappropriate use

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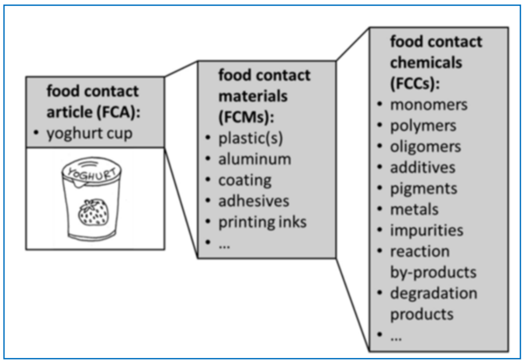
Oggi facciamo un salto nell'America Latina con una preparazione semplice, scenografica e buona. Sto parlando del **Lomo al Trapo** ovvero filetto bovino avvolto in un canovaccio.

Per **prima cosa** mettiamo a bagno il pezzo di lenzuolo in una bacinella con il liquido scelto (birra o vino)

Come realizzare coppe di cioccolato con palloncini

FCM: Food Contact Materials



distribution

Analytical Technique
Test method
Testing conditions

Picture source: Environmental Health PerspectivesVol. 125, No. 9 Scientific Challenges in the Risk Assessment of Food Contact Materials

FCM: Food Contact Materials

MIGRATING SUBSTANCES

- **IAS** (Intentionally Added Substances): specifically added during the manufacturing process of materials, having a function in the manufacturing process or in the final product.

- Row materials – starting substances
- Monomers
- Additives
- Solvents
- Colourings

- NIAS (Non intentionally added substances): impurities in the substances used, reaction intermediate formed during the production process, or products of reaction or decomposition.

- Additives degradation
- Polymer degradation
- Residues
- Neoformed products
- Impurities

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FCM: Food Contact Materials …. and PFAS

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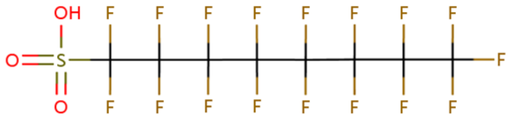
More than 4500 manufactured substances that meet the definition of PFAS

- Perfluoroalkylated (PFAS) substances consist of a large group of substances a fully fluorinated hydrophobic alkyl chain of varying length (4-16 C atoms) and a hydrophilic end [R, F(CF2)nR]
- Within PFAS groups, two major substances are reported and studied: Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonic acid (PFOS):
- PFOA - C8HF15O2

- PFOS - C8F17SO3-
- PFAS have high compound stability.



- PFAS are bioaccumilative, persistent and toxic.

PFAS in FCM: sample preparation and targeted&untargeted testing

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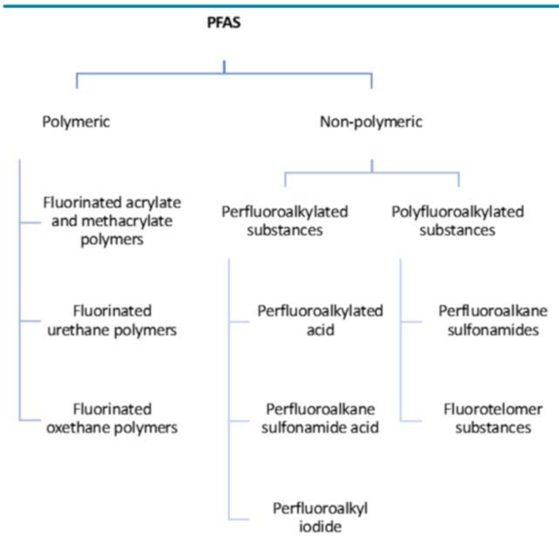
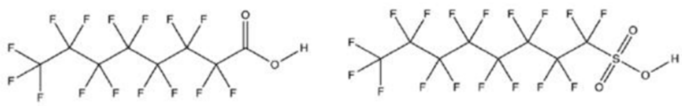
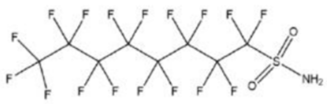


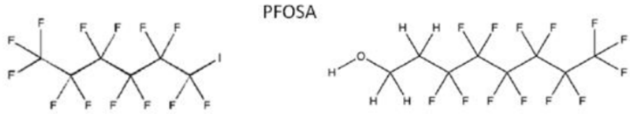
Figure 1. Classification of perfluoroalkylated and polyfluoroalkylated substances [2].



PFOA PFOS



PFOSA



PFHxI 6:2 FTOH

S.P. Perfluoroalkyland PolyfluoroalkylSubstancesin the Environment: Terminology, Classification, and Origins. Integr. Environ. Assess. Manag. 2011

Received: 7 December 2021 | Revised: 21 April 2022 | Accepted: 25 April 2022
DOI: 10.1002/ajim.23362

REVIEW ARTICLE

AMERICAN JOURNAL OF INDUSTRIAL MEDICINE WILEY

Historical and current usage of per- and polyfluoroalkyl substances (PFAS): A literature review

Linda G. T. Gaines PhD, PE

U.S. Environmental Protection Agency, Washington, District of Columbia, USA

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Email: gaines.linda@epa.gov

Abstract

Background: Per- and polyfluoroalkyl substances (PFAS) have uniquely useful chemical and physical properties, leading to their extensive industrial, commercial, and consumer applications since at least the 1950s. Some industries have publicly reported at least some degree of information regarding their PFAS use, while other industries have reported little, if any, such information publicly.

Methods: Publicly available sources were extensively researched for information. Literature searches were performed on key words via a variety of search mechanisms, including existing PFAS use and synthesis literature, patent databases, manufacturers' websites, public government databases, and library catalogs. Additional searches were conducted specifically for suspected or known uses.

Results: PFAS have been used in a wide variety of applications, which are summarized into several industries and applications. The expanded literature search yielded additional references as well as greater details, such as concentrations and specific PFAS used, on several previously reported uses.

Conclusions: This knowledge will help inform which industries and occupations may lead to potential exposure to workers and to the environment.

Carbon tetrafluoride, the simplest perfluorocarbon, was first produced in 1886. Other PFCs have been made since at least the 1930s. PFAS with functional groups have been made since at least the 1940s and since at least the 1950s have been used in various industries and products due to their unique properties. The first fluoropolymer patent was filed in 1934. Polytetrafluoroethylene (PTFE) was first synthesized in 1938, and due to its unique properties, was used in the Manhattan Project to separate UF6 isotopes.
In 1946, DuPont commercialized PTFE.

TABLE 3 Coating products or materials coated with PFAS

Agricultural glass and plastic covers (ex. greenhouses)
Automotive finishes
Caulks
Cellulose
Cements
Ceramics
Chemical processing industry equipment such as ducts, reactors, impellers, tanks, pipes, and fasteners
Clearcoats
Cookware/bakeware (household, industrial, commercial)
Dryer drums (commercial)
Fishing rods and reels
Floor waxes
Glass (automobile windshields, automobile headlights, bathroom mirrors, eyeglasses, etc.)
Guns
Grouts
Inks
Metals
Musical instrument strings
Natural stones
Paint
Piano parts including pins and knuckles
Pigments
Plastics and elastomers
Polishes
Resins
Sealers
Sewing machine presser feet
Ski wax (snow skis, snowboards)
Sports Equipment strings (tennis racquet, etc.)
Stains (floor, wood, etc.)
Varnish
Waxes
Wood

Abbreviation: PFAS, per- and polyfluoroalkyl substances.

Plastics and elastomers

Coating products or materials coated with PFAS

Packaging, paper, and cardboard

TABLE 9 Packaging, paper, and cardboard

Anticorrosion liner
Baking paper
Butter wrappers
Carbonless forms
Coated raw paper
Folding cartons
Food plates, bowls, etc.
General liner and flute
Kraft paper
Masking papers
Microwave popcorn bag susceptors
Neutral liner
Neutral white role paper
Paper combined with metal
Pet food bags
Pizza boxes
Paper food straws
Raw paper for plaster board
Take-out food containers and food wraps
Wallpaper
Wood-containing paper

Received: 6 October 2020 | Revised: 6 January 2021 | Accepted: 2 February 2021

DOI: 10.1111/1541-4337.12726

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Comprehensive REVIEWS WILEY

Per- and polyfluoroalkyl substances and their alternatives in paper food packaging

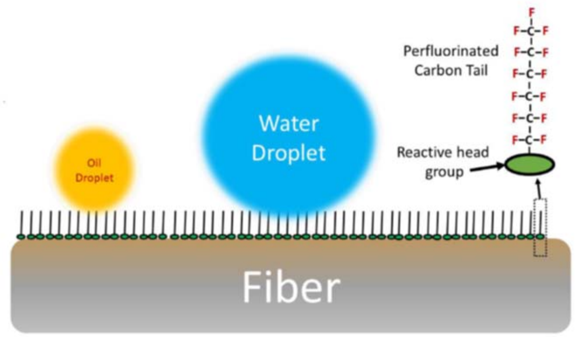


FIGURE 2 Schematic of PFAS coating and orientation on the surface of fibers. PFAS chemicals tend to coat the surfaces of fibers, including fibers located internally when internal sizing containing PFAS is used such as with molded pulp paper packaging

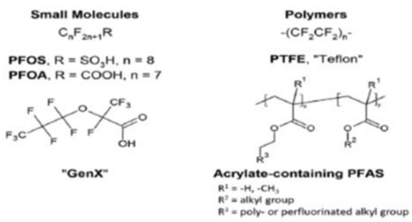


FIGURE 1 Chemical structure of selected PFAS

Regulatory Enforcement

L 372/32 EN Official Journal of the European Union 27.12.2006

DIRECTIVE 2006/122/ECOF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 12 December 2006

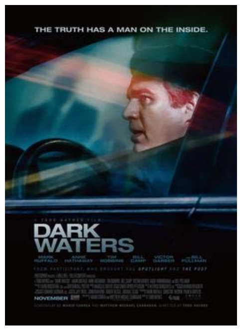
amending for the 30th time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates)

(Text with EEA relevance)

Early studies focused on perfluoroalkyl acids (PFAA), like perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA).

In **2006** the European Commission limited the use of these substances, in the same year US EPA launched a global stewardship program inviting companies to reduce PFOA. Following these regulations that limited their use, industries searched for alternative fluorinated compounds, like perfluoroalkyl sulfonates and perfluoroalkyl ether carboxylic acids (e.g., GenX, ADONA and F-53B)

*In **1999**, Robert Bilott sued E.I. du Pont de Nemours and Co, better known as DuPont, on behalf of a West Virginia farmer whose cows were dying. Over the course of that lawsuit, Bilott discovered that DuPont had been using a chemical called PFOA in the production of Teflon for decades, while quietly studying its effects on lab animals and factory workers.*



EPA Document #: EPA/600/R-08/092

METHOD 537. DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

Version 1.1
September 2009

FOOD CONTACT CENTER

Regulatory Enforcement on FCM

U.S. FOOD & DRUG ADMINISTRATION

August 5, 2021

Dear Manufacturers, Distributors, and Users of Fluorinated Polyethylene Food Contact Articles:

The U.S. Food and Drug Administration (FDA) is directing this letter to manufacturers, distributors, and food manufacturers that use fluorinated polyethylene food contact articles as a reminder that only certain fluorinated polyethylene containers are authorized for food contact use. Specifically, FDA's regulation authorizing the use of fluorinated polyethylene in contact with food is listed in Title 21 of the Code of Federal Regulations Part 177.1615 (21 CFR 177.1615).[1] This regulation stipulates that fluorinated polyethylene containers for food contact use may only be manufactured by modifying the surface of the molded container using fluorine gas in combination with gaseous nitrogen as an inert diluent. The regulation does not authorize fluorination in the presence of water, oxygen, or gases other than nitrogen.

DGCCRF	*Fiche MCDA n°4 (V02 – 01/01/2019)* Aptitude au contact alimentaire des matériaux organiques à base de fibres végétales destinés à entrer en contact avec des denrées alimentaires	Page 1/18

9. PFOA Acide perfluorooctanoïque (PFOA, CAS 335-67-1) LMS = ND (LD = 0,05 µg/kg d'aliment) (10)	Présence d'un revêtement/vernis imperméabilisant contre l'eau ou l'huile

COMMISSION REGULATION (EU) 2021/1297

of 4 August 2021

amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council as regards perfluorocarboxylic acids containing 9 to 14 carbon atoms in the chain (C9-C14 PFCAs), their salts and C9-C14 PFCA-related substances

PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER

News 3
13.09.2021
REGULATION
Released by
California Legislature

CALIFORNIA LEGISLATURE PASSES BILL TARGETING PFAS IN FOOD PACKAGING AND COOKWARE

The bill prohibits the sale of plant fibres-based food packaging that contains PFAS that has been intentionally added to have a functional or technical in the product, or is present in the product at or above 100 parts per million

The California legislature passed AB 1200, known as the California Safer Food Packaging Cookware Act of 2021, on September 7, 2021, and was enrolled and presented to the governor for signature on September 13, 2021. The bill bans the use of perfluorinated and polyfluorinated alkyl substances (PFAS) in food packaging composed in substantial part of paper, paperboard, or other materials derived from plant fibres. It also requires warning labels on cookware to which PFAS was intentionally added. Similar to other state laws on PFAS in food packaging, the California bill defines PFAS as "fluorinated organic chemicals containing at least one fully fluorinated carbon atom." It also requires, as of January 1, 2024, manufacturers of cookware that contains any chemical on the state's Green Chemistry list of chemicals of concern to list the presence of those chemicals on the product label, and to post this information on the internet, along with a link to the authoritative lists for the relevant substances.

THE PEOPLE OF THE STATE OF CALIFORNIA DO ENACT AS FOLLOWS:

SECTION 1. Chapter 12.5 (commencing with Section 108945) is added to Part 3 of Division 104 of the Health and Safety Code, to read:

CHAPTER 12.5. Juvenile Products

108945. For purposes of this chapter, the following definitions apply:

(a) "Adult mattress" means a mattress other than a crib mattress or toddler mattress.

(b) "Regulated perfluoroalkyl and polyfluoroalkyl substances" or "regulated PFAS" means either of the following:

(1) PFAS that a manufacturer has intentionally added to a product and that have a functional or technical effect in the product, including, but not limited to, the PFAS components of intentionally added chemicals and PFAS that are intentional breakdown products of an added chemical that also have a functional or technical effect in the product.

(2) The presence of PFAS in a product or product component at or above 100 parts per million, as measured in total organic fluorine.

LIMIT ON TOTAL FLUORINE...

Ministry of Environment and Food of Denmark
Danish Veterinary and Food Administration

Ban on fluorinated substances in paper and board food contact materials (FCM)

Indicator value

The Danish Veterinary and Food Administration has introduced an indicator value that can help the industry assess whether organic fluorinated substances have been added to paper and board. The indicator value is 20 microgram organic fluorine per gram of paper. This corresponds to 10 microgram organic fluorine per square decimeter of paper, when the paper has a weight of 0.5 gram per square decimeter. Content below the indicator value is considered as unintentional background pollution. So, companies can use the value to ensure that organic fluorinated substances have not been added to the paper.

PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER

EN 13432 standard "Packaging - Requirements for packaging recoverable through **composting** and **biodegradation** - Test scheme and evaluation criteria for the final acceptance of packaging"

A.1.2 Heavy metals and other toxic and hazardous substances

The concentration of any substance listed in Table A.1, present in packaging materials and whole packaging, shall not exceed the value given in that table.

Table A.1 - Maximum element content of packaging material and whole packaging

Element	mg/kg on dry substance	Element	mg/kg on dry substance
Zn	150	Cr	50
Cu	50	Mo	1
Ni	25,0	Se	0,75
Cd	0,5	As	5
Pb	50	F	100
Hg	0,5		

F limit 100 ppm

Vaschetta in cartoncino denominato : "FC210462.01"

Prova	Risultato	UdM
Fluoro *UNI EN15408:2011 (Fluorine)* *Prova effettuata presso Innovhub SSI Area Combustibili*	1062	mg/kg

F result >1000 ppm

BIODEGRADABLE PRODUCTS INSTITUTE

Home » Certification » Fluorinated Chemicals (PFAS)

Fluorinated Chemicals (PFAS)

The BPI Certification Scheme states that organic fluorinated chemicals, such as perfluorinated and polyfluorinated substances, cannot be present in formulas for BPI Certified items. BPI's rules for fluorinated chemicals are as follows:

1) The product formula must not contain fluorinated chemicals — as evidenced by safety data sheets for all ingredients.

2) Test results from a BPI-Approved lab showing a maximum of 100ppm total organic fluorine.

3) A statement of no intentionally added fluorinated chemicals, signed by the manufacturer.

Statement… declaration of NON INTENTIONAL USE

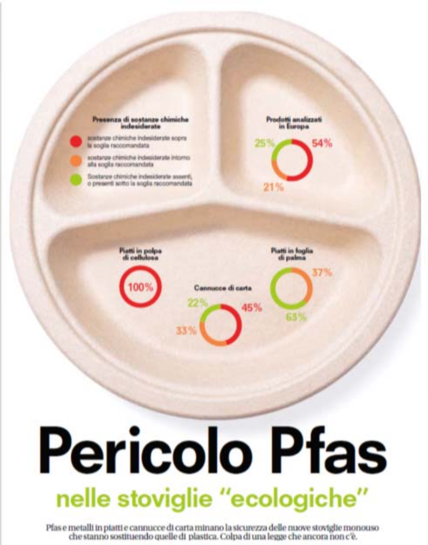
TÜV AUSTRIA	TÜV AUSTRIA	OK compost INDUSTRIAL
	OK compost INDUSTRIAL Certification Scheme	
	Doc Ref: PD-BA-TABE-CERT-BIO-CS-001_OKI_EN	
	Edition: F (2022-01-01)	

8.1.4. Chemical characteristics

255 As specified in EN 13432 the total Fluorine concentration of the product must not exceed 100ppm. To further restrict the use of per-fluorinated or fluorinated chemicals each applicant must sign a self-declaration that no PFAS (as defined by the OECD, see §12.4.) are added to their product.

PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER



Altroconsumo 2021 – referred to BEUC Study

"Pfas" testing…..

Stoviglie monouso	CARATTERISTICHE: Compostabile	Biodegradabile	LE NOSTRE ANALISI: Pfas	Cloropropanoli	Alluminio e altri metalli	Ammine aromatiche	Pesticidi	Bisfenolo A	GIUDIZIO GLOBALE
PIATTI IN POLPA DI CELLULOSA									
DUNI, 10 bio plates	✓		★	★★★★★	★★★★★	n.d.	★★★★★	n.d.	★
ECOTABLE, piatti 100% fibra naturale 12 pezzi			★	★★★★★	★★★★★	n.d.	★★★★★	n.d.	★
ESSELUNGA, piatti piani in polpa di cellulosa 15 pezzi	✓	✓	★	★★★★★	★★★★★	n.d.	★★★★★	n.d.	★
NATURAL BIBO, 15 piatti biodegradabili compostabili	✓	✓	★	★★★★★	★★★★★	n.d.	★★★★★	n.d.	★
VALE LA NATURA, piatti piani eco 25 pezzi	✓	✓	★	★★★★★	★★★★★	n.d.	★★★★★	n.d.	★
CANNUCCE IN CARTA COLORATE									
TALKING TABLES, 30 paper straws		✓	★★★★★	★★★★★	★★★★★	★★★★★	n.d.	★★★★★	★★★★★
SAMURAI, 24 cannucce trendy, in carta			★★★	★★★★★	★★★	★★★★★	n.d.	★★★★★	★★★
ECO TABLEWARE, paper straws	✓	✓	★★★★★	★★★★★	★	★★★★★	n.d.	★★★★★	★
STRAWIX BIODEGRADABLE, 25 cannucce in carta		✓	★	★★★★★	★★★	★★★★★	n.d.	★★★★★	★
PIATTI IN FOGLIE DI PALMA									
ECOGREENWARE, Biodegradable & Natural plates	✓	✓	n.d.	n.d.	★★★★★	n.d.	★★★★★	★★★★★	★★★★★
TALKING TABLES, Tropical palm leaf plates 6 pezzi		✓	n.d.	n.d.	★★★★★	n.d.	★★★★★	★★★★★	★★★★★
BIOZOYG, Palmware 25 Pezzi	✓	✓	n.d.	n.d.	★★★★★	n.d.	★★★	★★★★★	★★★

★★★★★ ottimo; ★★★★ buono; ★★★ accettabile; ★★ mediocre; ★ pessimo

n.d. non disponibile. L'analisi non è stata eseguita.

Alerts on the packaging market

"Pfas" testing?

TOF testing!

The test investigated the presence of fluorinated compounds in paper straws and the moulded fibre products (41 total samples). The TOF analysis was performed through a modified version of European standard DIN EN ISO 10304-1 (D20). In the analysis the material is burned, and the organic fluorine converted to hydrogen fluoride which is then collected in a liquid that removes inorganic fluoride and thereafter analysed with ion chromatography.

Fluorinated compounds

Of the 41 products analysed for fluorinated compounds, 27 (66%) exceeded the Danish indicator value. The highest concentration of organic fluorine measured was 2,800 mg/kg – or 140 times above the indicator value. All the moulded fibre products – as well as some paper straws – exceeded the indicator value. These results thus suggests that fluorinated compounds were used, for example to make the products water- and/or fat repellent. Seven paper straws contained organic fluorine close to the reference value; indicating that fluorinated compounds were either used intentionally in printing inks or were present as a contaminant at high levels in e.g. recycled materials.

The present test did not explore the presence of specific PFAS compounds in the sampled SUP alternatives. Previous investigations have however identified specific PFAS compounds in paper and board food packaging through combined use of TOF analysis and targeted methods. In 2017, five consumer organisations detected[23] the presence of PFOA and its six-carbon cousin compounds perfluorohexanoate acid (PFHxA) and perfluorobutanoic acid (PFBA) in 31 paper samples; all exceeding the Danish TOF indicator value. Recent U.S. research[24] measured both short- and long-chain PFAS compounds in 36 out of 38 paper and other plant-based straws. Among the most frequently detected compounds in that study were also PFOA, PFBA, and PFHxA. Migration analysis showed that approximately two-thirds of the total extractable PFAS leached from the paper straws into cold water.

Targeted analysis is only able to detect those PFAS compounds for which analytical standards are available; thousands of PFAS are in commercial use, however, implying that targeted analyses are prone to 'overlook' many compounds present in the food packaging material. For example, a recent European wide investigation[25] by eight civil society organisations found that targeted analysis could assign less than 1% of the total organic fluorine present in sampled disposable food packaging and tableware to the investigated 55 PFAS compounds. TOF analyses in contrast can detect all PFAS, including unknown precursor compounds, and typically also operates with lower limits of detection. Researchers at the Danish Technological University have thus concluded that these methods are suitable for non-targeted detection of PFAS in paper and board material.[26]

I test che ha condotto per il Salvagente il professor Massimo Chiari, come abbiamo anticipato, ***non misura i Pfas specifici.*** *La tecnica che ha usato nel suo laboratorio dell'Infn si chiamata "Emissione di raggi gamma indotta da particelle" (Pige), e può determinare la quantità totale di fluoro in un certo prodotto.*

Attualmente, del resto, sono ***4.730*** *le molecole censite che fanno parte della famiglia dei per-fluoro-alchilici, di varia natura, a catena lunga e catena corta.* ***Sarebbe stato complicato*** *testare ogni sostanza per definire precisamente l'elemento utilizzato.*

PFAS in FCM: sample preparation and targeted&untargeted testing

*"Currently, **moreover, 4,730 molecules are surveyed that are part of the family of per-fluoro-alkyls, of various kinds, with long and short chains.** It would have been complicated to test each substance to precisely define the compound used"*

Sometimes, also with untargeted testing LC Q TOF, we do not detect PFAS content in paper samples having total fluorine>1000ppm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Environmental Science & Technology Letters

Letter

pubs.acs.org/journal/estlcu

Fluorinated Compounds in U.S. Fast Food Packaging

Laurel A. Schaider, Simona A. Balan, Arlene Blum, David Q. Andrews, Mark J. Strynar, Margaret E. Dickinson, David M. Lunderberg, Johnsie R. Lang, and Graham F. Peaslee

Silent Spring Institute, Newton, Massachusetts 02460, United States
California Department of Toxic Substances Control, Sacramento, California 95814, United States
Green Science Policy Institute, Berkeley, California 94709, United States
Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, United States
Environmental Working Group, Washington, D.C. 20009, United States
National Exposure Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States
Chemistry Department, Hope College, Holland, Michigan 49423, United States
Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee 37831, United States
Department of Physics, University of Notre Dame, Notre Dame, Indiana 46556, United States

Supporting Information

One explanation for these findings is that much of the total fluorine in these samples was present as inorganic fluorine or non-PFAS organofluorine compounds…..
Another explanation is that our extraction and LC/TOF MS analyses failed to capture a substantial portion of the PFASs present in the samples. For instance, FTOHs have been among the most frequently detected PFASs in FCMs,43 but they are volatile and form adducts with LC modifiers, making their measurement erratic as determined by LC/MS.

PFAS in FCM: sample preparation and targeted&untargeted testing

Plastics and elastomers


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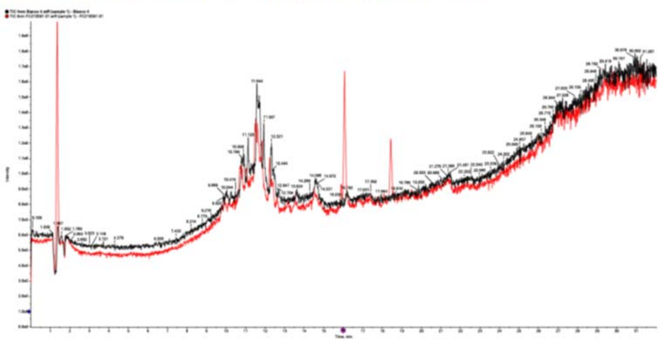
RAPPORTO DI PROVA N° FC210592.01	Date: 30/11/2021
Cliente/Customer	*Food Machines Producer*
Materiale / Material	Plastica / Plastic
Descrizione Campione / Sample Description	TEFLON DURO
Codice Articolo / Item Code	---
Campionamento / Sampling	A cura del cliente / by customer
Data di arrivo / Arrival Date	23/11/2021
Data inizio prove/Start test date	26/11/2021
Data fine prove/End test date	29/11/2021
PASS/FAIL	PASS

Modalità negativa

FIG. 1 Overlay TIC, Bianco Solvente EtOH 95 % (nero), FC210591.01 EtOH 95 % (rosso)



Nelle analisi di screening non e' stata riscontata la presenza di sostante perfluorurate

Before discussing analytical approach,
let's talk about **sample preparation**…

FOOD CONTACT CENTER

PD CEN/TS 15968:2010

BSI Standards Publication

Determination of extractable perfluorooctanesulphonate (PFOS) in coated and impregnated solid articles, liquids and fire fighting foams. Method for sampling, extraction and analysis by LCqMS or LC-tandem/MS

bsi.

Quoted from CEN/TS 15968:2010

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association and supports essential requirements of REACH (1907/2006), annex XVII, designation 53 [1]

Procedure

5 Principle

The analytes listed in Table 1, after extraction with methanol, are determined by liquid chromatography with tandem mass spectrometric detection (LC-tandemMS) or liquid chromatography mass spectrometric detection (LC-qMS). The list of analytes is not comprehensive for all possible PFOS derivates.

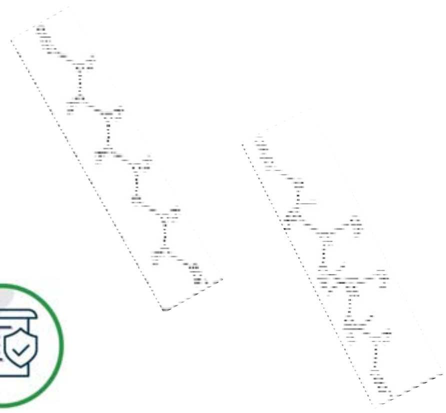
FOOD CONTACT CENTER

CEN/TS 15968:2010

Analysis instrument

- LC-tandem MS (triple quadrupole MS; ion-trap MS)
- LC-qMS (single quadrupole MS; clean-up is necessary)
- LC-TOF MS

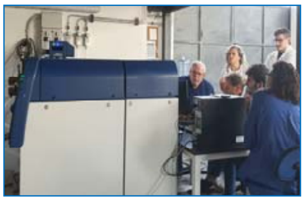
17



LC TOF MS
2010… unusual to find TOF in a Standard Method!

Anyway, if you have an LC – TOF MS, you also need a Library!

PFAS in FCM: instrumental library for untargeted testing






Università degli Studi Firenze

Ricerca Untarget di contaminanti in materiali destinati al contatto alimentare mediante tecniche LC-HRMS

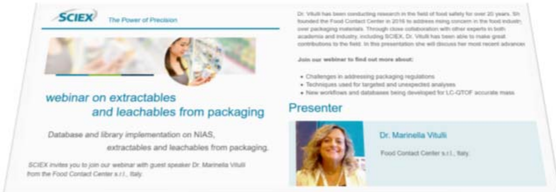
Relatore
Serena Orlandini

Correlatore
Marinella Vitulli

Candidato
Filippo Pratesi

Library and Project Successfully concluded in 2021

We continuously update the library…



PFAS in FCM: sample preparation and targeted&untargeted testing

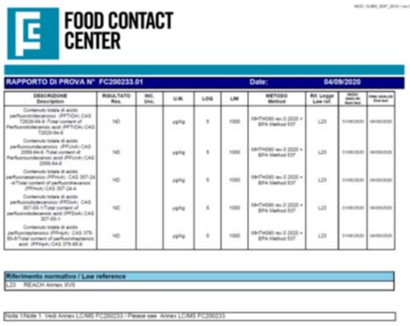
- textile / coated material
- plastic / non-coated material

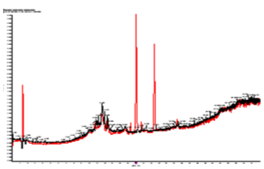
→ ultrasonic bath extraction (2 hrs, 60℃) → LC-MS-MS analysis

target

untarget

Extraction vs Migration





COMMISSION REGULATION (EU) 2021/1297

of 4 August 2021

amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council as regards perfluorocarboxylic acids containing 9 to 14 carbon atoms in the chain (C9-C14 PFCAs), their salts and C9-C14 PFCA-related substances

PM/Ref. N° (classe)	CAS RN	NAME	Molecular Formula	Massa esatta
PFCA	335-67-1	**Perfluorooctanoic acid**	$C_8HF_{15}O_2$	**414,068388**
PFCA	4234-23-5	Perfluoroundecanoic acid	$C_{11}HF_{21}O_2$	564,0909072
PFCA	141074-63-7	Perfluoropentadecanoic acid	$C_{15}HF_{29}O_2$	764,1209328
PFCA	57475-95-3	Perfluoroheptadecanoic acid	$C_{17}HF_{33}O_2$	864,1359456
PFCA (Perfluorinated Carboxylic Acid)	375-22-4	Heptafluorobutyric acid	$C_4HF_7O_2$	214,0383624
PFCA derivatives	2706-90-3	Pentanoic acid, nonafluoro- (TSCA, NDSL, ENCS, AICS)	C5HF9O2	264,0458688

PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER

2. Shall not, from 25 February 2023, be used in, or placed on the market in:
(a) another substance, as a constituent;
(b) a mixture;
(c) an article,
except if the concentration in the substance, the mixture, or the article is below 25 ppb for the sum of C9-C14 PFCAs and their salts or 260 ppb for the sum of C9-C14 PFCA-related substances.

Low Limits in a Mixture?????

Coating products or materials coated with PFAS

Substrates covered by FCM coatings

Coating	paper	Aluminium foil	Metal packaging	Plastic	Kitchen ware	Glass	Metal, plastic, concrete	Wood	Textiles
1			X						
2	X	X	X	X					
3	X			X					
4				X					
5					X				
6					X				
7	X								
8							X		
9		X							
10						X			
11			X						
12	X	X	X	X	X	X	X	X	X

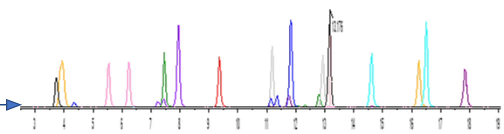
Source Slide: Peter Oldring

PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER

Dilution and purification

target

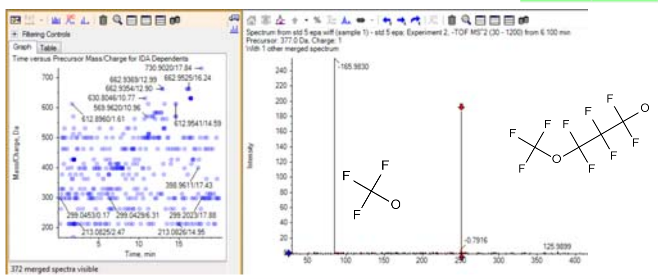


Analytical column Kinetex EVO 2.6 µm; 150 x 2.1 mm
EPA 537.1 Method
Eluent A: 20mM ammonium acetate
Eluent B: methanol

Name	Formula	Found At Mass (Da)
HFPO-DA GENX	C6HF11O3	284.97908
NEtFOSAA	C12H8F17NO4S	583.98363
NMeFOSAA	C11H6F17NO4S	569.96772
PFBS	C4HF9O3S	298.94323
PFDA	C10HF19O2	512.96004
PFDoA	C12HF23O2	612.95347
PFHpA	C7HF13O2	362.96978
PFHxS	C6HF13O3S	398.93688
PFHxA	C6HF11O2	312.97323
PFNA	C9HF17O2	462.96365
PFOS	C8HF17O3S	498.93055
PFOA	C8HF15O2	412.96645
PFTA	C14HF27O2	712.94678
PFTrDA	C13HF25O2	662.9506
PFUnA	C11HF21O2	562.95655
11Cl-PF3OUdS	C10HClF20O4S	630.88961
9Cl-PF3ONS	C8HClF16O4S	530.89608
ADONA	C7H2F12O4	376.96907

untargeted

Analytical column Kinetex F5 2.6 µm; 150 x 2.1 mm



Tof Experiment Parameters

CUR	35
GS1	50
GS2	55
ISVF	4500
TEM	400

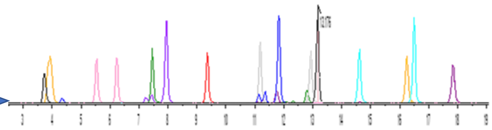
PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER

Waste water

Dilution

target



Analytical column Kinetex EVO 2.6 µm; 150 x 2.1 mm
EPA 537.1 Method
Eluent A: 20mM ammonium acetate
Eluent B: methanol

Name	Formula	Found At Mass (Da)
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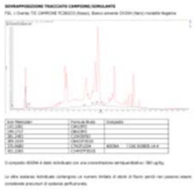
NEtFOSAA	C12H8F17NO4S	583.98363
NMeFOSAA	C11H6F17NO4S	569.96772
PFBS	C4HF9O3S	298.94323
PFDA	C10HF19O2	512.96004
PFDoA	C12HF23O2	612.95347
PFHpA	C7HF13O2	362.96978
PFHxS	C6HF13O3S	398.93688
PFHxA	C6HF11O2	312.97323
PFNA	C9HF17O2	462.96365
PFOS	C8HF17O3S	498.93055
PFOA	C8HF15O2	412.96645
PFTA	C14HF27O2	712.94678
PFTrDA	C13HF25O2	662.9506
PFUnA	C11HF21O2	562.95655
11Cl-PF3OUdS	C10HClF20O4S	630.88961
9Cl-PF3ONS	C8HClF16O4S	530.89608
ADONA	C7H2F12O4	376.96907

untargeted

Analytical column Kinetex F5 2.6 µm; 150 x 2.1 mm



LIMITS

Fascicolo Iter
DDL S. 2392

Misure urgenti per la riduzione dell'inquinamento da sostanze poli e perfluoroalchiliche (PFAS) e per il miglioramento della qualità delle acque destinate al consumo umano

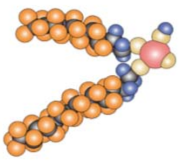
PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER

Packaging, paper, and cardboard

Figure 5.7 Single sided migration test of paper and board.

Figure 5.8 Double sided extraction of a food contact material: a) The sample is cut in strips b) the weight of the tube is recorded c) the weight of the paper is recorded.



DEPARTMENT OF BASIC SCIENCES AND ENVIRONMENT
FACULTY OF LIFE SCIENCES
UNIVERSITY OF COPENHAGEN

Polyfluorinated surfactants in food packaging of paper and board

By Xenia Trier

Supervisors:
Jan H. Christensen, Ass. Professor, KU-LIFE
Kit Granby, Senior Researcher, DTU-Food

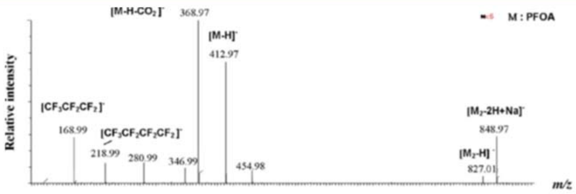
5. Migration of PFS from food paper and board

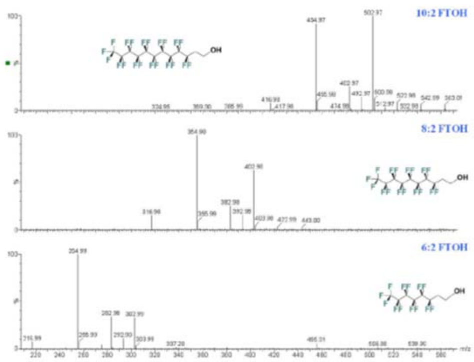
The use of PFS in paper and board packaging is of interest from a food safety point of view (Forrest 2007), because the consumer has a high risk of exposure, as mentioned in the Introduction. Briefly, these include that food paper and board packaging has a European market share of approximately 20% (Lange et al. 2006, van der Putte et al. 2010); that the packaging is single-use; due to the relatively high initial concentrations of PFS, and because several of the PFS are small molecules, which give them a higher potential be transferred (i.e. migrate) to the food.

To better understand the migration behaviour of PFS from paper and board into food and beverages, this chapter describes the paper and board material (5.1), why and how PFS are used in paper and board (5.2), the theories behind migration processes (5.3), European and US legislation (5.4), and choice of migration conditions for compliance and enforcement testing (5.5). Finally, the results of a survey made on PFS from 74 samples of paper and board sampled in 2009 from Danish, Swedish and Canadian fast food and retail stores are presented (**paper 4**).

PFAS in FCM: sample preparation and targeted&untargeted testing

FOOD CONTACT CENTER





Negative-ion electrospray ionisation (ESI–) coupled to mass spectrometry (MS) is a popular detection method for the smaller anionic poly- and perfluorinated surfactants (PFS), such as the PFCAs. So far, the FTOHs have mainly been analysed by gas chromatography (GC)-MS ,2, but GC is not suitable for the analysis of residual FTOHs in the fluoropolymers, both because thermal degradation of the polymers can form artefact FTOHs3 and because polymers often are not sufficiently volatile for GC.

Common name
FTOH x:2 fluorotelomer alcohols

Also GC-MS Studies on Paper&Board

MIGRATION OF FLUORINATED TELOMER ALCOHOLS (FTOH) FROM FOOD CONTACT MATERIALS INTO FOOD AT ELEVATED TEMPERATURES

Fengler R, Schlummer M, Gruber L, Fiedler D, Weise N

Fraunhofer Institute for Process Engineering and Packaging IVV, Dep. Products Safety and Analysis, Giggenhauser Str. 35, D-85354 Freising, Germany

Materials and methods

At first, fluorine-positive muffin papers were identified by using sliding spark spectroscopy and Headspace GC/EI-MS as described elsewhere[4]. Then, methanol extracts of the muffin papers were subjected to FTOH specific analysis based on GC/CI-MS[4,5] and quantified with isotope-labeled standards of 4:2-, 6:2-, 8:2- and 10:2-FTOHs.

PFAS in FCM: a case History

FOOD CONTACT CENTER

OFD#EDJDVVH#SXOS#DQG#SRO\SURS\OHQH PHDW#WUD\#

Goal of study: Compare two product system
Functional unit: 1 items of packaging
Products system:
- Polypropylene (PP) 100% virgin tray, with reference flow 23.10g
- Bagasse tray, with reference flow 44.45g

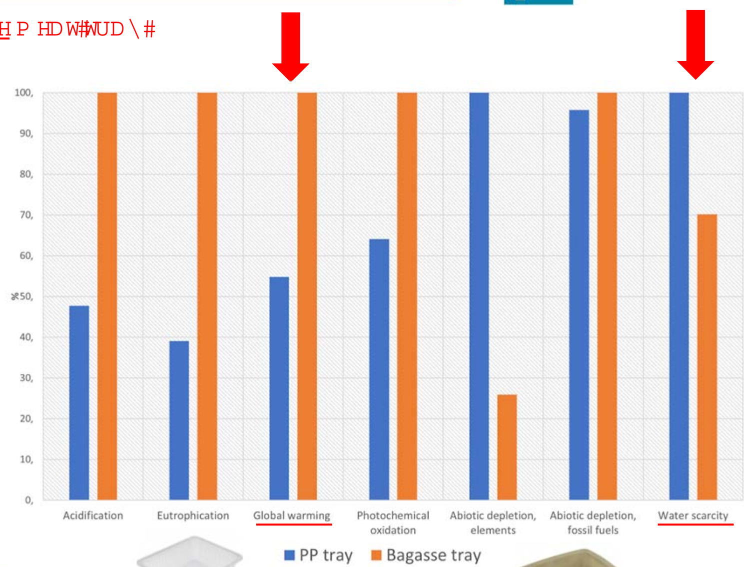
Boundary conditions: LCA cradle to gate
Geographical boundaries: Global
Database: Ecoinvent 3.7.1
Data quality: secondary data from database for each materials and processes
Software: SimaPro 9.2

Characterization factor: EPD® THE INTERNATIONAL EPD® SYSTEM

Reference document:

Single-use plastic tableware and its alternatives: Recommendations from Life Cycle Assessments

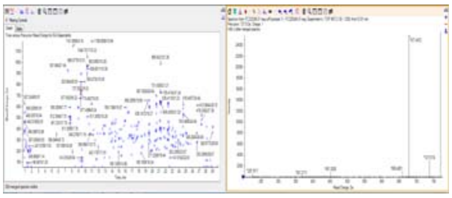
https://www.lifecycleinitiative.org/library/

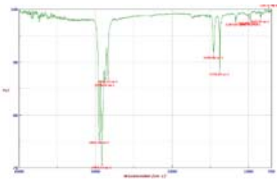


PFAS in FCM: a case History

WHVWLQJ#SRO\SURS\OHQH PHDW#WUD\#

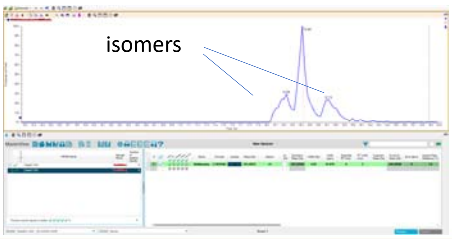
Comparison blank/simulant EtOH 10%, 10days 40°C



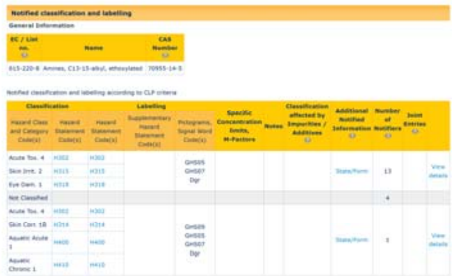


Risk Assessment:

Only traces of non cancerogenic substances



Amines, C13-15-alkyl, ethoxylated
CAS 70955-14-5



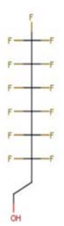
PFAS in FCM: a case History

WHVWLQJ#EDJDVVH#SXOS PHDW#WUD\#

DESCRIZIONE Description	RISULTATO Res.	INC. Unc.	U.M.	LOQ
Metanale (Formaldeide) in acido acetico 3%/Methanal(Formaldehyde) in acetic acid 3%mg/dm2)	0,04		mg/dm²	0,01
Migrazione specifica di 23 Elementi Pesanti in acido acetico 3% - Tecnica ICP/MS				
Migrazione specifica di alluminio in acido acetico al 3% / Specific migration of aluminium in acetic acid 3%	5,55	±1,67	mg/kg	0,01
Migrazione specifica di piombo in acido acetico 3% / Specific migration of lead in acetic acid 3%	0,01	±0,003	mg/kg	0,005

2. Screening HS GC/MS FC210462.03

C8H5F13O



Retention Time (min)	Identification	Match (%)	Semiquantitative Content mg/kg (ppm)	CAS
15.0	3-Phenylpentane (**int standard**)	95	0,1	538-68-1
10.6	**1H,1H,2H,2H-Perfluorooctan-1-ol**	86	1,7	647-42-7
11.1	2,4-Dimethylfuran	86	21,0	3710-43-8
11.6	2-Furanmethanol	96	2,0	98-00-0
14.1	Ethyl methacrylate	86	2,7	97-63-2

EN 13432 standard "Packaging - Requirements for packaging recoverable through **composting** and **biodegradation** - Test scheme and evaluation criteria for the final acceptance of packaging"

2 Heavy metals and other toxic and hazardous substances

.... concentration of any substance listed in Table A.1, present in packaging materials and whole packaging, shall not exceed the value given in that table.

Table A.1 - Maximum element content of packaging material and whole packaging

Element	mg/kg on dry substance	Element	mg/kg on dry substance
Zn	150	Cr	50
Cu	50	Mo	1
Ni	25,0	Se	0,75
Cd	0,5	As	5
Pb	50	F	100
Hg	0,5		

F limit 100 ppm

Vaschetta in cartoncino denominato : "FC210462.01"

Prova	Risultato	UdM
Fluoro *UNI EN15408:2011 (Fluorine)* *Prova effettuata presso Innovhub SSI Area Combustibili*	1062	mg/kg

F result >1000 ppm

Risk Assessment:

large amount of fluoro compounds!

PFAS in FCM: a case History

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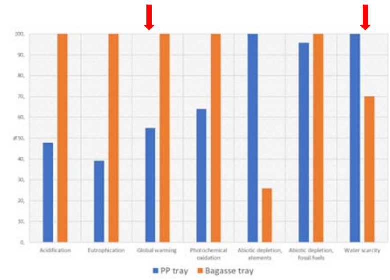
P hdw#sdfndjlqj#wud|#SR O\SUR S\OHQ H dqg#EDJDVVH#SXOS

The environmental impact categories closest to stakeholder interest are **Global Warming** (CO_2 contributions) and **Water Footprint**.
The **bagasse pulp** provides a **very high** contribution to **Global Warming** because its production involves an intense use of energy.
The use of **water**, on the other hand, **is lower** because bagasse is **a waste** from the processing of sugarcane*.

VIQHUJ\#ULVN#DVVHVVPHQW#) #GHFIVIRQ#PDNIQJ#

LCA results:



*the use of water is allocated to the sugarcane production and not to the bagasse waste

PFAS in FCM: a case History

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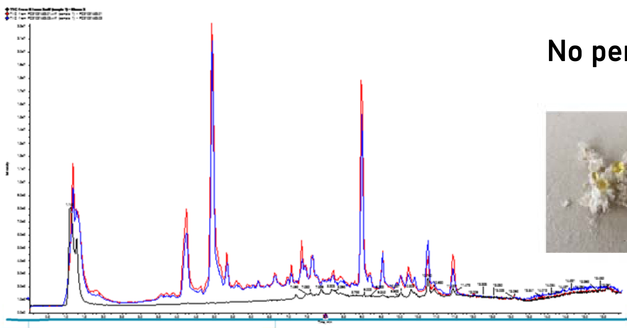
Vaschetta in cartoncino denominato : "FC210462.01"

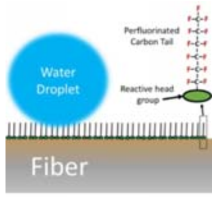
Prova	Risultato	UdM
Fluoro *UNI EN15408:2011 (Fluorine)* *Prova effettuata presso Innovhub SSI Area Combustibili*	1062	mg/kg

F result >1000 ppm

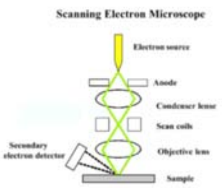
Screening LC-HRMS Perfluoro Compounds
FIG. 1 Overlay TIC, Bianco Solvente EtOH 95 % (nero), FC210462.01 EtOH 95 % (rosso), FC210462.01 EtOH 95 % (rosso)

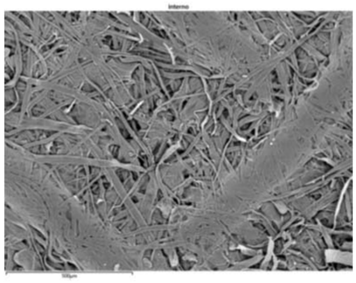


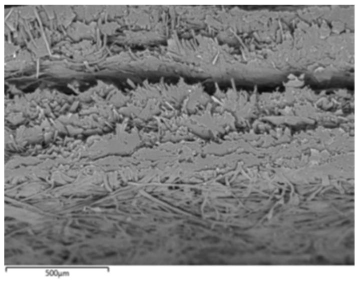
No perfluorocompounds in the LC Q TOF screening!



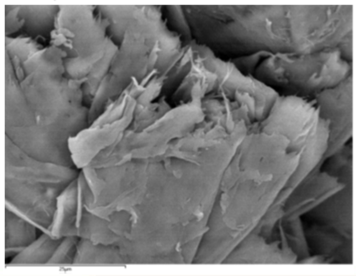
PFAS in FCM: a case History







Etichetta spettro	C	O	Mg	Si	Ca	Totale
1	52.75	46.65	0.23	0.25	0.13	100.00

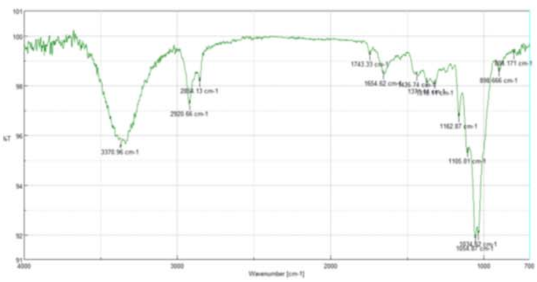


Carbon and Oxygen ratio tipycal of Cellulose

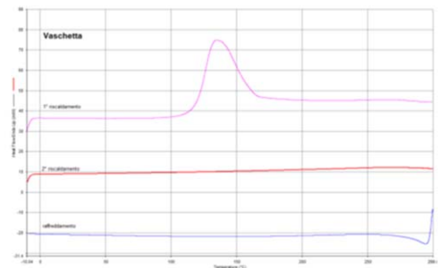
Internal SEM: structure similar to Viscose…

PFAS in FCM: a case History

Figura 4: Spettro FT/IR-ATR del campione di vaschetta
Lo spettro è attribuibile a quello della sola cellulosa, non si osservano bande di assorbimento attribuibili ad altre componenti organiche/inorganiche.



Una frazione del campione di vaschetta è stata sottoposta ad **analisi DSC** impiegando il seguente programma termico:
- I° riscaldamento da -10 °C a 300°C, 20 °C/min in N2
- Raffreddamento da 300 °C a -10 °C, -20 °C/min in N2
- II° riscaldamento da -10 °C a 300 °C, 20 °C/min in N2



FT IR and DSC typical of Cellulose

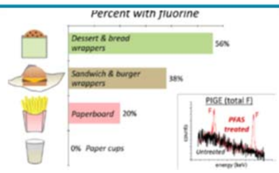
In Figura è riportato il termogramma DSC ottenuto.
Figura Termogramma DSC per il campione di vaschetta
L'analisi DSC mostra, per il campione di vaschetta, solo una fusione nel primo riscaldamento dovuto alla cellulosa (linea rosa in Figura 3), non sono evidenti altre transizioni termiche nel secondo riscaldamento (linea rossa in Figura 3).

PFAS in FCM: a case History

FOOD CONTACT CENTER

Fluorinated Compounds in U.S. Fast Food Packaging

Laurel A. Schaider, Simona A. Balan, Arlene Blum, David Q. Andrews, Mark J. Strynar, Margaret E. Dickinson, David M. Lunderberg, Johnsie R. Lang, and Graham F. Peaslee



However, some samples with high levels of total fluorine according to PIGE did not contain elevated levels of PFASs according to LC/MS analyses. Four samples with a total F concentration of >200 nmol/cm2 contained peak areas for known and/or unknown PFASs that were at or below levels detected in six samples with a total F concentration below the LOD. ***One explanation for these findings is that much of the total fluorine in these samples was present as inorganic fluorine or non-PFAS organofluorine*** *compounds. Another explanation is that* ***our extraction*** *and LC/TOF MS analyses failed to capture a substantial portion of the PFASs present in the samples. .....*

→ **T**otal **O**xidizable **P**recursors **A**ssay

PFAS in FCM: a case History

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Many Literature Papers....

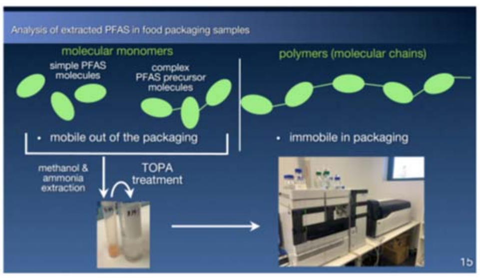
When the samples with high total fluorine were tested for 28 specific PFAS in Phase 2, these 28 PFAS did not appear in most cases. This indicates that other members of the PFAS family are responsible for the Phase 1 results. A TOPA analysis confirmed the likely presence of unknown PFAS 'precursors' and other 'polymeric' PFAS. While the identity of these unknown PFAS cannot be easily determined, unidentified PFAS should be treated in the same way as known PFAS and steps taken to transition them out of packaging.

Environmental Sciences Europe

COMMENT — Open Access

Digging deep—implementation, standardisation and interpretation of a total oxidisable precursor (TOP) assay within the regulatory context of per- and polyfluoroalkyl substances (PFASs) in soil

Bernd Göckener, Frank Thomas Lange, Lukas Lesmeister, Emine Gökçe, Hans Ulrich Dahme, Nicole Bandow and Annegret Biegel-Engler



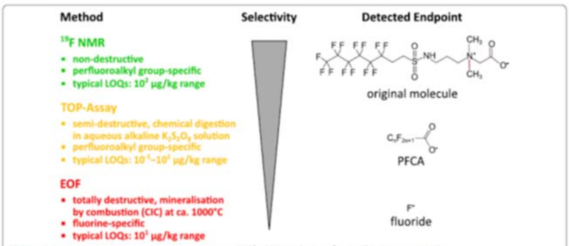


Fig. 1 Scheme representing current sum parameter approaches for PFASs and organofluorine determination in soils

PFAS in FCM: a case History

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•Screening in LC/QTOF dopo dopo idrolisi basica, e screening LC/QTOF dopo trattamento con Permanganato di Potassio.

Per "attaccare" le catene di composti fluorurati in maniera mirata, dopo confronto con specialisti di materiali e analitica, sono state programmate analisi previo pretrattamento.
L'analisi TOP (Total Oxidizable Precursors) si basa sull' ossidazione della matrice in analisi.
Nel caso in cui all' interno della matrice siano presenti dei precursori di composti perfluorurati, il trattamento con un agente ossidante porta alla loro conversione in acidi perfluorocarbossilici o perfluorosolfonici, i quali possono essere determinati successivamente mediante analisi mirate.
L' analisi TOP consente la determinazione semi-quantitativa degli analisi ricercati poichè risulta difficile ottenere un'ossidazione riproducibile di tali composti a causa della possibile presenza nel campione di altre sostanze che possono prevenire l'ossidazione dei composti bersaglio, influenzando così l'identificazione dei precursori e l'accurata quantificazione dei PFAS nascosti.

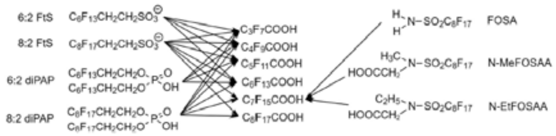
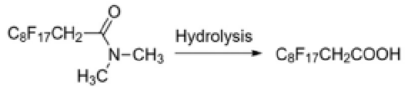


Fig1. Formazione dei prodotti di ossidazione a partire da diversi precursori (Houtz e Sedlak, 2012).

L'analisi THP "Total Hydrolyzable Precursors" invece prevede il trattamento del campione con metanolo e idrossido di sodio con lo scopo di idrolizzare i precursori dei PFAS. Lo studio dei database disponibili mostra che gran parte dei composti organofluorurati brevettati sono dei derivati di semplici PFAS con l'aggiunta un legame con l'atomo ossigeno, come ad esempio i polimeri con catene laterali di fluorotelomeri.
È noto che i legami esterei che si vengono a formare sono suscettibili all'idrolisi la quale porta alla liberazione dei dei rispettivi acidi perfluorocarbossilici o perfluorosolfonici che possono a questo punto essere analizzati mediante analisi target.
In conclusione l'analisi TOP e l'analisi THP possono costituire dei metodo alternativi e complementari per valutare i PFAS nascosti.



PFAS in FCM: a case History

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Total Oxidizable Precursors (TOP): Estrazione in ambiente acquoso e trattamento con KMnO4
Modalità negativa

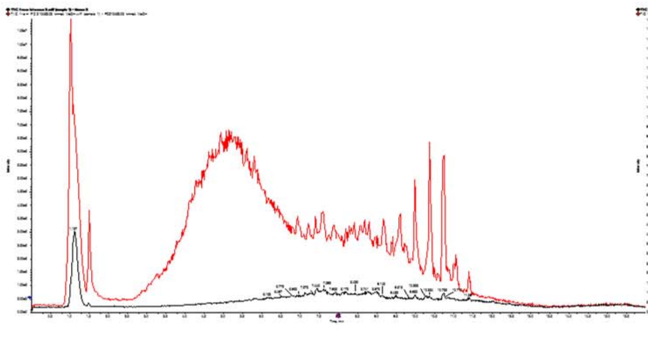


FIG. 3 Overlay TIC, Bianco Processo (nero), FC210462.03 (rosso).
E' stata riscontrata la presenza di Perfluoroheptanoic acid cas 375-85-9 in concentrazione semiquantitativa di 3.2 ug/Kg, Perfluorohexanoic acid cas 307-24-4 in concentrazione pari a 82.0 ug/Kg.
E' stata riscontrata la presenza di 6:2 Fluorotelomer unsaturated carboxylic acid in concentrazione semiquantitativa di **70.6 ug/Kg.**

Total Hydrolyzable Precursors (THP) : Estrazione e idrolisi in ambiente basico con metanolo.
Modalità negativa

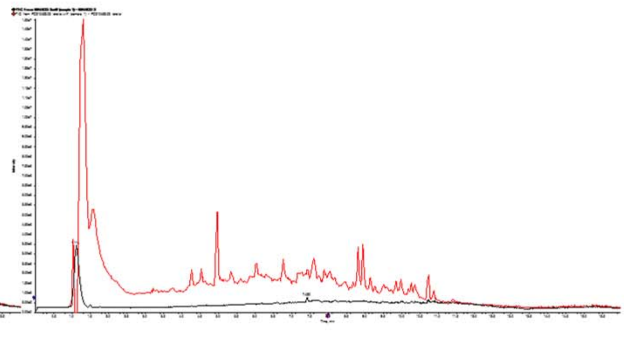
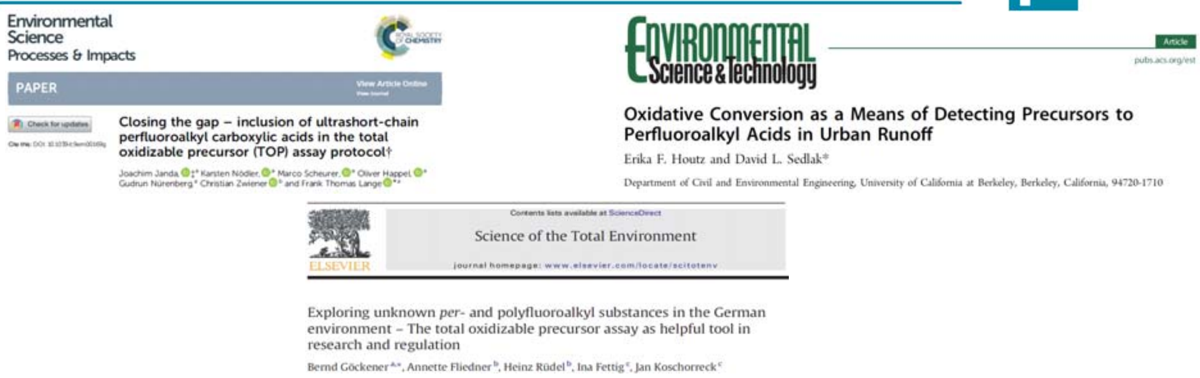


FIG. 4 Overlay TIC, Bianco Processo (nero), FC210462.03 (rosso).
Non e' stata riscontrata la presenza di sostanze perfluorurate generate con il trattamento di idrolisi
.
E' evidente che le estrazioni forzate in ambiente acquoso e in ambiente basico con metanolo non hanno fornito dati utili, mentre il trattamento con permanganato di potassio ha evidenziato presenza di composti perfluorati. Le quantità ad ogni modo non sono elevate, e non sono comparabili al valore di fluoro totale evidenziato.

PFAS in FCM: a case History


Environmental Science Processes & Impacts

PAPER

Closing the gap – inclusion of ultrashort-chain perfluoroalkyl carboxylic acids in the total oxidizable precursor (TOP) assay protocol†

Joachim Janda, Karsten Nödler, Marco Scheurer, Oliver Happel, Gudrun Nürenberg, Christian Zwiener and Frank Thomas Lange

Environmental Science & Technology

Article

pubs.acs.org/est

Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff

Erika F. Houtz and David L. Sedlak*

Department of Civil and Environmental Engineering, University of California at Berkeley, Berkeley, California, 94720-1710

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

ELSEVIER

Exploring unknown *per*- and polyfluoroalkyl substances in the German environment – The total oxidizable precursor assay as helpful tool in research and regulation

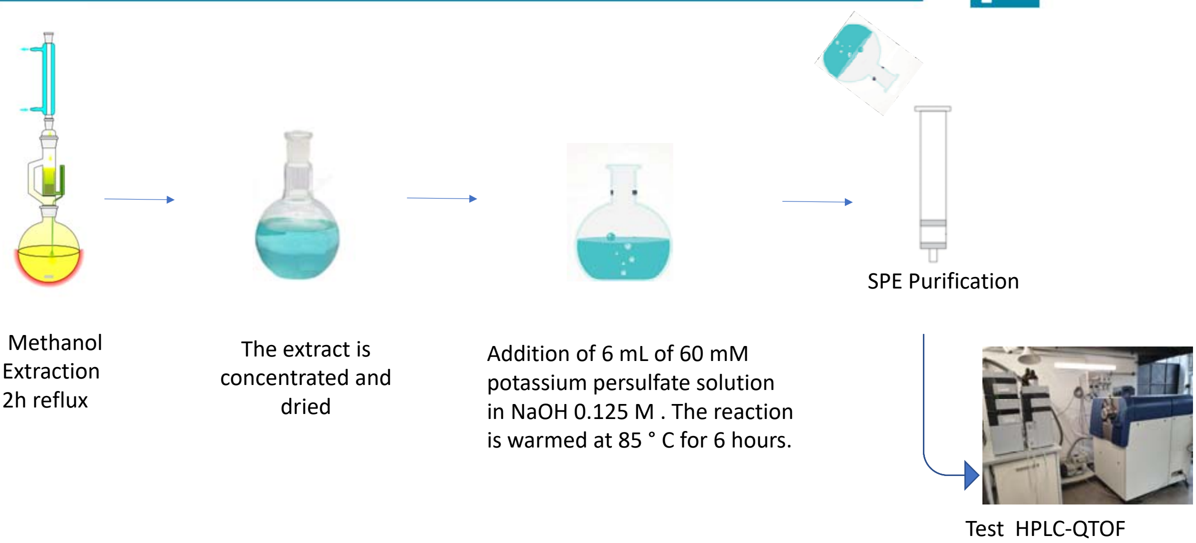
Bernd Göckener[a,*], Annette Fliedner[b], Heinz Rüdel[b], Ina Fettig[c], Jan Koschorreck[c]

Hydroxyl radical was produced by thermolysis of **persulfate (S_2O_8)2**- under basic pH conditions.
At pH values above 12, thermolysis rapidly converts persulfate into sulfate radical ($SO_4^{\cdot-}$), which is then quickly converted into ·OH

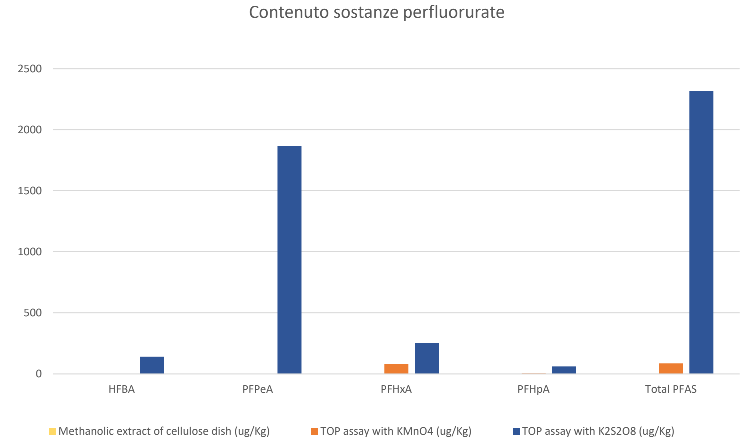
$$S_2O_8^{2-} + \text{heat} \rightarrow 2SO_4^{\cdot-}$$

$$SO_4^{\cdot-} + OH^- \rightarrow SO_4^{2-} + {}^{\cdot}OH$$

PFAS in FCM: a case History



	Methanolic extract of cellulose dish (ug/Kg)	TOP assay with KMnO4 (ug/Kg)	TOP assay with K2S2O8 (ug/Kg)
HFBA	ND	ND	140
PFPeA	ND	ND	1864
PFHxA	ND	82	252
PFHpA	ND	3	60
Total PFAS	ND	85	2316



Methanolic extract of cellulose dish (ug/Kg) TOP assay with KMnO4 (ug/Kg) TOP assay with K2S2O8 (ug/Kg)

Risk Assessment on food contact materials

"I would like to be honest here... I have tested my products in different labs, and for same services I obtained very different results....."

Customers

Can we manage this situation?
Can we do something to harmonize testing, defining standard protocols, supported by statistical analysis?

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EFFECTIVE PROFICIENCY TEAM

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OUR MANIFESTO

Sometimes in analytics and in testing we face problems that seem to be difficult to solve.

We strongly believe that the best way to approach such cases is **statistical problem solving**.

TWO MAIN REASONS:

- «robust» answers to complex problems
- lower costs and less time to get solutions

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ABOUT US:

- We are a **NET of professionals working in different organizations** like Universities, Accredited Labs, National Accreditation Bodies, Public and Private Companies.
- We started years ago **sharing technical and analytical problems, knowledge and solutions** - by the years we created an **«informal NET».**
- The majority of **our works are reserved** and cannot be published, due to the confidentiality agreements, but still **we can share a great number of «lessons learned».**
- In 2017 we decided to make the informal net **visible through a web site** and open it to contribution from other colleagues.

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TOOLS:

- We work **by projects** and every project has its own **team of professional** selected for the specific topic.
- The setting of the project always refers, when possible, to legislation and applicable standards (e.g. for interlaboratory studies we refer to ISO 17043 and ISO 13528).
- When samples are requested, as for interlaboratory trial, we provide **real samples** coming from industrial productions.
- **Anonymity of participants** in interlaboratory trials is covered by a **software specifically designed**.

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CASE HISTORY

CUSTOMER: Public Authority
SAMPLES: mineral oils in soils
PROBLEM: the Official Standard Method was unfitted, in terms of measurement uncertainty, to define the conformity of soils to limits reported in legislation
SOLUTION: enhancement of the analytical method followed by the validation of the method with an interlaboratory trial (compulsory to maintain accreditation on the specific test)
COMMUNICATION OF RESULTS: reserved to the Public Authority personnel only

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CASE HISTORY

CUSTOMER: Private Company (food manufacturer)
SAMPLES: residual solvent in flexible packaging
PROBLEM: investigate the differences between declaration of the 14 suppliers of flexible packaging and the measurements performed, on the incoming goods, by the quality control laboratory of the Company
SOLUTION: interlaboratory trial with a set of samples manufactured on purpose for the trial with a specific level of residual solvent
COMMUNICATION OF RESULTS: the final report was discussed in a meeting between the suppliers and the QC laboratory of the Company

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INTERNATIONAL STANDARD ISO 4531
Second edition 2022-04

Vitreous and porcelain enamels — Release from enamelled articles in contact with food — Methods of test and limits

Émaux vitrifiés — Libération depuis les articles émaillés en contact avec les aliments — Méthode d'essai et limites

COLLABORATIVE TRIALS – METALS

PROBLEM: investigate the performances of the analytical method, such as uncertainty value, developed as Annex in an ISO Standard Draft – quantification of metals in food contact simulants PROTOCOL: multi-method with a multi-level analysis of repeatability
SAMPLES: baking / grilling ware and appliances for food contact – samples produced on purpose for the scope of this trial
COMMUNICATION OF RESULTS: a complete anonymous report, in conformity with ISO 17043 and ISO 13528, will be given to all participants – single method results will be processed with a correlation analysis if possible (it depends on the number of participants)
PARTICIPANT FEE: none (free of charge)

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WHAT NEXT?

PFAS?.... are you interested?

e-mail info@proficiencyproblemsolving.com

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Food & Non Food Materials and Packaging

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Italian Mass Spectrometry
Society

IMaSS Emerging Contaminants Day

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022
Aula Magna U.E.1 - Polo Didattico Navile
Via della Beverara 123/1

Andrea PERISSI

Sensitive PFAS analysis from Edible Produce on Xevo TQ-XS after QuEChERS extraction

Sensitive PFAS analysis from Edible Produce on Xevo TQ-XS after QuEChERS extraction

Andrea Perissi, MS Sales Specialist – Waters

Environmental release and contamination of per- and polyfluorinated alkyl substances (PFAS) has resulted in contamination of a variety of food sources. Different type of commodities needs different approach.

Cultivating produce using PFAS contaminated water and soils can lead to the uptake of these compounds into the edible fruits and vegetables portions of plants. Thus, it is beneficial to have a straightforward method to monitor the occurrence of PFAS in produce. For this work, the FDA C-010.01 method based on the QuEChERS extraction method was implemented for extraction of PFAS using DisQuE dispersive solid phase extraction (dSPE) products followed by highly sensitive LC-MS/MS analysis on ACQUITY UPLC I-Class PLUS coupled to Xevo TQ-XS.

Complex food commodities such as fish, meat, edible offal, and eggs require a comprehensive sample extraction and clean up. To accommodate these types of samples, an alkaline digestion and extraction was implemented followed by Weak Anion Exchange (WAX) SPE to produce a suitable sample for analysis. High sensitivity LC-MS/MS analysis was performed on an ACQUITY UPLC I–Class PLUS coupled to Xevo TQ-XS. The method was evaluated in six different commodity types including salmon, tilapia, ground beef, beef liver, beef kidney, and chicken eggs.

This approach proved to be accurate, sensitive, and robust for a range of 30 PFAS compounds of varying chemistry classes to match the challenging concentrations published in reports by the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA).

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Sensitive PFAS analysis from Edible Produce on Xevo TQ-XS after QuEChERS extraction

Andrea Perissi
MS Sales Specialist Waters Italy

Agenda

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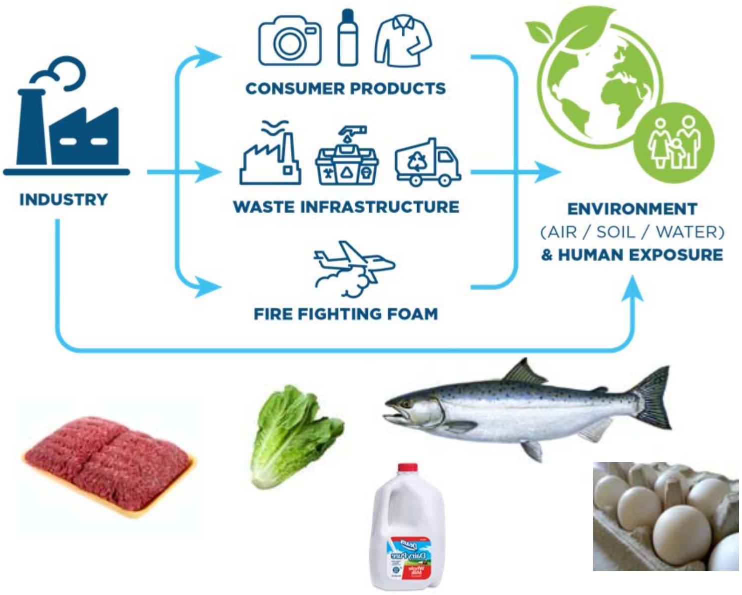
- Why are we looking for PFAS in different food matrixes?
- In which direction are the regulatory bodies moving?
- Which Instrument configuration Waters suggest to reach the analytical target?
- Do we need different approach for different commodities?

Per- and Polyfluoroalkyl Substances (PFAS)



- PFAS can enter in the environment throughout many routes
- Contaminated water,soil and biosolids are often used in food production
- PFAS uptake and bioaccumulation in food supply is a risk

Regulatory Scenario

- To date, most of the analytical efforts have been focused on PFAS throughout the environmental matrixes,. However the estimation of PFAS exposure for food sources is rapidly increasing in food safety evaluation
- Regulatory measures are in progress within Europe to protect/ limit consumer exposure to PFAS
- Certain food commodities are more susceptible of PFAS contamination (milk, eggs, fish)
- Maximum Levels (MLs) for certain PFAS in food are being estabilished using ALARA (As Low As Reasonably Achievable) approach

FDA Method C-010-01

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Determination of 16 Perluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Version 2019 (2019)

Author: Susan Genualdi and Lowri deJager

CFSAN/ORS reviewers: Tim Begley, Gregory Noonan

- Single laboratory validated in lettuce, milk, bread, fish
- QuEChERS based method with dSPE and/or SPE clean up
- 16 PFAS included:
  - C4 – C10 carboxylates
  - C4 – C8 sulfonates
  - GenX, ADONA, 9Cl-PF3ONS (F53B major), 11ClPF3OUdS (F53B minor)

FDA Method C-010-01

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Table 3. Sample preparation conditions based on food commodity type

Commodity	Amount of sample used:	mL of water added:	mL of CH_3CN added:	Concentrate with nitrogen?
Fruits/vegetables	5.0 g	5	10	no
Breads	5.0 g	15	10	no
Milk	5.0 mL	5	10	yes -take 5 mL of extract to 0.5 mL
Cheese	1.0 g	5	10	no
Other Dairy	5.0 g	5	10	no
Meat	5.0 g	5	10	no

EURL analytical guidlines for food (may 2022: https://eurl-pops.eu/)

- May 2022 European Reference Laboratory (EURL) for halogenated POPs in feed and food (Freiburg Germany) issued recomandation for the routine monitoring PFAS in food chain. https://eurl-pops.eu/

Matrix	PFOS	PFOA	PFNA	PFHxS
Eggs,Crustaceans & Molluscs	≤ 0.30	≤ 0.30	≤ 0.30	≤ 0.30
Fish Meat & Meat of Terrestrial Animals	≤ 0.10	≤ 0.10	≤ 0.10	≤ 0.10
Edible Offal of Terrestrial Animals	≤ 0.50	≤ 0.50	≤ 0.50	≤ 0.50
Fish Oil	≤ 0.50	≤ 0.50	≤ 0.50	≤ 0.50
Fruits	≤ 0.010	≤ 0.010	≤ 0.005	≤ 0.015
Vegetables	≤ 0.010	≤ 0.010	≤ 0.005	≤ 0.015
WildFungi	≤ 1.5	≤ 0.010	≤ 0.005	≤ 0.015
Food for Infants & Young Children (as ready to eat)	≤ 0.010	≤ 0.010	≤ 0.005	≤ 0.015
Milk	≤ 0.020	≤ 0.010	≤ 0.05	≤ 0.060

Required limits of quantification (LOQ) in µg.kg-1 for the four individual PFAS

PFAS solution for food analysis – Tested configuration

Xevo TQ-XS

Acquity I Class PLUS

PFAS Analysis Kit

Waters‘ Total Application Solution for PFAS

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New Technologies introduced in 2002

- Able to detect PFAS in every matrix further reducing Limit of Quantification

LC modifications for PFAS analysis

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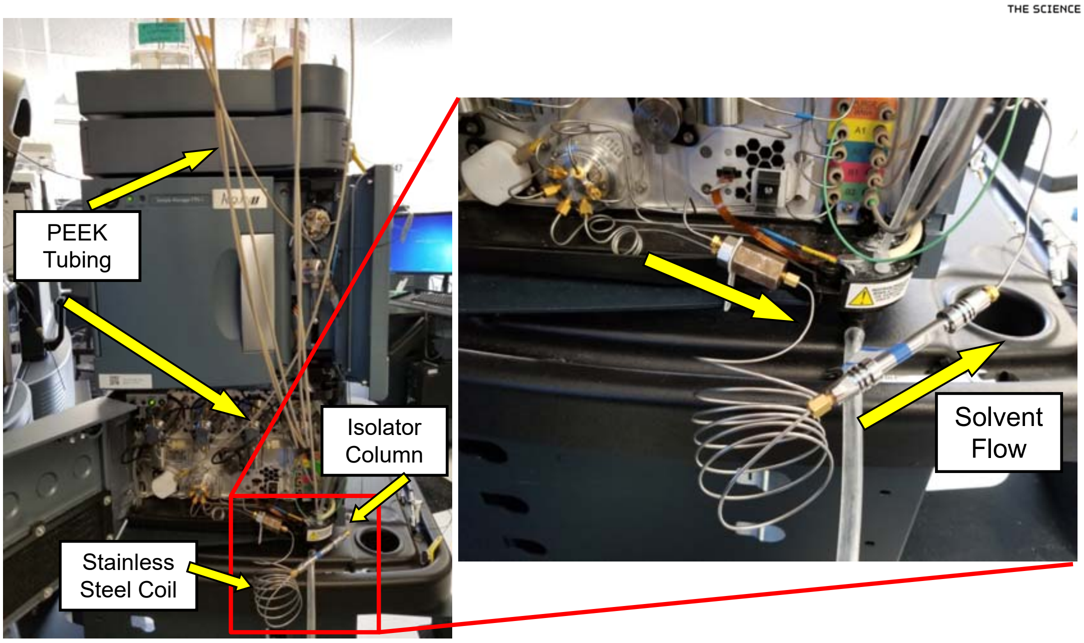
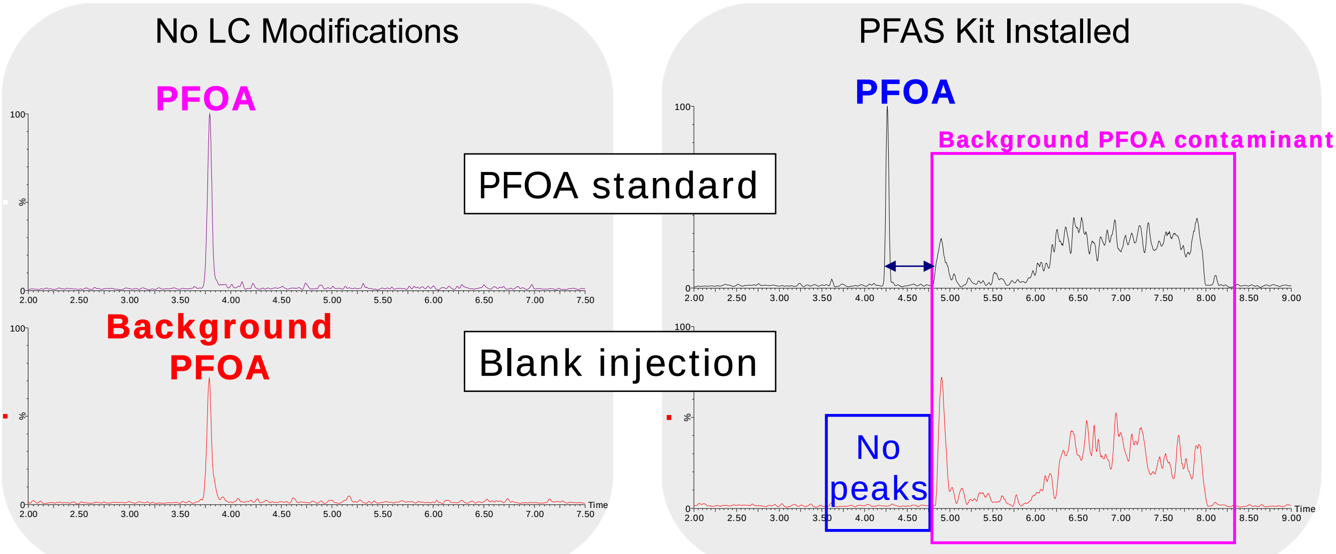


Benefits of LC modifications for PFAS analysis



Instrument Methods

Source Parameters

- Instrument: Xevo TQ-XS
- Ion Mode: ESI-
- Capillary Voltage: 0.5 kV
- Desolvation Temperature: 350°C
- Desolvation Flow: 900 L/hr
- Cone Flow: 150 L/hr

LC Method

- Instrument: Acquity I Class PLUS with **PFAS Kit**
- Column: ACQUITY BEH C18 2.1mm x 100 mm, 1.7 µm
- Mobile Phase A: Water + 2 mM ammonium acetate
- Mobile Phase B: Methanol + 2 mM ammonium acetate
- Injection Volume: 10 uL
- Gradient:

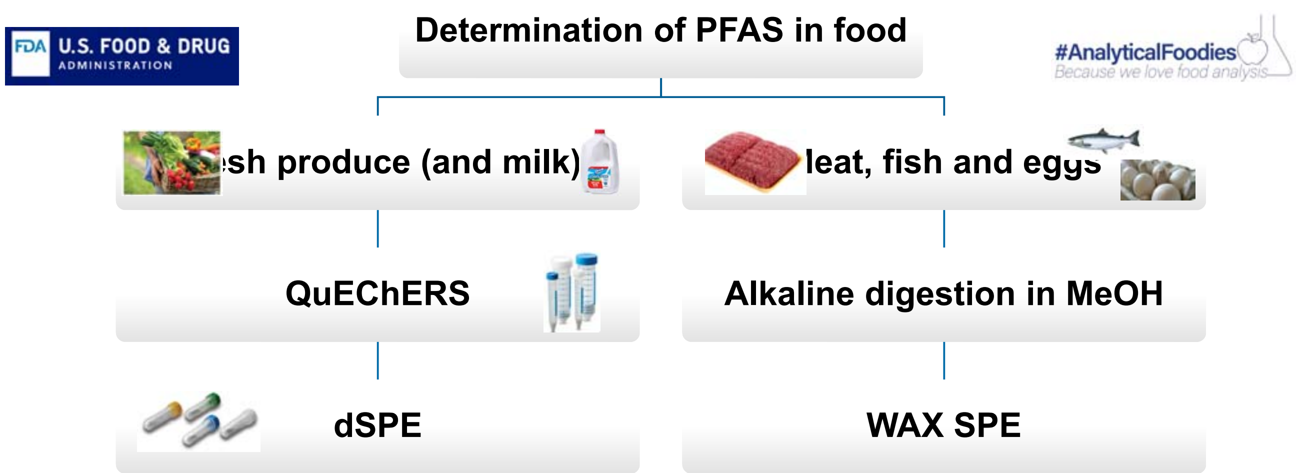
Time (min)	Flow (mL/min)	%A	%B
0	0.3	95	5
1	0.3	75	25
6	0.3	50	50
13	0.3	15	85
14	0.3	5	95
17	0.3	5	95
18	0.3	95	5
22	0.3	95	5

Method Development Strategy

QuEChERS, dSPE or OASIS WAX

- Starting from consolidated instrumenal method from PFAS analysis in environmental samples, we worked on sample prep for the different food commodities
- For vegetables we started from FDA method usinig Quechers extraction
- For fatty matrixes suggested by EURL as more critical for PFAS contaminaion we started with SPE approach already developed using OASIS WAX

Sample Preparation of Different Food Samples



Poll Question

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Produce	Commodity Class
Romaine Lettuce	High Water
Russet Potato	High Starch
Whole Carrot	Low Water
Strawberry	High Sugar
Cranberry	High acid and sugar

0.1 ng/g in potato matrix

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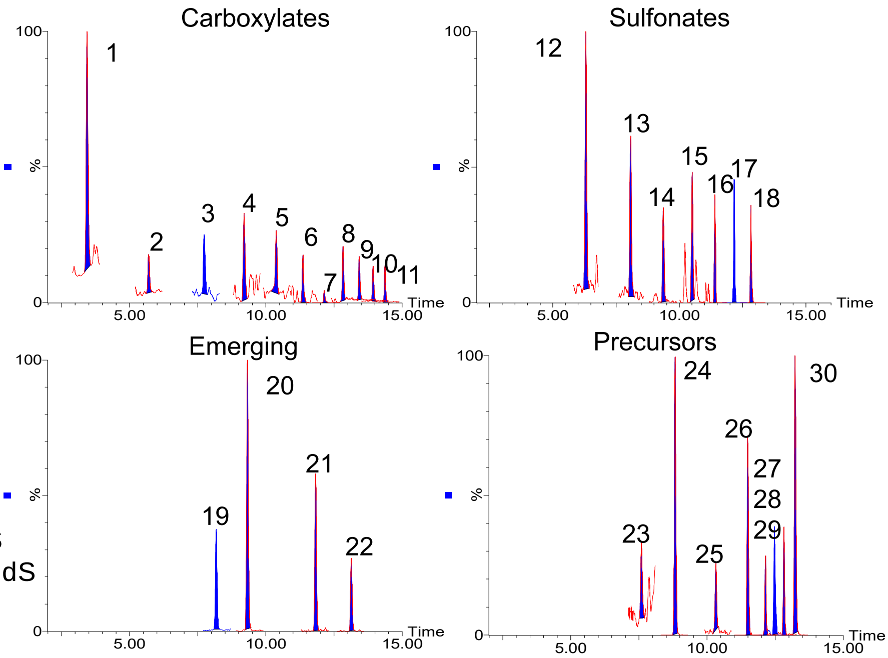
(1)PFBA
(2)PFPeA
(3)PFHxA
(4)PFHpA
(5)PFOA
(6)PFNA
(7)PFDA
(8)PFUnDA
(9)PFDoDA
(10)PFTriDA
(11)PFTreDA

(19)HFPO-DA
(20)ADONA
(21)9Cl-PF3ONS
(22)11Cl-PF3OUdS



(12)PFBS
(13)PFPeS
(14)PFHxS
(15)PFHpS
(16)PFOS
(17)PFNS
(18)PFDS

(23)4:2 FTS
(24)FBSA
(25)6:2 FTS
(26)FHxSA
(27)8:2 FTS
(28)NMeFOSAA
(29)NEtFOSAA
(30)FOSA

Isomers - 0.05 ng/g in matrix

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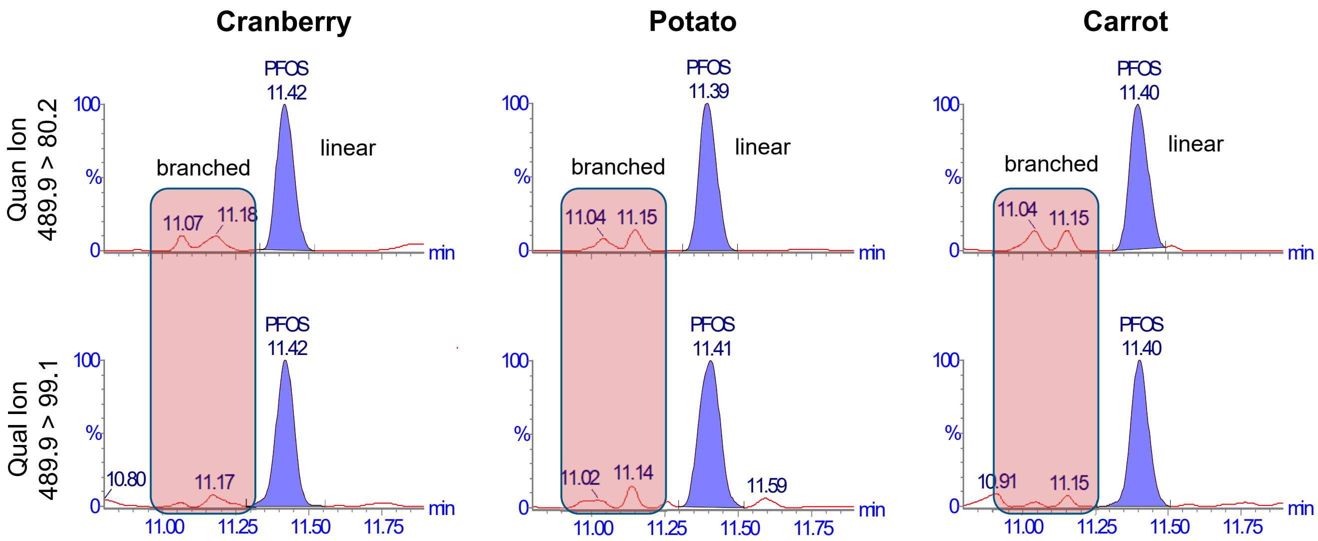

S/N for PFOS @ 0,05 ng/g give us enough margin to reach the latest EURL recommendation

Recovery and calculated concentration in each matrix

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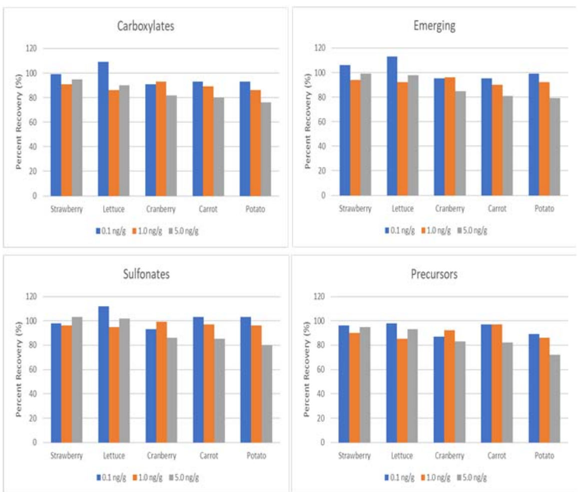

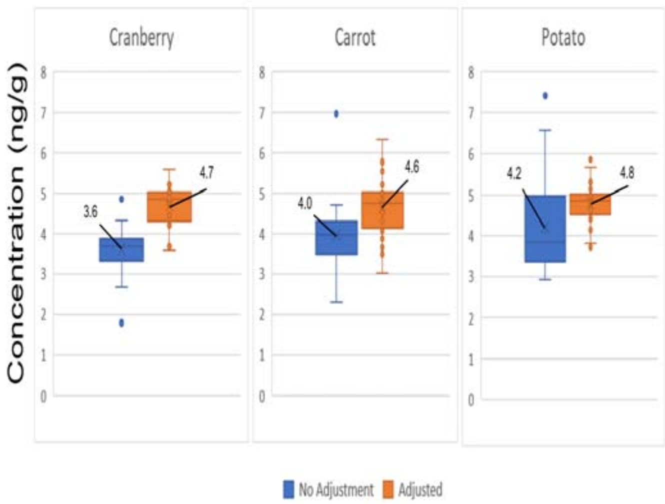

Increasing sample complexity

- Total Workflow for the Sensitive Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish, Meat, Edible Offal and Eggs
- Alkaline digestion followed by WAX SPE was implemented for extraction of complex food commodities such as fish, meat, edible offal, and eggs followed by analysis using Xevo TQ-XS. This approach proved to be accurate, sensitive, and robust for a range of 30 PFAS compounds of varying chemistry classes.
- One extraction method for multiple types of challenging matrices.
- Sensitive analysis on TQ-XS to reach detected levels in real samples compiled and published by EFSA and FDA.
- Use of the PFAS Analysis Kit to minimize system contamination.

Sample Preparation Food of Animal Origin

SPE sample enrichment with Xevo TQ-S micro

Application Note

Total Workflow for the Sensitive Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish, Meat, Edible Offal, and Eggs

Kari L. Organtini, Stuart Adams, Simon Hird, Renata Jandova

Waters Corporation

Oasis WAX cartridge
6 cc 150 mg (186009344)

Prepare samples (2 g)
Alkaline digestion in MeOH and dilution in water
pH adjust to < 6 → activates ion exchange sites in WAX sorbent when loaded

Condition Cartridge

Load Sample

Rinse Cartridge

Elute analytes
Eluent with pH > 8 → "turns off" ion exchange sites in WAX sorbent to release PFAS
Dry and dilute

Chromatogram of Salmon Extract Spiked at 0.1 ng/g

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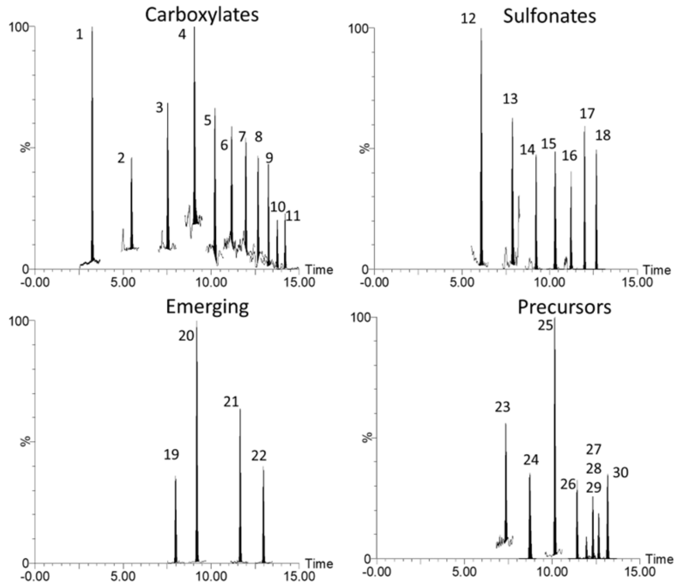

- Chromatogram shows the 30 PFAS evaluated in the method spiked into salmon extract at 0.1 ng/g (equivalent to 0.02 ng/mL injected on instrument)
- Peak assignments are located in the Appendix of the application note

Even though the extraction method results in 5x dilution of sample concentration, the sensitivity of the TQ-XS allows for trace level detection of PFAS in matrix samples

Confidence in Results Shown with NIST SRM 1947

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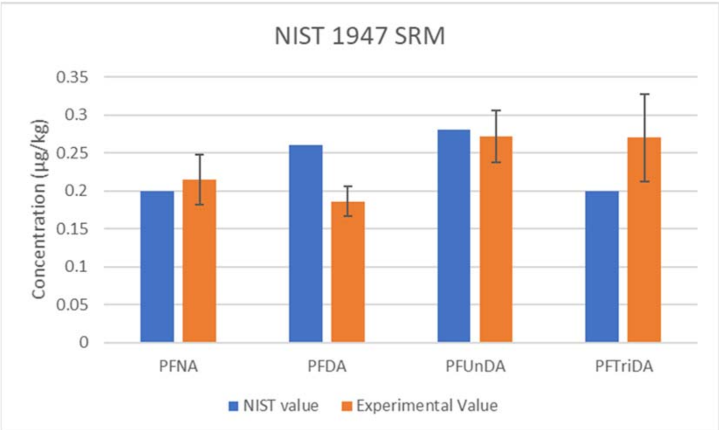

NIST SRM 1947 reports low level concentrations of 4 PFAS in fish tissue. The method presented in the application note produces very similar results which provides confidence of method accuracy.

- NIST SRM 1947 is a standard reference material consisting of fish tissue where four PFAS have been identified
- The figure shows the comparison of the values determined using the application note method (orange) compared to the values reported by NIST (blue)
- Values are close to the results reported by NIST showing this method is accurate.

PFAS Detected in Food Samples

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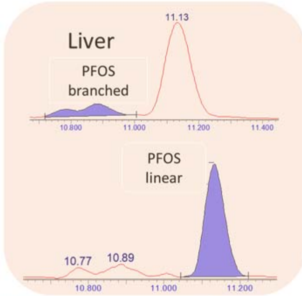
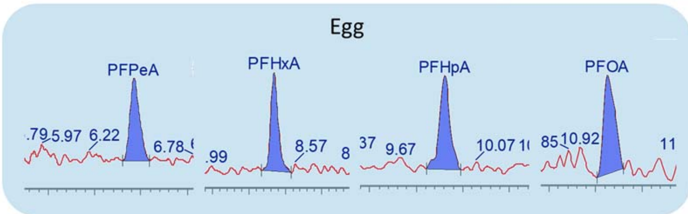
- Beef Liver and Chicken Eggs purchased from local grocery stores contained detectable levels of PFAS (shown in table and chromatograms)

PFAS	Liver (ng/g)	Egg (ng/g)
PFOS linear	0.52	-
PFOS branched	0.24	-
PFOS (total)	0.76	-
PFPeA	-	0.18
PFHxA	-	0.25
PFHpA	-	0.29
PFOA	-	0.13



The method was applied to real unknown samples with detection of PFAS in two types of food samples.



Conclusions

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- An alkaline digestion extraction followed by WAX SPE clean-up is provided to extract PFAS from challenging food samples of meat, fish, edible offal, and eggs.
- Sensitive and accurate analysis of extracts was performed using the Xevo TQ-XS allowing for detection and quantitation limits in the sub-ng/g range.
- Considering the last EURL recommendation SPE Approach should be evaluate also for vegetables in order to decrease limit of quantification.
- Utilization of the isotope dilution method allows for accurate calculation of PFAS concentration in samples without having to use matrix matched calibration curves.
- Five PFAS were detected in two different food samples purchased from local grocery stores.

Application Notes on PFAS Analysis

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Drinking Water

Routine Determination of Per- and Polyfluoronated Alkyl Substances (PFAS) in Drinking Water by Direct Injection Using UPLC-MS/MS to Meet the EU Drinking Water Directive 2020/2184 Requirements

Evaluation of the Performance of a UPLC-MS/MS Method for the Determination of PFAS in Drinking Water, for Checking Compliance with the EU Drinking Waters Directive, Using an Interlaboratory Study

Environmental Samples

A Large Volume Injection Technique Using Simplified Sample Preparation for Perfluorinated Alkyl Substance (PFAS) in Soils in Accordance with ASTM 7968

Large Volume Direct Injection Method for the Analysis of Perfluorinated Alkyl Substances (PFAS) in Environmental Water Samples in Accordance with ASTM 7979-17

Environmental Samples

An Alternative Ionization Technique for Perfluorinated Alkyl Substance (PFAS) Analysis: Evaluating UniSpray for Water and Soil Samples

UniSpray

Improved Sensitivity for the Detection of Per- and Polyfluorinated Alkyl Substances in Environmental Water Samples Using a Direct Injection Approach on Xevo™ TQ Absolute

Analysis of Legacy and Emerging Perfluorinated Alkyl Substances (PFAS) in Environmental Water Samples Using Solid Phase Extraction (SPE) and LC-MS/MS

Approaches to Non-targeted Analyses of Per- and Polyfluoroalkyl Substances (PFAS) in Environmental Samples

Food

QuEChERS Extraction of Per- and Polyfluoroalkyl Substances (PFAS) from Edible Produce with Sensitive Analysis on Xevo TQ-XS

Total Workflow for the Sensitive Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish, Meat, Edible Offal, and Eggs

Serum and Cosmetics

Extracting and Analyzing PFAS from Human Serum

Trace Level Analysis of Perfluoroalkyl Substances in Solid Cosmetics Following Methanol Extraction

Webinars and Websites

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www.waters.com/PFAS

www.waters.com/UniSpray

Oasis WAX for PFAS Analysis

Italian Mass Spectrometry Society

IMaSS Emerging Contaminants Day

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022
Aula Magna U.E.1 - Polo Didattico Navile
Via della Beverara 123/1

Marco FONTANA

Sviluppo e validazione di metodi per il monitoraggio PFAS su campioni di aeriformi

SVILUPPO E VALIDAZIONE DI METODI PER IL MONITORAGGIO PFAS SU CAMPIONI DI AERIFORMI

Relatore
Dott. Marco Fontana
Laboratorio Nord Ovest
ARPA Piemonte
marco.fontana@arpa.piemonte.it

SVILUPPO E VALIDAZIONE DI METODI PER IL MONITORAGGIO PFAS SU CAMPIONI DI AERIFORMI

Arpa Piemonte – Agenzia Regionale per la Protezione Ambientale

Marco Fontana
Paolo Fornetti
Gabriella Mele
Enrica Pipino
Simona Possamai
Antonella Salzarulo
Nicola Santamaria
Valentina Serafino

arpav – Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto

Alessandro Benassi
Caterina Cecchinato
Gianni Formenton
Massimiliano Prenzato
Antonio Uguaglianza
Francesca Zanon

Samples analyzed in ARPA Piemonte and ARPA Veneto 2021-2022

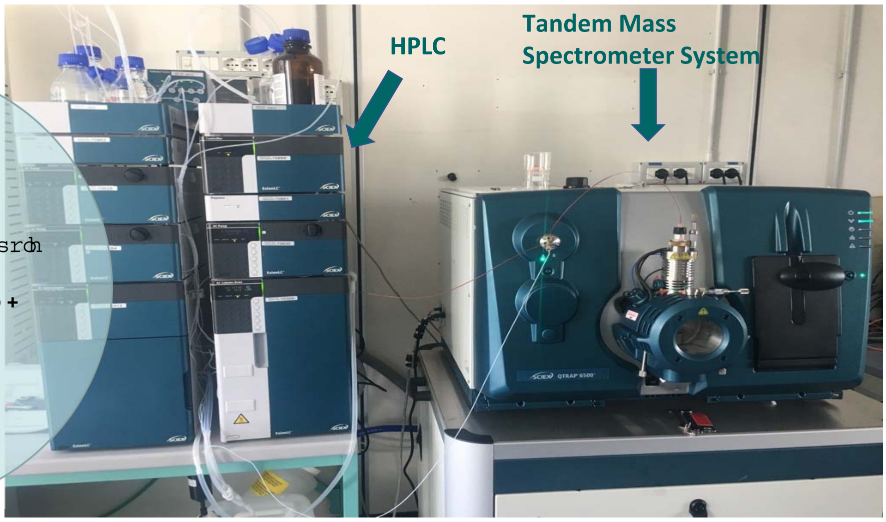
	ARPA Piemonte		ARPA Veneto
Matrix	**2021**	**2022***	**2021**
Drinking water	130	140	1271
Groundwater	240	430	2855
Surface waters	740	390	
Wastewater	130	22	319
Soils – Waste - Sludge	-	validation study	113
Depositions	30	12	-
High volume echo puf	15	8	5
Air sampling tubes	-	20	-
Serum	-		8774
MOCA - Reach	-		137
Food	-		19

*data at 1st september 2022

The Instrument

OF0PV2PV wulsoh#txdguxsroh

Ab Sciex Triple Quad 6500 +

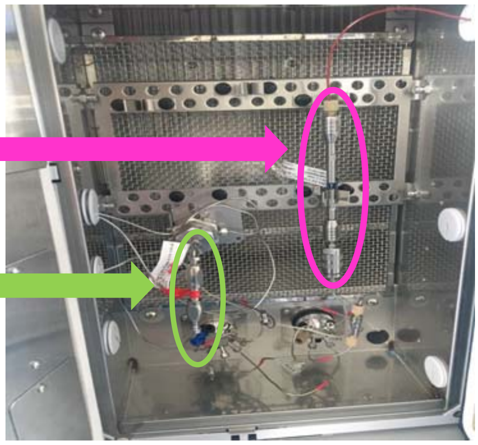


Liquid Chromatography System

The system provides a reverse phase C18 column was used to separate the target analytes in the LCsystem and solvents from the target analytes in the analytical sample. This column was placed between the solvent mixing chamber and the injector sample loop

ANALITIC COLUMN

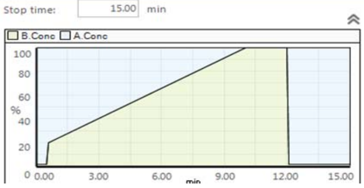
PFAS DELAY COLUMN



Liquid chromatography

Wkh#v|vwhp #xvhv d#elqdu| judglhqwz lwk#wz r p reloh#skdvhv=#Z dwhu#dqg#P hRK2FK6FQ# 4=4/#erwk 53p P #dp p rqlxp dfhwdwh

Stop time: 15.00 min



Flow: 0.4000 mL/min
A.Conc 98.0 %
B.Conc 2.0 % B.Curve 0

Pressure limits:
Minimum: 0 bar Maximum: 800 bar

Flow program
Flow program / Simple

	Time	Flow	A.Conc	B.Conc	B.Curve
1		0.4000	98.0	2.0	0
2	0.50	0.4000	98.0	2.0	0
3	0.60	0.4000	80.0	20.0	0
4	10.00	0.4000	0.0	100.0	0
5	12.00	0.4000	0.0	100.0	0
6	12.10	0.4000	98.0	2.0	0
7					

Compressibility settings

Autopurge settings

Fkurp dwrjudsklf frqglwlrqv

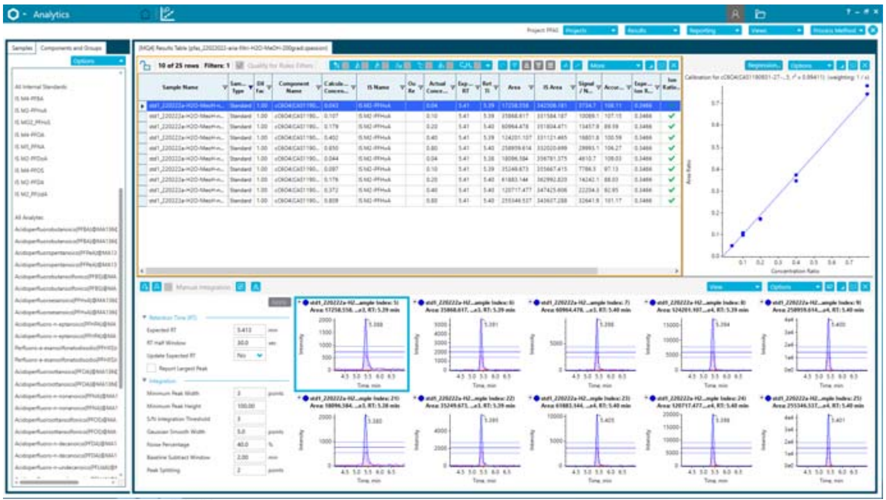
Froxp q#SKHQRP HQH[#Oxqd#Rp hjd#F4;#6pp #Srodu433#i# OF#Froxp q 433#[#5#p p

Delay Column

Luna Omega 3 µm polar C18

Iorz #3/7#p o2p lq

Calibration curve



SIDV
9#fdoleudwlrq ohyhov
Uhsruwlqj#udqjh#3134031 73#
øj2O#

fF9R7#h#DGYQ5#
8#fdoleudwlrq ohyhov
Uhsruwlqj#udqjh#3137031;3#
øj2O#

Lqmhfwlrq#yroxph#:3#øo

Dffhswdeoh lrq udwlr#63(

Analytes and labeled internal standard

10 perfluoroalkyl carboxylic acids

5 perfluoroalkylsulfonates

1 chloro perfluoropoliether carboxilate

Analytes	Labeled internal standards
PFHxA	$^{13}C_2$-PFHxA
PFOA	$^{13}C_4$-PFOA
PFOS	$^{13}C_4$-PFOS
PFDA	$^{13}C_2$-PFDA
PFUdA	$^{13}C_2$-PFUnA
PFBA	$^{13}C_4$-PFBA
PFPeA	$^{13}C_3$-PFPeA
PFBS	$^{13}C_2$-PFHxA
PFHxS	$^{18}O_2$-PFHxS
PFHpA	$^{13}C_4$-PFOA
PFHpS	$^{13}C_4$-PFOS
PFNA	$^{13}C_5$-PFNA
PFDoA	$^{13}C_2$-PFDoA
PFBS	$^{18}O_2$-PFHxS
cC6O4	$^{13}C_2$-PFHxA
ADV_	$^{13}C_5$-PFNA

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues
Bologna, 15-09-2022

The Stockholm Convention on POPs (Persistent Organic Pollutants) has been updated with the introduction of PFOA and PFOS among the substances defined as Persistent Organic Pollutants

Chemical	Pesticides	Industrial chemicals	By-products
Alpha Hexachlorocyclohexane	+		+
Beta Hexachlorocyclohexane	+		+
Chlordecone	+		
Commercial pentabromodiphenyl ether		+	
Commercial octabromodiphenyl ether		+	
Hexabromobiphenyl		+	
Lindane	+		
Pentachlorobenzene	+	+	+
Perfluoroctane sulfonic acid, its salts and perfluorooctane sulfonyl fluiride (PFOs)		+	

International Agency Research on Cancer

World Health Organization

IARC has classified PFOA in group 2B, or as a possible human carcinogen.

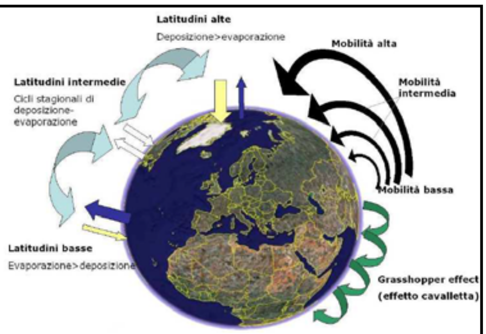
Due to growing concerns about the potential impact on health and the environment, the production and use of long-chain PFAS has decreased in favor of short-chain ones.In place of PFOA and PFOS, new molecules such as GenX, (HFPO-DA), ADONA, cC6O4 have been introduced to the market. They are PFAS whose chemical structure has been modified by inserting oxygen between the perfluorinated chains.

WHAT ABOUT PFAS IN THE AIR?

The average concentrations of PFOA in air samples collected in urban areas are in the range 0,00154 ÷ 0,152 ng/m^3

Higher average concentrations, 0,101÷0,552 ng/m^3, with maximum values equal to 0,919 and 0,828 ng/ m^3 were measured respectively in Oyamazaki, Japan, and Hazelrigg, United Kingdom, attributing in the latter case the values to the emissions of a fluoropolymer production plant located 20 km downwind of semi-rural communities.

Much higher PFOA concentrations, 70÷170 ng/m^3, were measured in 6 of the 28 air samples collected at the perimeter of the DuPont Washington Works fluoropolymer manufacturing plant, near Parkersburg, West Virginia (US).



There are no Italian or European reference values

There are no official approved methods for sampling and analysis

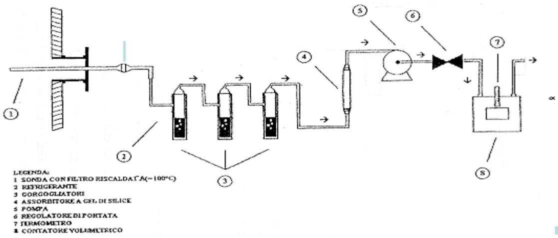
For some molecules there are no certified materials and analytical standards (ADV-N2)

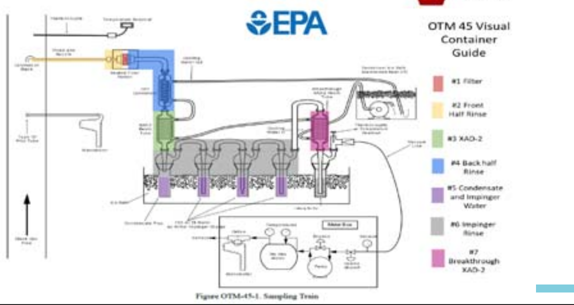
Arpa Piemonte Agenzia Regionale per la Protezione Ambientale
Sistema Nazionale per la Protezione dell'Ambiente
Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues
Bologna, 15-09-2022

HOW SAMPLING NEW PFAS IN THE AIR???

ARPA Piemonte and ARPA Veneto are investigating different ways of monitoring aeriforms:

- **atmospheric depositions**
- **high volume sampling**
- **sorbent tubes**
- **atmospheric emissions (Arpa Veneto)**





WET AND DRY DEPOSITION

Collected with HDPE deposimeters in accordance with UNI EN 15841 (Normalized method for the determination of arsenic, cadmium, lead and nickel in atmospheric depositions)

You can evaluate the mass of pollutant that in a period of time is transferred from the atmosphere to the soil, vegetation, water.

TOTAL DEPOSITION - (wet+dry)

Concentration = [$\mu g * m^{-2}$ day^{-1}].

Advantage:

- long period of observation of exposure
- availability of analytical methods for water analysis
- no sample extraction procedures

Disadvantage:

- low sensitivity
- accumulation data and not exposure data

WET AND DRY DEPOSITION

BEFORE SAMPLING: Rinsing the deposimeters with ultrapure Millipore water and keeping a control aliquot
SAMPLING PERIOD: campaigns of approximately 30 days

SAMPLE RECOVERY: a volume of approximately 500ml of ultrapure Millipore HPLC water was chosen for sample recovery from deposimeters.
STORAGE: in polypropylene containers at a temperature <4 ° C.
ANALYSIS: with the analytical technique LC-MS / MS triple quadrupole (Reports ISTISAN 19/7 ISS.CBA.052.REV00).

WET AND DRY DEPOSITION

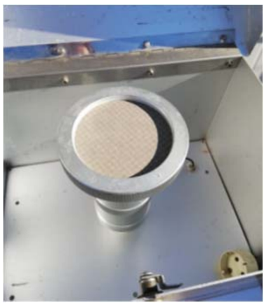
Tests carried out in duplicate in Piedmont exposed area during all the 12 months in 2021 have returned information about the presence of site-specific PFAS compounds (in particular cC_6O_4 and ADV-N2), while they did not reveal the presence of other PFAS, if not traces of PFOA.

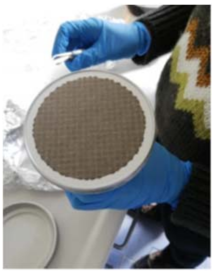
	ADV_N2	PFOA	cC6O4
	µg/(m2d)	µg/(m2d)	µg/(m2d)
MIN	0,08	<LOQ	0,16
MED	0,58	0,02	2,20
MAX	1,26	0,04	5,33

Piedmont not exposed area did not reveal the presence of any PFAS (<LOQ).

In 2022 the monitoring stations were duplicated to assess the dispersion of pollutants in different directions.

HIGH VOLUME SAMPLING





SUPPORTS:

- Quartz-fiber filters
- PUF pre-cleaned cartridge

BEFORE SAMPLING:
PUF adsorbent plugs are subjected to 1-hour sonication in methanol

SAMPLING PERIOD AND CONDITION: campaigns of approximately 24 hours at 250 l/min

SAMPLE RECOVERY:

- Quartz-fiber filters: 30' sonication in methanol at ambient temperature
- PUF pre-cleaned cartridge: ASE extraction with methanol

STORAGE: in polypropylene containers at a temperature <4 ° C.

ANALYSIS: dilution 50:50 with water and direct injection with the analytical technique LC-MS / MS triple quadrupole (Reports ISTISAN 19/7 ISS.CBA.052.REV00).

HIGH VOLUME SAMPLING

In accordance with other method like EPA TO9A (specific for PCDD/PCDF).

Advantage:

- high flow
- long period of observation

Disadvantage:

- power supply
- cartridge preparation

CONCENTRATION IN AIR

Concentration = [ng*m^{-3}].

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues
Bologna, 15-09-2022

Cooperation
Arpa Piemonte – Arpa Veneto

April 2022

AREA	SAMPLING	METHANOL EXTRACTION
PIEMONTE EXPOSED AREA	Arpa – Piemonte	Filter sonication
		Puf ASE
	Arpa - Veneto	Filter sonication
		Puf ASE
PIEMONTE «NOT EXPOSED AREA»	Arpa – Piemonte	Filter sonication
		Puf ASE
	Arpa - Veneto	Filter sonication
		Puf ASE

June 2022

AREA	SAMPLING	METHANOL EXTRACTION
VENETO EXPOSED AREA	Arpa – Piemonte	Filter sonication
		Puf ASE
	Arpa - Veneto	Filter sonication
		Puf ASE
VENETO NOT EXPOSED AREA	Arpa – Piemonte	Filter sonication
		Puf ASE
	Arpa - Veneto	filter
		Puf ASE

COOPERATION Arpa Piemonte – Arpa Veneto

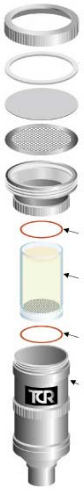
RESULTS

AREA	SAMPLING VOLUME m³	cC6O4	cC6O4 %	cC6O4 ng/m³	ADV–N2	ADV–N2 %	ADV–N2 ng/m³	OTHER PFAS	OTHER PFAS ng/m³
PIEMONTE EXPOSED AREA	390	FILTER	97	**1,7-6,0**	FILTER	100	**1,11**	FILTER +PUF	<0,1
		PUF	3		PUF	0			
PIEMONTE «NOT EXPOSED AREA»	390	FILTER	58	0,03-0,21	FILTER	100	<0,1	FILTER +PUF	<0,1 (Traces PFOA - PFNA - PFDA)
		PUF	42		PUF	0			
VENETO EXPOSED AREA	250	FILTER	31	<0,05-0,32	FILTER	100	<0,1	FILTER +PUF	<0,1
		PUF	69		PUF	0			
VENETO NOT EXPOSED AREA	200	FILTER	35	<0,05-0,6	FILTER	100	<0,1	FILTER +PUF	<0,1
		PUF	65		PUF	0			

HIGH VOLUME SAMPLING: VALIDATION STUDY

24h Sampling Recovery on high volume sampler

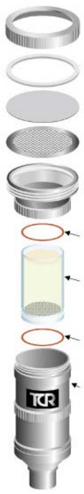
Isotopical labeled surrogates RECOVERY



Fortified filter with labeled surrogate-24hours sampling	R% AVERAGE FILTER	R% AVERAGE PUF	TOTAL RECOVERY %
M-PFPeA	0	62	**62**
M-PFHxA	0	42	**42**
M-PFHpA	0	42	**42**
13C8_PFOA	14	36	**50**
M-PFNA	45	7	**52**
M-PFDA	59	0	**59**
IM-PFUdA	70	0	**70**
M3_PFBS	94	0	**94**
M_PFHxS	83	0	**83**
13C8_PFOS	82	0	**82**
M2-4:2 FTS	158	0	**158**
M2-6:2FTS	152	0	**152**
M2-8:2FTS	155	0	**155**

HIGH VOLUME SAMPLING: VALIDATION STUDY

cC6O4 Sampling Recovery for high volume sampling



Experimental conditions	Filter cC6O4 recovery range %	Puf cC6O4 recovery range %	Total recovery range %
Filter fortified* with cC6O4 solution on glass fiber filter Methanol extraction in ASE for PUF	**8-15**	**62-80**	**70-95**

*50 ng fortified

cC6O4 distribution after 24 hours sampling between filter and polyurethane foam (PUF) in a real sample: 97% filter – 3% PUF.

AIR SAMPLING TUBES

SUPPORTS:
Air sampling tubes: SKC 226-30-16 (XAD-2/ Glass Fiber Filter) with expiry date April 2025 and lot 13769.

SAMPLING PERIOD AND CONDITION: campaigns of approximately 6-24 hours at 1 l/min

SAMPLE RECOVERY:
30’ sonication in methanol at ambient temperature

STORAGE: in polypropylene containers at a temperature <4 ° C.

ANALYSIS: dilution 50:50 with water and direct injection with the analytical technique LC-MS / MS triple quadrupole (Reports ISTISAN 19/7 ISS.CBA.052.REV00).

CONCENTRATION IN AIR Concentration = $[ng*m^{-3}]$.

AIR SAMPLING TUBES: VALIDATION STUDY

To simulate trapping of cC6O4 acid from air, known amounts of cC6O4 acid in 10 µl methanol were added to the GFF/XAD-2 air sampling tubes. After drying for approximately 5 minutes, suction of air during 360 minutes at 1,0 l/min took place.
Each section of the tube was extracted with 5 ml methanol and was sonicated for 20 minutes

cC6O4 Recovery for Air sampling tubes

Front section cC6O4 recovery range %	Back section cC6O4 recovery range %	Total recovery range %	CV %
120-140 %	0	120-140%	6 %

*1 ng fortified (10µl 100 µg/l)

AIR SAMPLING TUBES

In accordance with other occupational safety method.

Advantage:

- No power supply
- cartridge preparation

Disadvantage:

- low flow
- short period of observation

CONCENTRATION IN AIR

Concentration = [ng*m^{-3}].

Arpa Piemonte – Agenzia Regionale per la Protezione Ambientale

arpav – Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto

THANKS FOR YOUR ATTENTION

Traditional and “Emerging” PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues
Bologna, 15-09-2022

Italian Mass Spectrometry
Society

IMaSS Emerging Contaminants Day

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022
Aula Magna U.E.1 - Polo Didattico Navile
Via della Beverara 123/1

Lorenzo ZINGARO

Analysis of Perfluoroalkyl Substances in Human Hair by Agilent LC/MS Accurate Mass Spectrometry Q-TOF: A rapid biomonitoring screening method using the Agilent 1290 Infinity II LC and the Agilent 6546 Q-TOF

Agilent
Trusted Answers

Analysis of Perfluoroalkyl Substances in Human Hair by Agilent LC/MS Accurate Mass Spectrometry Q-TOF

Lorenzo Zingaro
Agilent Technologies Product Specialists

RA44781.4501388889

Aim of the study

Rapid biomonitoring screening for PFAS determination

Which matrix ?

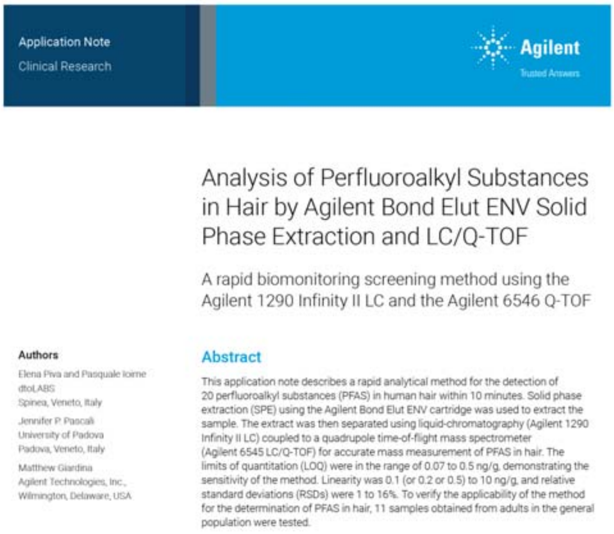
- ✓ Easy way to obtain samples
- ✓ Compared with biological fluids
- ✓ Compounds and matrix stability

Hair

Hair is gaining popularity in biomonitoring assessments of human exposure to organic pollutants

Gottardo, R. *et al.* Broad Spectrum Toxicological Analysis of Hair Based on Capillary Zone Electrophoresis-Time-of-Flight Mass Spectrometry, *J. Chrom. A* **2007**, 1159, 190–197.
Palumbo, D. *et al.* Novel Zwitterionic Hilic Stationary Phase for the Determination of Ethyl Glucuronide in Human Hair by LC/MS/MS, *J. Chrom. B* **2018**, 1100–1101 33–38.
Li, J. *et al.* Development of Extraction Methods for the Analysis of Perfluorinated Compounds in Human Hair and Nail by High Performance Liquid Chromatography Tandem Mass Spectrometry, *J. Chrom. A* **2012**, *1219*, 54–60.
Alves, A. *et al.* New Approach for Assessing Human Perfluoroalkyl Exposure Via Hair, *Talanta* **2015**, *144*, 574–583.

PFAS in Hair



Application Note
Clinical Research

Agilent
Trusted Answers

Analysis of Perfluoroalkyl Substances in Hair by Agilent Bond Elut ENV Solid Phase Extraction and LC/Q-TOF

A rapid biomonitoring screening method using the Agilent 1290 Infinity II LC and the Agilent 6546 Q-TOF

Authors

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Abstract

This application note describes a rapid analytical method for the detection of 20 perfluoroalkyl substances (PFAS) in human hair within 10 minutes. Solid phase extraction (SPE) using the Agilent Bond Elut ENV cartridge was used to extract the sample. The extract was then separated using liquid-chromatography (Agilent 1290 Infinity II LC) coupled to a quadrupole time-of-flight mass spectrometer (Agilent 6545 LC/Q-TOF) for accurate mass measurement of PFAS in hair. The limits of quantitation (LOQ) were in the range of 0.07 to 0.5 ng/g, demonstrating the sensitivity of the method. Linearity was 0.1 (or 0.2 or 0.5) to 10 ng/g, and relative standard deviations (RSDs) were 1 to 16%. To verify the applicability of the method for the determination of PFAS in hair, 11 samples obtained from adults in the general population were tested.

Read the full story in application note
5994-4237EN

- ✓ 20 perfluoroalkyl substances (PFAS)
- ✓ Solid phase extraction (SPE)
- ✓ Fast separation (UHPLC)
- ✓ Biomonitoring screening (QTOF)

In collaboration with:

dto Excellence for innovators — Elena Piva - dtoLABS Spinea, Veneto

Jennifer Pascali - UniPadova

Compounds Tested

11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	perfluoro-*n*-hexanoic acid (PFHxA)
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	perfluoro-3-methoxypropanoic acid (PFMPA)
perfluoro-*n*-butanoic acid (PFBA)	perfluoro-4-methoxybutanoic acid (PFMBA)
perfluoro-1-butanesulfonic acid (PFBS)	perfluoro-*n*-nonanoic acid (PFNA)
perfluoro-*n*-decanoic acid (PFDA)	perfluoro-1-octanesulfonic acid (PFOS)
perfluoro-*n*-dodecanoic acid (PFDoA)	perfluoro-*n*-octanoic acid (PFOA)
perfluoro(2-ethoxyethane)sulfonic acid (PFEESA)	perfluoro-*n*-pentanoic acid (PFPeA)
perfluoro-1-heptanesulfonic acid (PFHpS)	perfluoro-1-pentanesulfonic acid (PFPeS)
perfluoro-*n*-heptanoic acid (PFHpA)	perfluoroundecanoic acid (PFUnA)
perfluoro-1-hexanesulfonic aci (PFHxS)	dodecafluoro-3H-4,8-dioxanonanoic acid (ADONA)

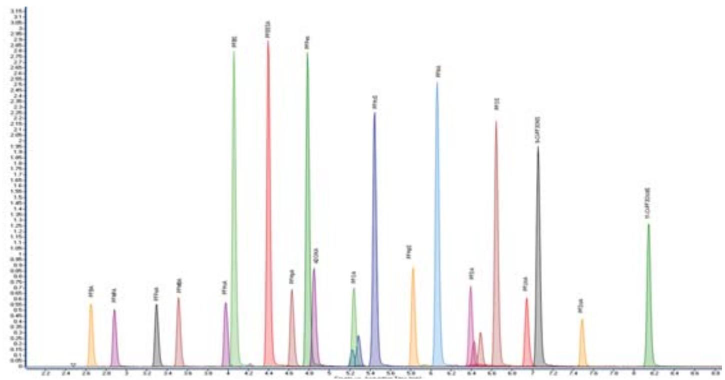
UHPLC – QTOF

Agilent

HPLC Method

Column:	Poroshell 120 EC-C18 column, 2.1 × 100 mm, 1.9 µm
Delay Column	Agilent delay column, C18 4.6 × 30 mm
Solvent A:	Water with 20 mM ammonium acetate
Solvent B:	Acetonitrile with 0.1% formic acid
Flow Rate:	0.300 ml/min
Inj	20 µL
Post Time	3 min

1290 Infinity II



Gradient:

Time [min]	A [%]	B [%]
0.00	93	3
1.00	75	25
9.00	15	85
10.00	3	97
12.00	3	97

Agilent

QTOF Method

Polarity	Negative Mode
Scan Mode	100 to 1000 m/z at 2 spectra/sec rate
Reference masses	112.9855 and 980.0163 m/z

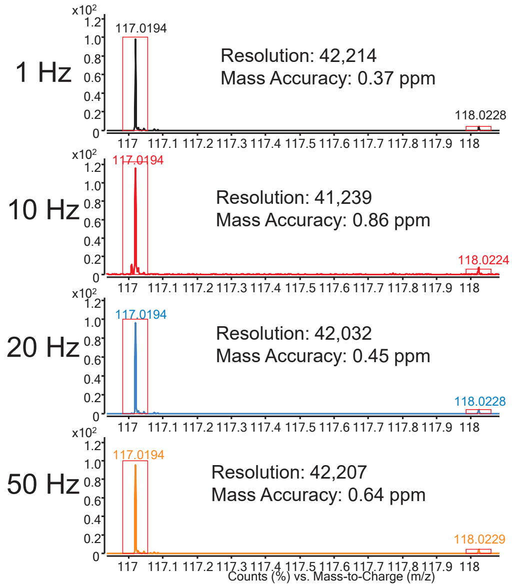
- ✓ Simultaneous Hi Resolution, Extended Dynamic Range (10Ghz)
- ✓ Higher resolution (>60k @2722*m/z*, >30k @118*m/z*)
- ✓ DIA Quadrupole-resolved All-Ions (Q-RAI)
- ✓ Capillary gate valve

Agilent 6546

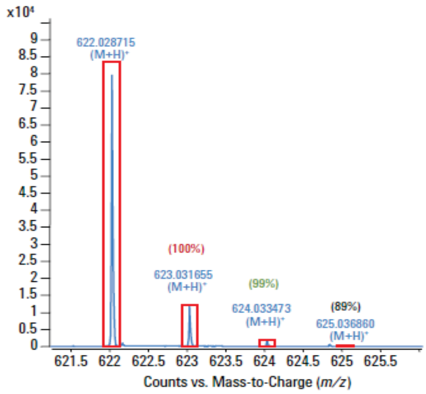
Compound Name	RT (min)	Formula	Exact Mass	Exact Mass [M-H]⁻
PFBA	2.64	$C_4HF_7O_2$	213.9865	212.9792
PFMPA	2.87	$C_4HF_7O_3$	229.9814	228.9741
PFPeA	3.28	$C_5HF_9O_2$	263.9833	262.9760
PFMBA	3.50	$C_5HO_3F_9$	279.9782	278.9709
PFHxA	3.97	$C_6HF_{11}O_2$	313.9801	312.9728
PFBS	4.07	$C_4HF_9O_3S$	299.9503	298.9430
PFEESA	4.40	$C_4HF_9O_4S$	315.9452	314.9379
PFHpA	4.63	$C_7HF_{13}O_2$	363.9769	362.9696
PFPeS	4.80	$C_5HF_{11}O_3S$	349.9471	348.9398
ADONA	4.86	$C_7H_2F_{12}O_4$	377.9762	376.9689
PFOA	5.26	$C_8HF_{15}O_2$	413.9737	412.9664
PFNA	5.35	$C_9HF_{17}O_2$	463.9705	462.9632
PFHxS	5.51	$C_6HF_{13}O_3S$	399.9439	398.9366
PFHpS	6.10	$C_7HF_{15}O_3S$	449.9407	448.9334
PFDA	6.42	$C_{10}HF_{19}O_2$	513.9673	512.9600
PFOS	6.68	$C_8HF_{17}O_3S$	499.9375	498.9302
PFUnA	6.99	$C_{11}HF_{21}O_2$	563.9641	562.9568
9Cl-PF3ONS	7.15	$C_8HClF_{16}O_4S$	531.9029	530.8956
PFDoA	7.54	$C_{12}HF_{23}O_2$	613.9609	612.9537
11Cl-PF3OUdS	8.21	$C_{10}HClF_{20}O_4S$	631.8965	630.8892

QTOF 6546

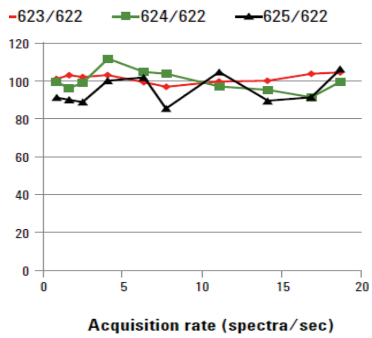
Resolution independent of acquisition rate



Isotopic Fidelity performance



A study on the Agilent Q-TOF showed a consistently low isotope ratio accuracy (RIA) error.



The RIAs for 623/622 at 10 different acquisition rates were all within 5% of the expected value (RSD = 2.8%)

Sample Prep

Agilent

Sample Prep

The high sensitivity required to detect contaminants in hair, in the order of ng/g, we developed a method with solid-phase extractions SPE

200 mg, 6 mL Agilent Bond Elut ENV SPE cartridges

- ✓ Rinse the Hair sample with 10 mL of water and then double with 10 mL of acetone
- ✓ Cut and weight 100 mg hair sample
- ✓ Add labeled IS
- ✓ Add 2 ml Acetonitrile
- ✓ Ultrasonic bath for 45 minutes at 45 °C (two times)
- ✓ SPE clen-up
- ✓ Reconstituted sample in 500 µL water/methanol (90/10 v/v)
- ✓ Injection

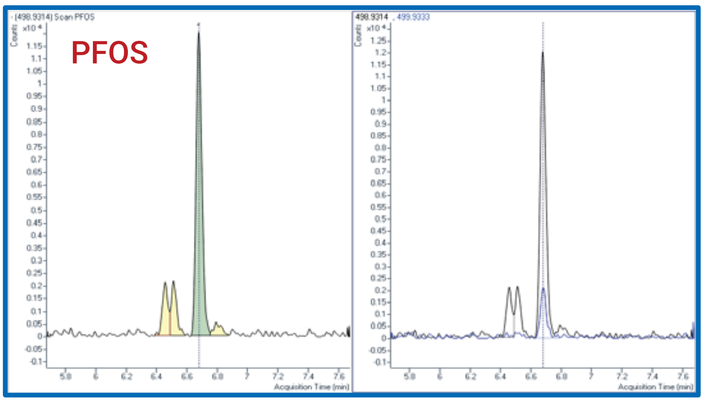
Agilent

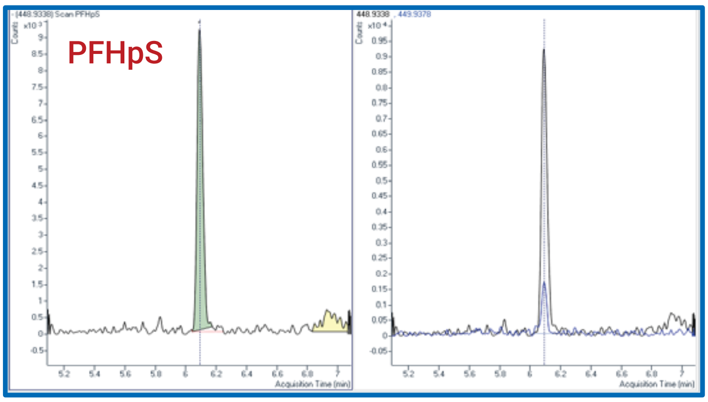
Results

Transitions

For each compounds :

- ✓ **Quantifier**
- ✓ **Qualifier**





Calibration curve, LOD and LOQ

The sensitivity, expressed as LOD and LOQ, was in the range:

- ✓ **LOD - 0.02 to 0.12 ng/g**
- ✓ **LOQ - 0.08 to 0.5 ng/g**

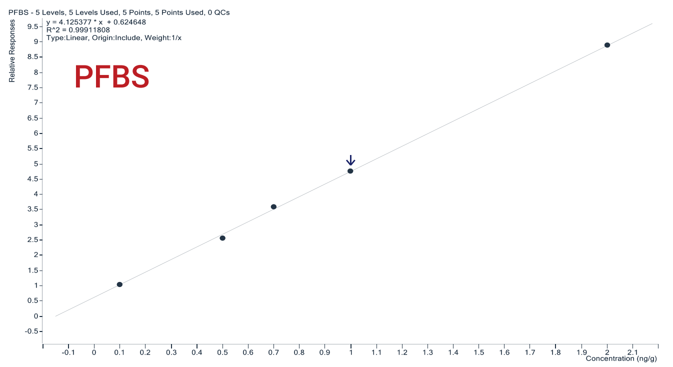
Compound	LOD ng/g	LOQ ng/g
PFBA	0.10	0.20
PFMPA	0.12	0.40
PFPeA	0.10	0.13
PFMBA	0.10	0.50
PFHxA	0.10	0.50
PFBS	0.02	0.10
PFEESA	0.02	0.07
PFHpA	0.1	0.24
PFPeS	0.02	0.08
ADONA	0.07	0.24

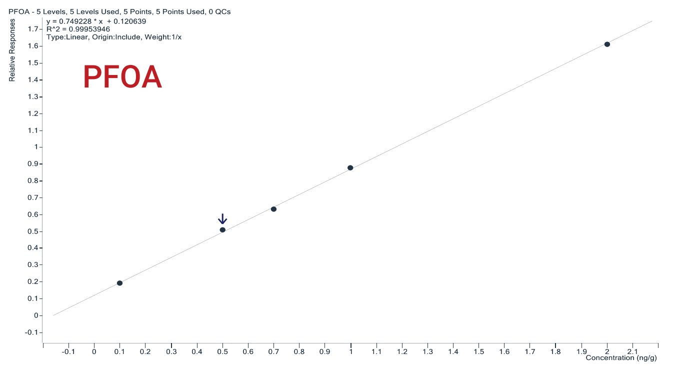
Compound	LOD ng/g	LOQ ng/g
PFOA	0.02	0.08
PFNA	0.05	0.15
PFHxS	0.02	0.05
PFHpS	0.02	0.08
PFDA	0.1	0.50
PFOS	0.02	0.08
PFUnA	0.09	0.29
9Cl-PF3ONS	0.02	0.08
PFDoA	0.09	0.30
11Cl-PF3OUdS	0.05	0.15

Calibration curve, LOD and LOQ

Calibration curve range was:

- ✓ **0.1 to 10 ng/g for PFBS, PFEESA, PFPeS, PFOA, PFHxS, PFHpS, PFOS, 9Cl-PF3ONS;**
- ✓ **0.2 to 10 ng/g for PFBA, PFPeA, PFNA, and 11Cl-PF3OUdS;**
- ✓ **0.5 to 10 ng/g for PFMPA, PFMBA, PFHxA, PFHpA, ADONA, PFUnA, and PFDoA.**





Intraday and interday accuracy

All intraday and interday recoveries were within 70 to 130% with RSDs <20%, indicating outstanding measurement stability.

Compound	Intraday Accuracy				Interday Accuracy			
	QC Low (0.7 ng/g)		QC High (1.5 ng/g)		QC Low (0.7 ng/g)		QC High (1.5 ng/g)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PFBA	104	13	118	7	98	13	102	14
PFMPA	102	12	106	9	102	10	97	11
PFPeA	108	4	101	9	102	11	95	11
PFMBA	92	8	99	5	96	10	88	9
PFHxA	109	16	102	6	105	12	93	9
PFBS	84	5	104	5	82	6	100	8
PFEESA	92	7	84	2	87	6	80	5
PFHpA	96	7	92	8	93	6	86	7
PFPeS	83	1	80	2	84	3	80	2
ADONA	106	16	104	15	112	9	98	12
PFOA	104	8	100	9	105	10	94	8
PFNA	107	10	108	8	99	10	97	13
PFHxS	81	3	83	3	83	3	84	2
PFHpS	82	2	84	4	80	5	80	5
PFDA	115	4	81	2	111	7	80	3
PFOS	112	3	112	4	108	7	104	7
PFUnA	100	12	101	10	97	16	102	11
9Cl-PF3ONS	108	8	84	4	100	7	80	5
PFDoA	99	12	102	10	100	13	94	11
11Cl-PF3OUdS	108	10	86	6	110	7	85	5

Conclusion

The study has shown the suitability of LC/Q-TOF for the determination of PFAS in human hair.

Fast and reliable method used Agilent Bond Elut ENV cartridges for SPE to prepare the sample extracts for analysis using an Agilent 6546 LC/Q-TOF.

Speed, sensitivity, linearity, and accuracy of the accurate-mass measurement of PFAS using LC/Q-TOF makes it suitable for routine biomonitoring studies.

The method could also be used for retrospective screening of samples for new members of the PFAS family, for example the emerging class of branched PFAS.

Thank You!

Questions?

Italian Mass Spectrometry
Society

IMaSS Emerging Contaminants Day

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022
Aula Magna U.E.1 - Polo Didattico Navile
Via della Beverara 123/1

Michele MAZZETTI
First investigation of per-and poly fluoroalkylsubstances (PFAS) in striped dolphin Stenella coeruleoalba stranded along Tuscany coast (North Western Mediterranean Sea)

IMaSS—Italian Mass Spectrometry Society

IMaSS Emerging Contaminants Days
Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues
Special focus: Novel Strategies for the Analysis of New Generation PFAS

FIRST INVESTIGATION OF PER-AND POLY FLUOROALKYLSUBSTANCES (PFAS) IN STRIPED DOLPHIN *STENELLA COERULEOALBA* STRANDED ALONG TUSCANY COAST (NORTH WESTERN MEDITERRANEAN SEA)

Michele Mazzetti[1], Letizia Marsili [3,4], Sara Valsecchi[2], Claudio Roscioli[2], Stefano Polesello[2], Paolo Altemura[1], Alessandro Voliani[1], Cecilia Mancusi[1,3]

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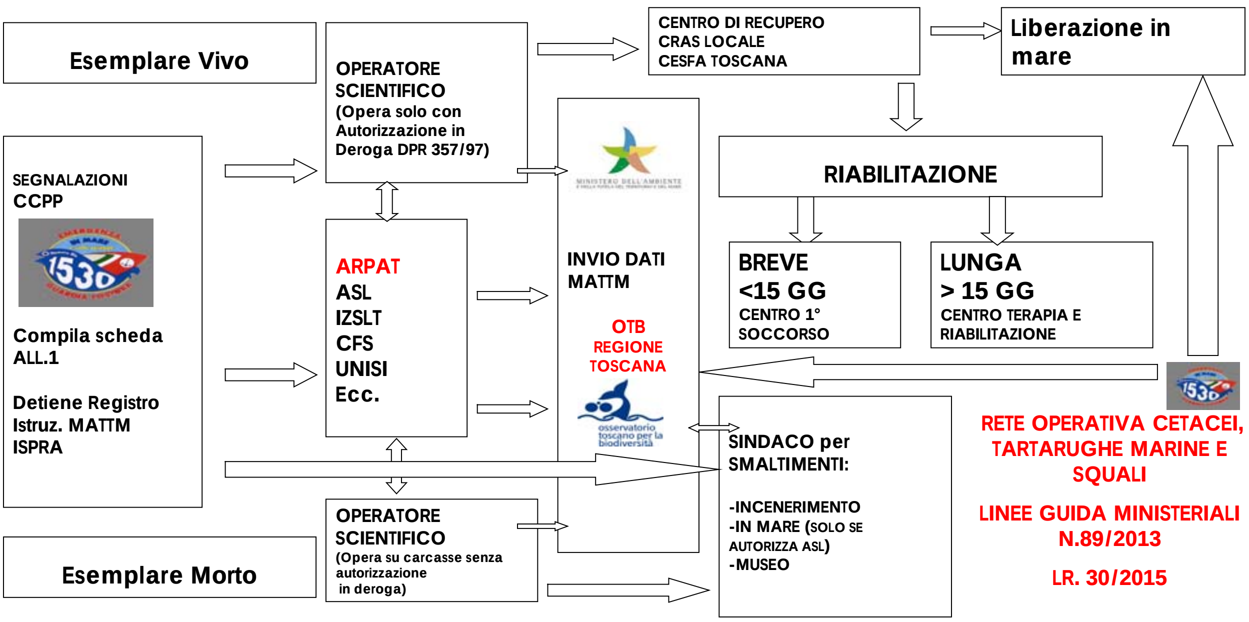
[4]CIRCE - Siena, Italy



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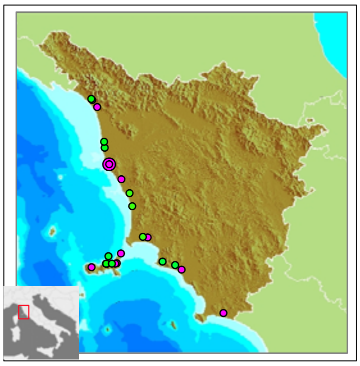
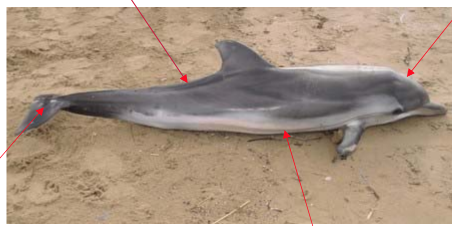
- **Sampling area**



- **Sample collection**



- **26 striped dolphins (*Stenella coeruleealba*)**
- **Period 2020-2021**

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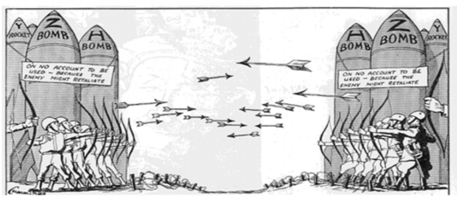
IMaSS Emerging Contaminants Days
Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues
Special focus: Novel Strategies for the Analysis of New Generation PFAS

Fluorinated organic compound: Background history

Industrial application of fluorinated organic compounds started (Chlorofluorocarbons (CFC) as refrigerants).

The major turning point in the history of industrial fluoroorganic chemistry was the beginning of the Manhattan Project for development of nuclear weapons in 1941.
The Manhattan Project triggered the need for highly resistant materials, lubricants, coolants and the development of technology for handling extremely corrosive fluoroinorganic compounds

The Cold War 1945-1991



After 1945, with the beginning of the Cold War, various defense programs provided a constant driving force for further development of the chemistry and use of organofluorine compounds.
In the 1950s and 60s more civilian applications of fluorinated pharmaceuticals and materials moved into the forefront

"Modern Fluoroorganic Chemistry Synthesis, Reactivity, Applications", P Kirsch, 2004 WILEY-VCH

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Fluorinated organic compound: Background history

Commercial Uses

Fluorinated surfactants can lower aqueous surface tension to less than 16 dynes/cm and function at very low concentrations (e.g., 100–500 mg/L or parts-per-million, ppm). They are effective in basic/acidic aqueous media and in organic solvents

- *superior wetting, spreading, and leveling properties for all types of surfaces. They give uniform film formation of coatings and eliminates pinholes and craters, even when applied to unclean surfaces*
- *extremely stable both chemically and thermally. Some of them are stable even in hot chromic acid, concentrated sulfuric acid or hydrofluoric acid*

- ***Aqueous Film-Forming Foams***
- ***Enhanced Oil Recovery***
- ***Coatings***
- ***Electroplating and Electrowinning***
- ***Electronics***
- ***Paper***
- ***Mining***
- ***Photographic Films***
- ***Fluoropolymer Polymerization Aid***
- ***Pesticide Application***

"Modern Fluoroorganic Chemistry Synthesis, Reactivity, Applications", P Kirsch, 2004 WILEY-VCH

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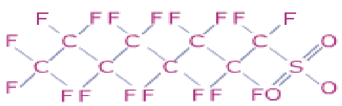
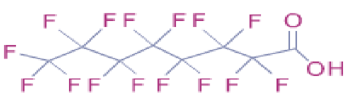
Perfluorinated organic compound: Environmental Regulatory Framework: Elements

IN EUROPEAN UNION



PFOS (Perfluorooctane sulfonate)

is classified as
- *as POP's (Reg. 757/2010) after PFOS was added to the Annex B of the Stockholm Convention in 2009*
- *Priority Substance for Water (Dir 2013/39/UE)*
- *Substance with Restriction limit as reported in Reg(EU) No 757/2010 amending Reg (CE) 850/2004*



PFOA (Perfluorooctanoic acid)

is classified as
- *Candidate in the SVHC List (Substances of Very High Concern) after MSC (Member States Commitee) identified in june 2013 PFOA as PBT.*
- *Substance with Restriction limits as reported in Reg. (UE) 2017/1000 (entry 68)*

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Perfluorinated organic compound: Environmental Regulatory Framework: Elements

DIRECTIVE 2013/39 of August 2013
Amending Dir. 2000/60 and 2008/15
(Rec. DLgs 172/2015)
ANNEX I
Environmental Quality Standard For Priority Substances and certain other pollutants
PART A: Environmental Quality Standard EQS

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
No	Name of substance	CAS number (1)	AA-EQS (2) Inland surface waters	AAC-EQS (2) Other Surface waters	MAC-EQS (2) Inland surface waters	MAC-EQS (2) Other Surface waters	EQS Biota (12)
			µg/L	µg/L	µg/L	µg/L	µg/Kg wet weight
(35)	Perfluorooctane sulfonic acid and its derivatives (PFOS)	1763-23-1	$6{,}5 \times 10^{-4}$	$1{,}3 \times 10^{-4}$	36	7.2	9.1

AA: annual average.
MAC: maximum allowable concentration.

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Perfluorinated organic compound: Environmental Regulatory Framework: Elements

DIRECTIVE 2013/39 of August 2013

Environmental Q

COMMISSION DIRECTIVE 2009/90/EC of 31 July 2009
Article 4
Minimum performance criteria for methods of analysis

Member States shall ensure that the minimum performance criteria for all methods of analysis applied are based on an uncertainty of measurement of 50 % or below (k = 2) estimated at the level of relevant environmental quality standards and a **limit of quantification equal or below a value of 30 % of the relevant environmental quality standards**.

Requested LOQ = $2{,}1 \times 10^{-4}$ µg/L **Requested LOQ = 3 µg/Kg**

(1)	(2)	(3					
No	Name of substance	CAS n (1					
			µg/L	µg/L	µg/L	µg/L	µg/Kg wet weight
(35)	Perfluorooctane sulfonic acid and its derivatives (PFOS)	1763-23-1	$6{,}5 \times 10^{-4}$	$1{,}3 \times 10^{-4}$	36	7.2	9.1

AA: annual average.
MAC: maximum allowable concentration.

OUTLINE

- Introduction
- **PFAS Methods – solid matrices**
- QuEChERS for PFAS and Applied Method
- Method Performance
- Results and Conclusion

Solid Matrix PFAS Methods

	ASTM D7968	EPA-821-R-11-007	537Ms / DoD
MATRIX	Soils	Sludge, Biosolids	Soils, Sediments, Biosolids, Tissues, etc
RL (ng/g)	0.025 - 0.75	0.25 - 10	various
PREPARATION	SLE (rotator) centrifuge, filter	digestion, incubation, SLE (shake), SPE	various
CLEAN-UP	Filtration	SPE WAX + filtration	various

SLE = Solid-Liquid Extraction
SPE = Solid Phase Extraction
WAX = Weak Anion Exchange

"Evaluation of QuEChERS Clean-up Sorbents for the Analysis of PFAS in Tissues and Biosolids
Syljohn Estil & Arnold Tesoro, LACSD Chemistry Research Group, August 2019

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Recent developments in Solid Matrix PFAS Methods

QuEChERS is considered accurate and highly productive at ultra trace levels. Yet, for the analysis of PFAS in food, this method is not widely applied compared to the straighfoward SLE and IPE methods.
Recently, a one step QuEChERS extraction and purification was found to be successful.

FDA U.S. FOOD & DRUG ADMINISTRATION

FDA Foods Program Compendium of Analytical Laboratory Methods: Chemical Analytical Manual (CAM)

METHOD NUMBER: C-010.01

POSTING DATE: 11/01/2019

POSTING EXPIRATION DATE: 10/31/2021

PROGRAM AREA: Chemical Contaminants

Determination of 16 Perluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Version 2019 (2019)

Author: Susan Genualdi and Lowri deJager

CFSAN/ORS reviewers: Tim Begley, Gregory Noonan

GLOSSARY

Analytical Methods

PAPER

Check for updates

Cite this: Anal. Methods, 2018, 10, 5715

Simultaneous determination of legacy and emerging per- and polyfluoroalkyl substances in fish by QuEChERS coupled with ultrahigh performance liquid chromatography tandem ma spectrometry†

Yan Gao, Qinghe Zhang,* Xiaomin Li, Xiuqin Li and Hongmei Li

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Instrumental Choice

Orbitrap Q-Exactive

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List of analytes

UNLABELLED

Perfluoro-n-hexanoic acid (PFHxA), Perfluoro-n-heptanoic acid (PFHpA), Perfluoro-n-octanoic acid (PFOA), Perfluoro-n-nonanoic acid (PFNA), Perfluoro-n-decanoic acid (PFDA), Perfluoro-n-undecanoic acid (PFUdA), Perfluoro-n-dodecanoic acid (PFDoA), Perfluoro-n-tridecanoic acid (PFTrDA), Perfluoro-n-tetradecanoic acid (PFTeDA), Perfluoro-n-hexadecanoic acid (PFHxDA), Perfluoro-n-octadecanoic acid (PFODA), Potassium perfluoro-1-butanesulfonate (L-PFBS), Sodium perfluoro-1-pentanesulfonate (L-PFPeS), Sodium perfluoro-1-hexanesulfonate (L-PFHxS), Sodium perfluoro-1-heptanesulfonate (L-PFHpS), Sodium perfluoro-1-octanesulfonate (L-PFOS), Sodium perfluoro-1-nonanesulfonate (L-PFNS), Sodium perfluoro-1-decanesulfonate (L-PFDS), Sodium perfluoro-1-dodecanesulfonate (L-PFDoS), perfluorooctanssulphonamide (FOSA)

LABELLED

Perfluoro-n-($^{13}C_4$)butanoic acid MPFBA, Perfluoro-n-($^{13}C_5$)pentanoic acid M5PFPeA, Perfluoro-n-(1,2,3,4,6-$^{13}C_5$)hexanoic acid M5PFHxA, Perfluoro-n-(1,2,3,4-$^{13}C_4$)heptanoic acid M4PFHpA, Perfluoro-n-($^{13}C_8$)octanoic acid M8PFOA, Perfluoro-n-($^{13}C_9$)nonanoic acid M9PFNA, Perfluoro-n-(1,2,3,4,5,6-$^{13}C_6$)decanoic acid M6PFDA, Perfluoro-n-(1,2,3,4,5,6,7-$^{13}C_7$)undecanoic acid M7PFUdA, Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid MPFDoA, Perfluoro-n-(1,2-$^{13}C_2$)tetradecanoic acid M2PFTeDA, Sodium perfluoro-1-(2,3,4-$^{13}C_3$)butanesulfonate M3PFBS, Sodium perfluoro-1-(1,2,3-$^{13}C_3$)hexanesulfonate M3PFHxS, Sodium perfluoro-1-($^{13}C_8$)octanesulfonate M8PFOS, Perfluoro-n-(2,3,4-$^{13}C_3$)butanoic acid M3PFBA, Perfluoro-n-(1,2-$^{13}C_2$)octanoic acid M2PFOAPerfluoro-n-(1,2-$^{13}C_2$)decanoic acid MPFDA, Sodium perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate MPFOS , $^{13}C_8$ perfluorooctansulphonamide ($^{13}C_8$ FOSA)

Analysis of PFOS in Fish: QUECHERs Extraction

Addition of 100 µl of 200 ng/ml methanolic solution of Extraction ILS ($^{13}C_8$-PFOS) to 2 g of fish omogeneate.

↓ equilbrate overnight at -18°C

Addition of 2 ml of water and 1 ceramic homogeneizer,

↓ vortex agitation for 1 minute

Addition of 10 ml of acetonitrile

↓ vortex agitation for 1 minute

Addition of 1 pouche of reagent mix for QUECHERs extraction compliance to EN15662

↓ vortex agitation for 1 minute

Centrifugation and collection of liquid phase

↓ equilbrate overnight at -18°C (fats precipitation)

Centrifugation (15 KG at -10ºC for 10 minutes), and Injection in UHPLC-HRMS system

RESIDUES AND TRACE ELEMENTS

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce

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Validation Study

JRC REFERENCE MATERIALS REPORT

CERTIFICATION OF THE MASS FRACTION OF PERFLUORALKYL SUBSTANCES (PFASs) IN FISH TISSUE (PIKE-PERCH): IRMM-427

Pikeperch, is a species of ray-finned fish from the family Percidae. It is found in freshwater and brackish habitats in western Eurasia.

	MASS FRACTION	
	Certified value (ng/g)	Uncertainty (ng/g)
Linear perfluorooctane sulfonate (L-PFOS) (1)	16(2)	1,7(2

1) As defined by using liquid chromatography mass spectrometry.
2) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory with a method of determination including liquid chromatography mass spectrometry. Sulfonates are expressed on an anion basis. The certified/ values and their uncertainties are traceable to the International System of Units (SI).
3) The uncertainty of the certified / indicative value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008

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Validation Study

Summary

Quantification Mode: ***SIDA***

Range: ***2 - 16 µg/Kg***

Uncertainty (conc. equal or higher than 9.2 µg/kg): ***50%***

Uncertainty (conc. less than 9,2 µg/kg): ***57 %***

LOQ: ***2 ng/g***

*Limit of repeatibiliy:****20%***

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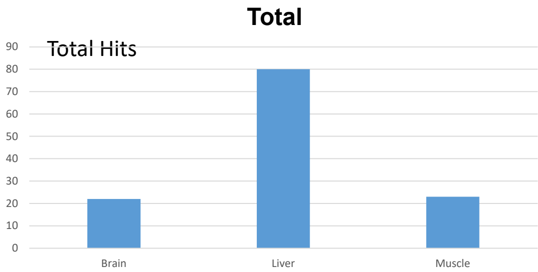
UNTARGET ANALYSIS - MASSLIST MATCH

High resolution chromatograms were processed by software Thermo Scientific COMPOUND DISCOVERER V. 3.3 SP1

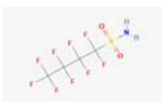
Main Filter: -0.10 <= Standard Mass Defect <= 0.15, Ritention time > 1 min

MASSLIST (more then 9000 compouds)
- Chemical list EPAPFAS: PFAS, S75S1, INSOL, INV, SRL, KEMI, OECD, STRUCT, TRIER
- NORMAN: FLUOROPEST, FLUOROPHARMA, NEXCH, RISKPFAS, NTREV Liu et al (2019). GLUEGE



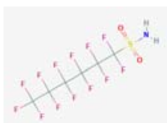
Untarget noteworthy compounds:

Perfluorobutanesulfonamide : **FBA** (Liver, blood):



C4H2F9NO2S
Monoisotopic mass:
298.96625

Perfluorohexanesulfonamide: **FHxSA** (liver, Blood, Muscle, Brain)



C6H2F13NO2S
Monoisotopic mass:
398.9599

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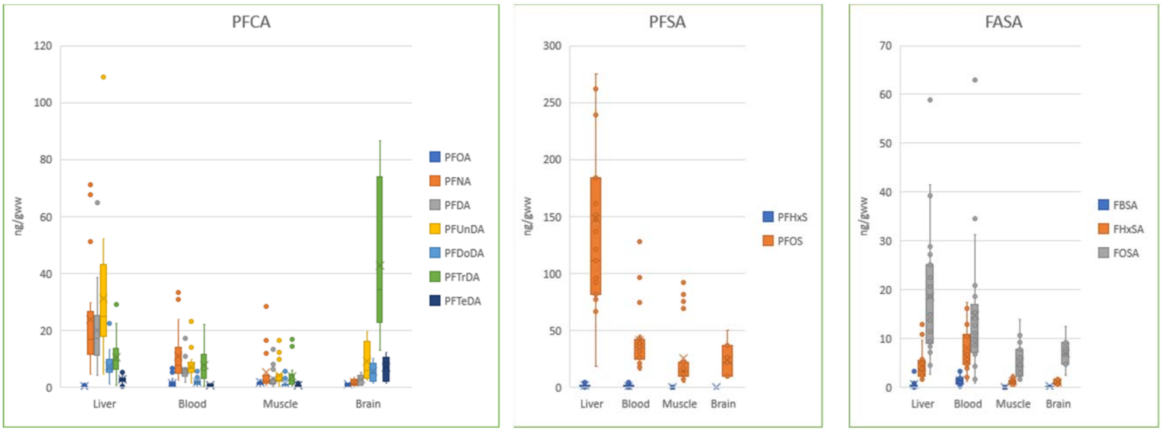
Concentrations of various analytes



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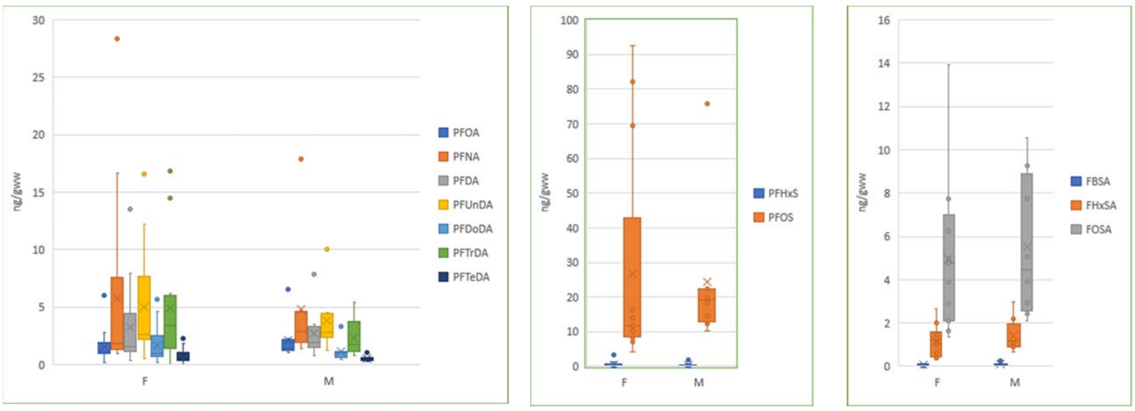
Concentrations of various analytes depending on sex of specimen



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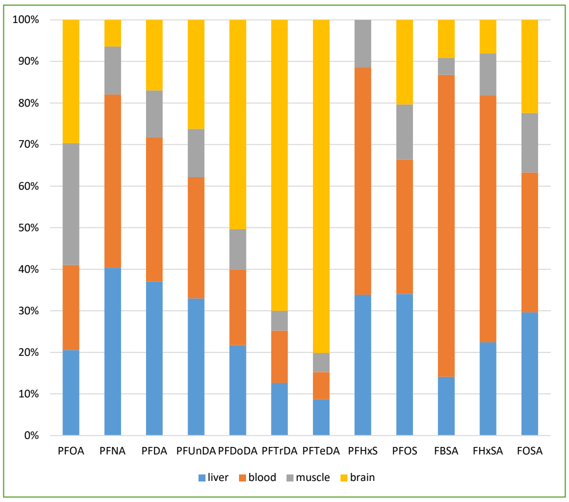
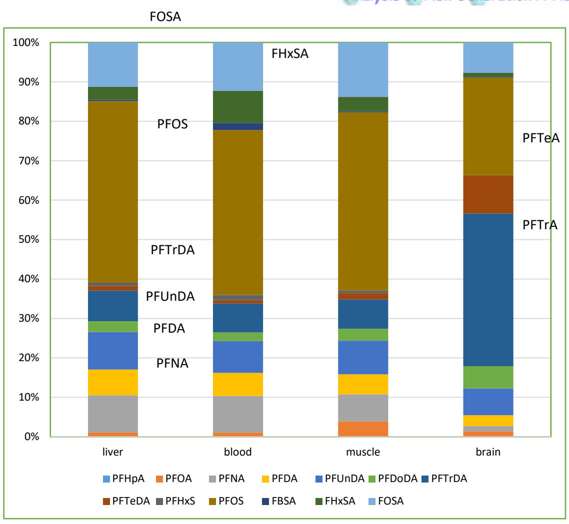
Tissue distribution of various analytes





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Comparison of Results with previous works

Compound	year	Location	Tissue	n	ng/gww	reference
PFOS	1991	Viareggio (North Thyrrenian Sea)	Liver	1	22.3	Kannan et al., 2002
	1991	Lecce (South Adriatic Sea)		3	16.3, 23.5, 40	Kannan et al., 2002
	2009-2018	Western Mediterranean Sea		29	1.2-246 (118)	Lopez-Berenguer et al., 2020
	2020-2021	*Tyrrhenian Sea*		*23*	*19-501 (37)*	*Present study*
ΣPFAS	2009-2018	Western Mediterranean Sea	Liver	29	8.6-523 (270)	Dassuncao et al., 2019.
	2020-2021	*Tyrrhenian Sea*		*23*	*48-827 (91)*	*Present study*
ΣPFAS	2009-2018	Western Mediterranean Sea	Muscle	29	4.8-75 (21)	Lopez-Berenguer et al., 2020
	2020-2021	*Tyrrhenian Sea*		*22*	*11-188 (31)*	*Present study*

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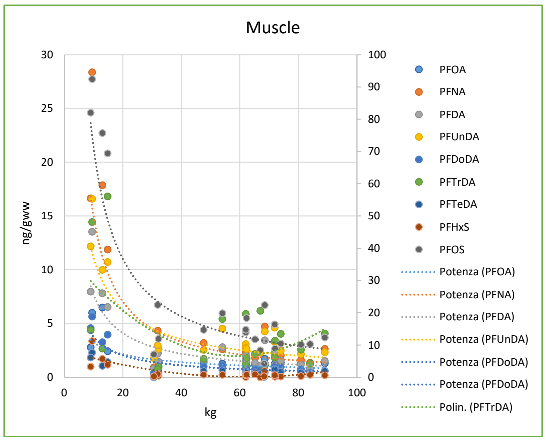
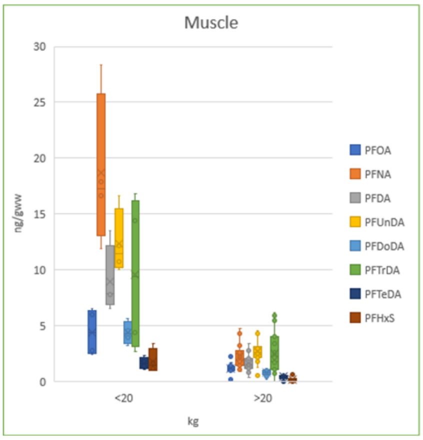
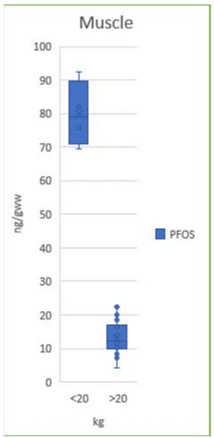
Concentrations of PFCAs and PFOS in muscle depending on weight of specimen







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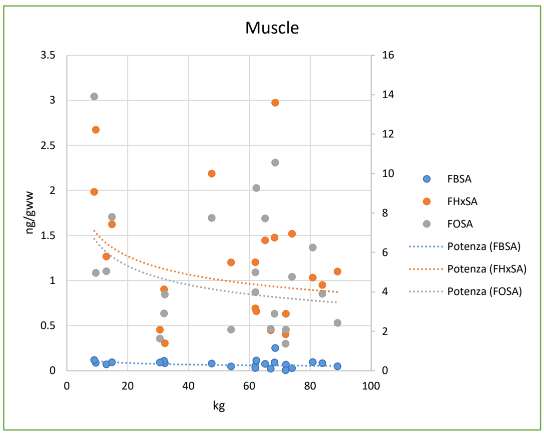
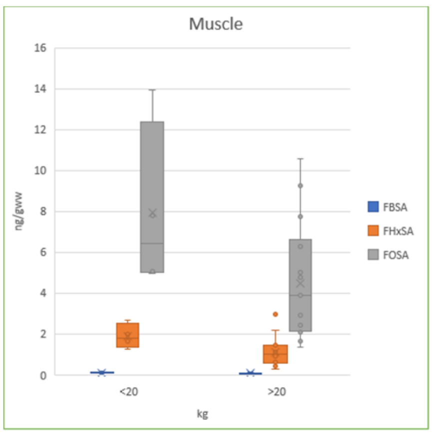
Concentrations of FASA in muscle depending on weight of specimen





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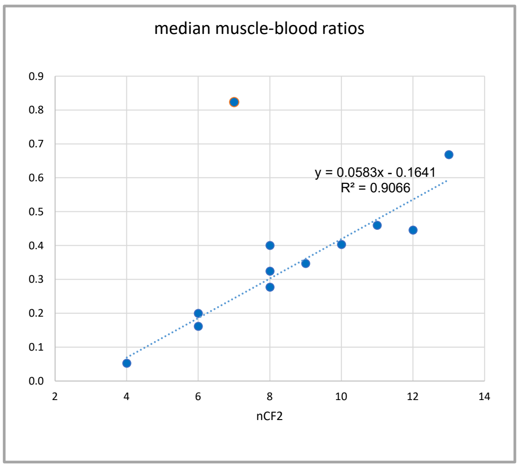
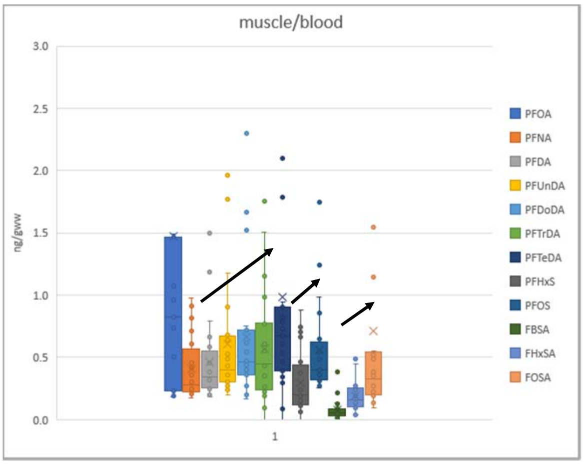
Muscle-Blood Ratios





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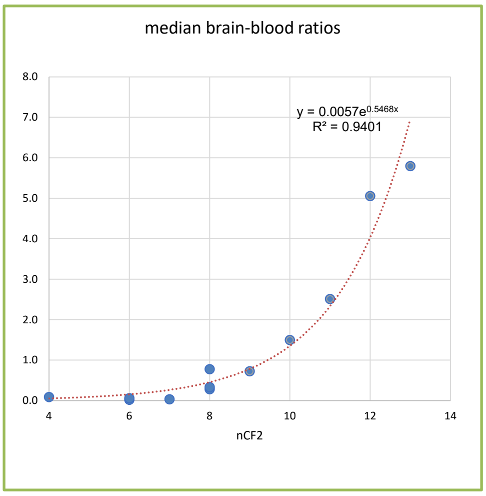
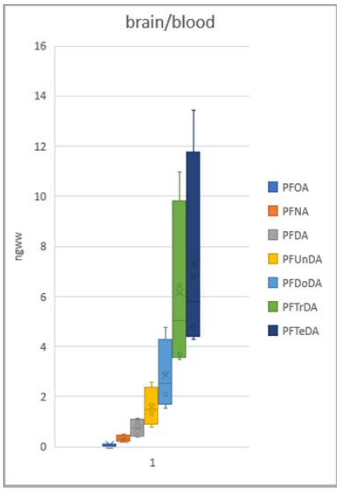
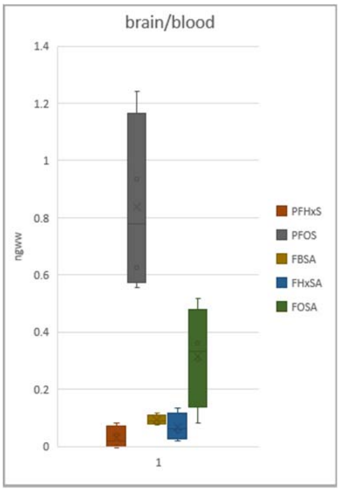
Brain-Blood Ratios







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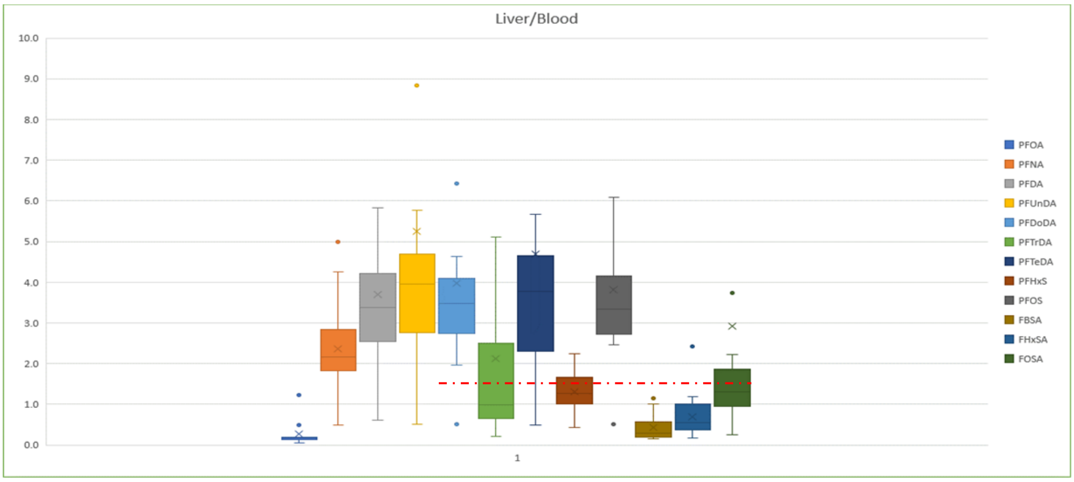
Liver-Blood Ratios



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Conclusions

- Striped dolphin is exposed to different type of contaminant (PFAS) and their accumulation was confirmed in some tissues (brain, muscle, liver, blood) with elevate concentrations
- The fluoroalkyl substances are potentially very dangerous for aquatic organisms, especially for apex predators: affect cellular functions, cause developmental and neuroendocrine anomalies.
- The intake of PFAS substances is probably due to diet and structure of food chain
- High concentrations of contaminants may have played an important role in causing, or at least in being a co-cause, of the death of dolphins
- Further in depth research is needed
- We'll propose to SNPA to adopt the shown analyses as a routine process also for dolphins and their preys (detection in biota as requested by Dgls 172/15)

Italian Mass Spectrometry Society

IMaSS Emerging Contaminants Day

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022
Aula Magna U.E.1 - Polo Didattico Navile
Via della Beverara 123/1

Damiano BRACCHITTA

L'analisi in routine dei PFAS, strategie analitiche per il miglioramento dei limiti di quantificazione

L’analisi in routine dei PFAS, strategie analitiche per il miglioramento dei limiti di quantificazione

Damiano Bracchitta (APPA, Trento)

National legislation

D.Lgs. 172/2015 from Directive 2013/39/UE

Tab. 1/A - Environmental quality standards for column of water and biota for priority substances

SQA-MA Internal surface waters
Perfluorottansolfonic acid (PFOS) and your salts CAS (1763-23-1)
$6.5*10^{-4}$ µg/l

Groundwater D.M. 06/07/2016 (from Directive 2014/80/UE) Tab.3

Groundwaters wich interacts with surface waters
Perfluorottansolfonic acid (PFOS) and your salts CAS (1763-23-1)
$6.5*10^{-4}$ µg/l

Limit Of Quantitation for Internal surface waters

DECRETO LEGISLATIVO 10 dicembre 2010, n. 219
LOQ ≤ 30% of Environmental quality standard annual average (SQA-MA).

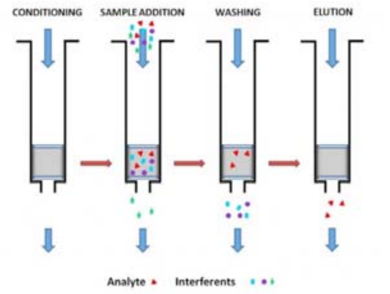
LOQ for PFOS is 0.0002 µg/l

How performe analysis of PFOS?

one possible way is:

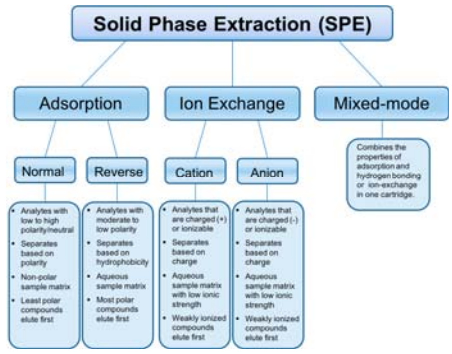
- UPLC coupled with mass spectrometry triple quadrupole like Shimadzu 8050
- 0.01 µg/l is LOQ reached with direct injection
- pre-concentration is require for reach 0.0002 µg/l

SPE
Solid Phase Extraction



Schematic representation of SPE clean-up procedure

Pictures from: https://separationmethods.com/product-category/sample-preparation/spe-cartridges/?orderby=reverse_alpha&filter_pore-size=60a



Pictures from: https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_%28Analytical_Chemistry%29/Analytical_Sciences_Digital_Library/Active_Learning/Contextual_Modules/Sample_Preparation/03_Solid-Phase_Extraction

How to achieve this LOQ?

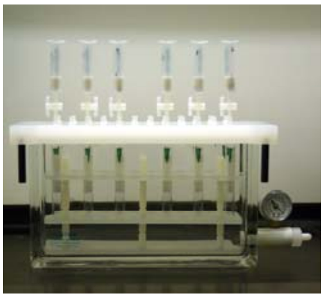
there are two ways

SPE offline

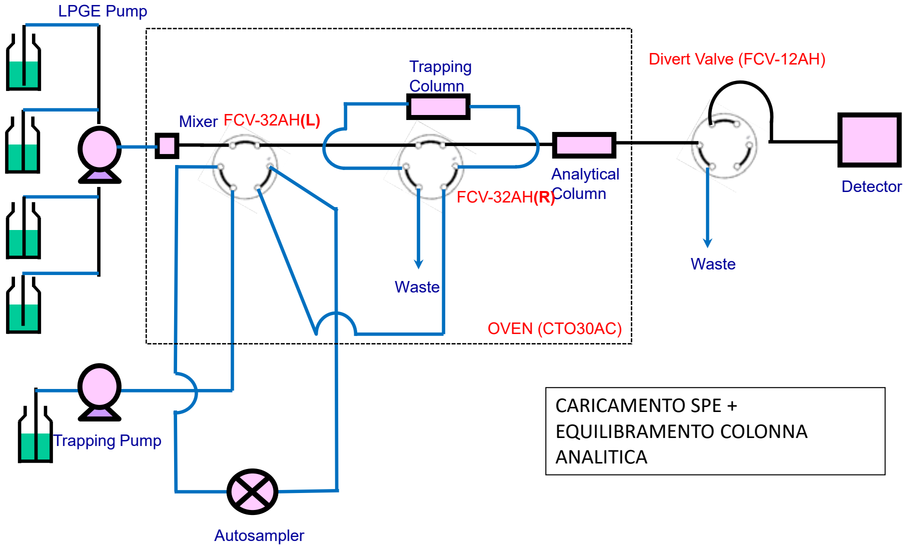
SPE online

SPE offline

- automated
- manual

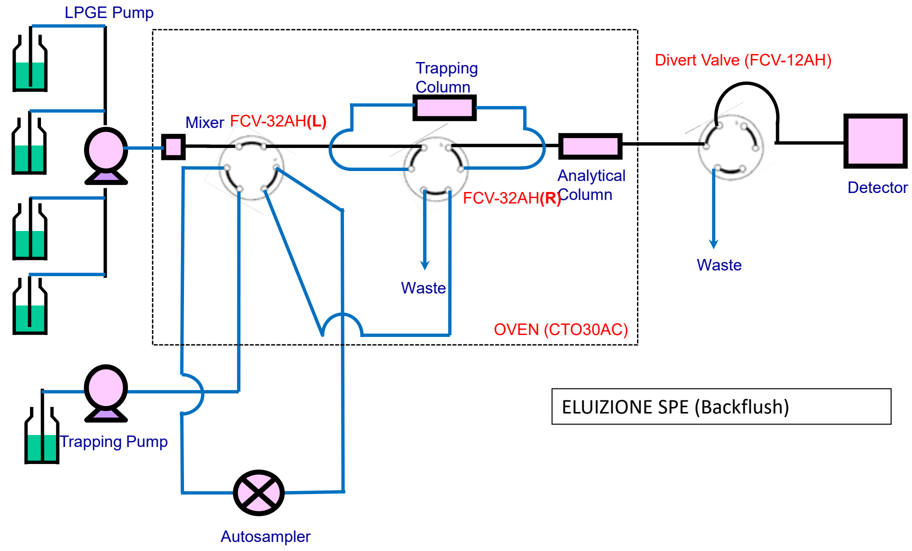


SPE online



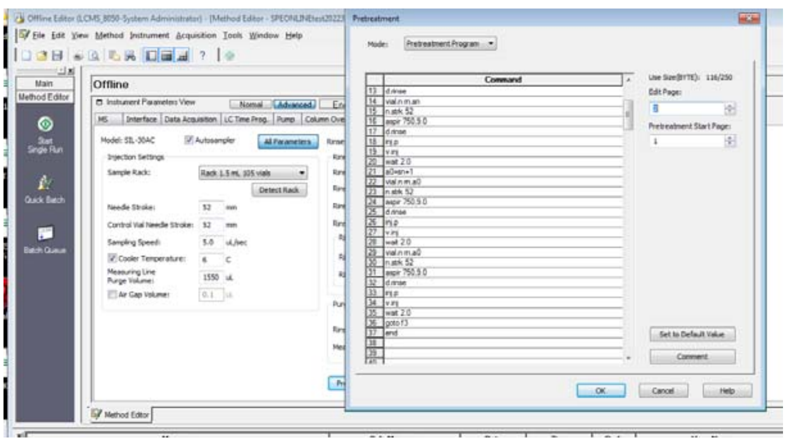
CARICAMENTO SPE + EQUILIBRAMENTO COLONNA ANALITICA

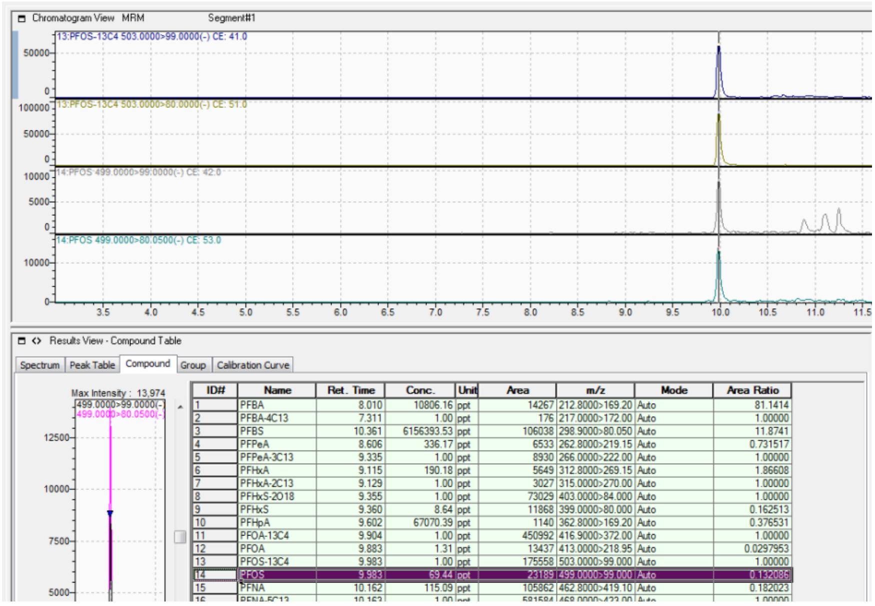
SPE online



Pretreatment

- example of pretreatment for PFOS SPEonline analysis





Troubleshooting

Low-temperature mineralization of perfluorocarboxylic acids

https://www.science.org/doi/10.1126/science.abm8868

Abstract

Per- and polyfluoroalkyl substances (PFAS) are persistent, bioaccumulative pollutants found in water resources at concentrations harmful to human health. Whereas current PFAS destruction strategies use nonselective destruction mechanisms, we found that perfluoroalkyl carboxylic acids (PFCAs) could be mineralized through a sodium hydroxide–mediated defluorination pathway. PFCA decarboxylation in polar aprotic solvents produced reactive perfluoroalkyl ion intermediates that degraded to fluoride ions (78 to ~100%) within 24 hours. The carbon-containing intermediates and products were inconsistent with oft-proposed one-carbon-chain shortening mechanisms, and we instead computationally identified pathways consistent with many experiments. Degradation was also observed for branched perfluoroalkyl ether carboxylic acids and might be extended to degrade other PFAS classes as methods to activate their polar headgroups are identified.

Thanks

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Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022

Aula Magna U.E.1 - Polo Didattico Navile

Via della Beverara 123/1

Sara VALSECCHI

Analytical strategies in LC-MS for the analysis of next generation PFAS

Analytical strategies in LC-MS for the analysis of "next generation" PFAS

new compounds – old problems

Sara Valsecchi (IRSA-CNR)
valsecchi@irsa.cnr.it

pubs.acs.org/est

Viewpoint

2021

A New OECD Definition for Per- and Polyfluoroalkyl Substances

Zhanyun Wang,* Andreas M. Buser, Ian T. Cousins, Silvia Demattio, Wiebke Drost, Olof Johansson, Koichi Ohno, Grace Patlewicz, Ann M. Richard, Glen W. Walker, Graham S. White, and Eeva Leinala

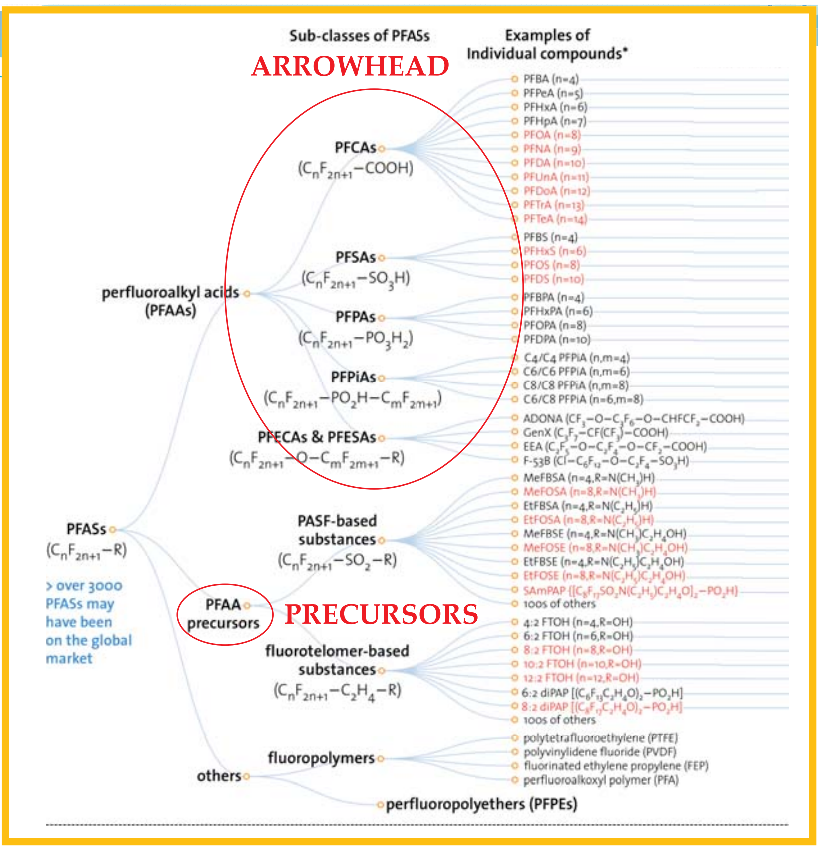
PFAS definition: "PFAS are defined as fluorinated substances that contain **at least one fully fluorinated methyl or methylene carbon atom** (**without any H/Cl/Br/I atom attached to it**), i.e., with a few noted exceptions, any chemical with at least a perfluorinated methyl group (−CF3)or a perfluorinated methylene group (−CF2−) is a PFAS". The "noted exceptions" refer to a carbon atom with a H/Cl/Br/I atom attached to it.

PFAS: Poly- and perFluoroAlkylic Substances

Browse PubChem: PFAS and Fluorinated Compounds in PubChem Tree

- PFAS and Fluorinated Compounds in PubChem 19,504,998
  - OECD PFAS definition 6,125,596
    - Molecule contains isolated CF2 606,618
    - Molecule contains isolated CF3 5,439,732
    - Molecule contains PFAS parts larger than CF2/CF3 189,410
      - Breakdown by isolated PFAS part count 189,410
      - Breakdown by isolated PFAS part type 189,410
  - Organofluorine compounds 19,355,749
  - Other diverse fluorinated compounds 109,082
  - PFAS and fluorinated compound collections 1,784,294
    - CompTox Chemicals Dashboard PFAS suspect lists 8,498
    - NORMAN-SLE PFAS suspect lists 6,040
    - OntoChem PFAS lists 1,777,168
    - Other fluorinated chemical content in PubChem 1,674

Industrial products
Pharmaceuticals
Pesticides



Wang *et al*., *Environ. Sci. Technol.* 2017, 51, 2508–2518

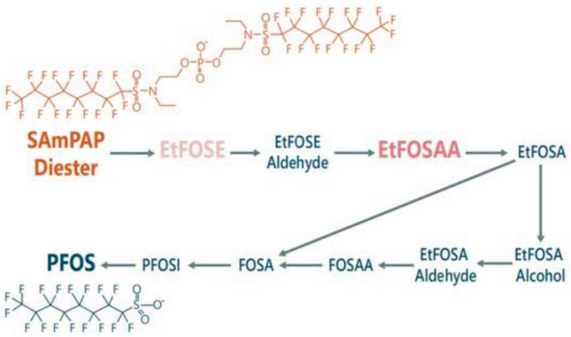
PFAS precursors

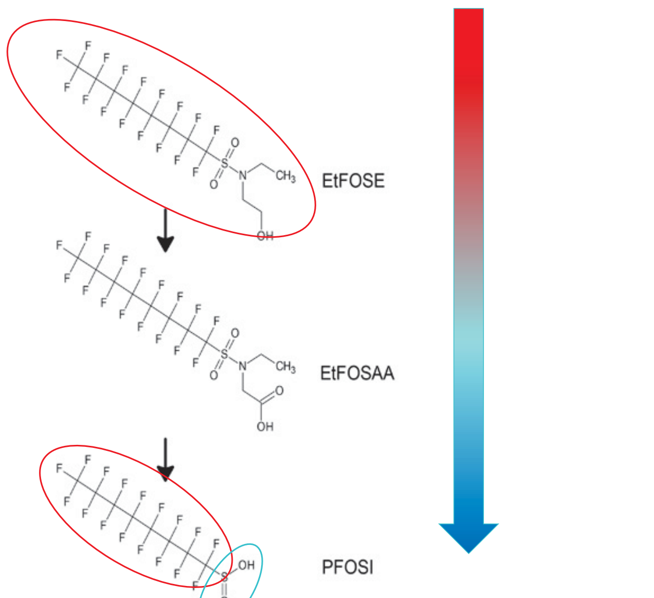
PRECURSORS

PRECURSORS	SEMI-STABLE INTERMEDIATES	ARROWHEAD
PFAS precursors	PFAS stable degradation intermediates	PFAS final degradation products

Lipophilicity / volatility

SAmPAP diester (Paper Industry)
Perfluorooctane sulfonamidoethanol-based phosphate





Hydrophilicity/solubility

PFCA-PFSA

Perfluoroalkylsulphonate	
TFMS	CF3SO$_3^-$
PFEtS	$C_2F_5SO_3^-$
PFPrS	$C_3F_7SO_3^-$
PFBS	$C_4F_9SO_3^-$
PFHxS	$C_6F_{13}SO_3^-$
PFOS	$C_8F_{17}SO_3^-$

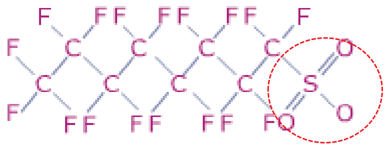
Ultra-short chain PFAS (TFMS, PFEtS, PFPrS)

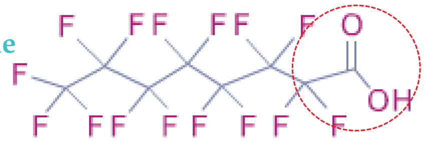
Acids / NO bioaccumulable

Surfactants / bioaccumulable

Perfluoroalkylcarboxylate	
TFA	CF_3COOH
TFPrA	C_2F_5COOH
PFBA	C_3F_7COOH
PFPeA	C_4F_9COOH
PFHxA	$C_5F_{11}COOH$
PFHpA	$C_6F_{13}COOH$
PFOA	$C_7F_{15}COOH$
PFNA	$C_8F_{17}COOH$
PFDA	$C_9F_{19}COOH$
PFUnA	$C_{10}F_{21}COOH$
PFDoA	$C_{11}F_{23}COOH$

Ultra-short chain PFAS (TFA, TFPrA)


PFOS (Perfluorooctane sulfonate)


PFOA (Perfluorooctanoic acid)

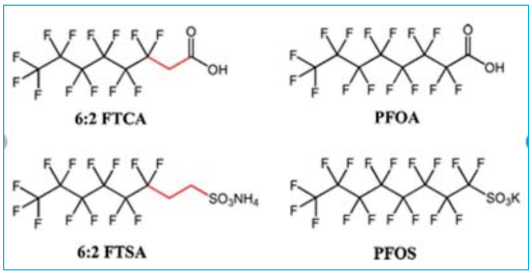
Next Generation PFAS: Shortening the fluorinated carbon chain


PFOS

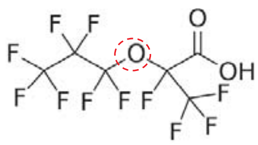

PFBS

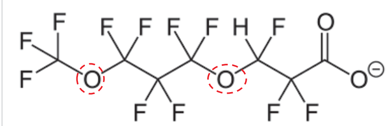
- Shorter-chain homologues
  - POSF-based derivatives → PHxSF-based derivatives, PBSF-based derivatives
  - PFOA → PFHxA

- Replace some fluorinated carbon atoms with non-fluorinated carbon atoms
  - PFOA → 6:2 FTCA



- Add oxygen atoms between fluorinated carbons (ethers: similar)
  - ADONA, GenX





Analytical Strategies

One by one PFAS	Bulk organofluorine measurement
LC-MS & GC-MS & SFC-MS ANALISI TARGET ESI(+/-) WIDE SCOPE SCREENING	Non-specific chemical methods TOF and TOP Assay
LC-HRMS & GC-HRMS NON-TARGETED ANALYSIS	EBM *Effect Based Methods* Bioassays, Biomarker, Ecological indicators

Effect Based Methods

Interferenze da antagonisti
No selettività: metodi di screening

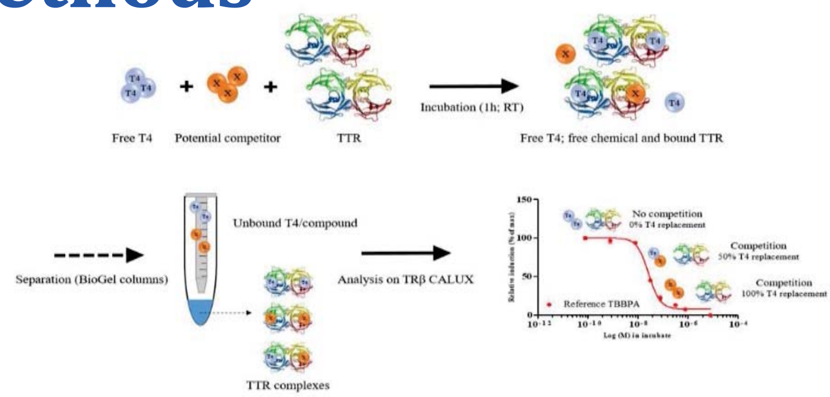


Table 3
Potency factors of tested PFASs compounds in the TTR-TRβ CALUX® bioassay.

Compound	Potency factor IC_{50}-based (PFOA = 1)	Potency factor PC_{80}-based (PFOA = 1)
PFBA	0.0012	**0.0018**
PFPeA	0.048	**0.080**
PFHxA	0.16	**0.19**
PFHpA	1.3	**1.4**
PFOA	1.0	**1.0**
PFNA	0.48	**0.32**
PFDcA	0.12	**0.12**
PFBS	0.10	**0.052**
PFHxS	2.5	**1.6**
PFHpS	2.0	**1.0**
PFOS	3.0	**2.0**
H4PFOS (6:2 FTS)	0.033	**0.019**
PFOSA	1.2	**0.72**

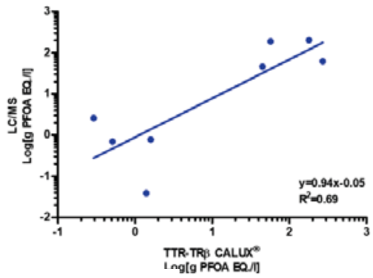
Diagram of the PFAS CALUX® bioassay. Free T4, potential competitor chemical and TTR are incubated as a mixture for 1h at room temperature. Unbound T4 and competing chemical are separated from bound TTR complexes using BioGel

Effect Based Methods

Environment International 157 (2021) 106791

Developing potency factors for thyroid hormone disruption by PFASs using TTR-TRβ CALUX® bioassay and assessment of PFASs mixtures in technical products

Peter A. Behnisch[a], Harrie Besselink[a], Roland Weber[b], Wolfram Willand[c], Jun Huang[d], Abraham Brouwer[a,e,*]



4 Aqueous film-forming foams/Aqueous firefighting foams (AFFF)
2 Chromium mist suppressants (CMS)

Table 6

Correlation between PFOA-equivalents measured directly in the TTR-TRβ CALUX® assays and LC-MS-based PFOA equivalents calculated using PC_{80}-based relative potency factors from TTR-TRβ CALUX® for AFFF surfactants and CMS applications.

	TTR-TRβ CALUX® (g PFOA eq./L)	LC-MS (g PFOA eq./L)	Ratio TTR-TRβ CALUX®/LC-MS (-)
Aqueous Fire Fighting Foams (AFFF) surfactants	45		0.98
VF-368 (2013)			
		46	
VF-570 (2013)	57	190	0.29
VF-9128 (2019)	1.6	0.8	2.0
VF-368 (2013) (after TOP assay)	270	62	4.4
VF-570 (2013) (after TOP assay)	180	203	0.89
VF-9128 (2019) (after TOP assay)	0.51	0.70	0.73
Chromium mist suppressants (CMS)			
FUMETROL® 21	1.4	0.039	36
FUMETROL® 21 (after TOP assay)	0.29	2.6	0.11

Metodi chimici aspecifici

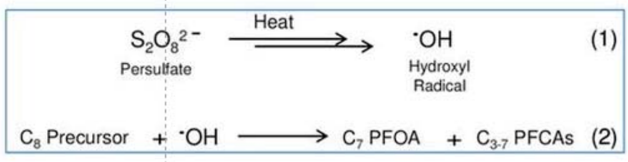
- **Fast, cheap**
- **No identification of the substance (no selectivity)**
- **Low sensitivity (heavily contaminated samples)**
- **Poor extraction efficiency for very polar PFAS**
- **It also measures fluorinated compounds no PFAS**

- TOF Assay (Total OrganoFluorine)
  - EOF (Extractable OrganoFluorine)

  SPE/solvent extraction + CIC (Combustion Ion Chromatography) (~0.1 µg F/L)

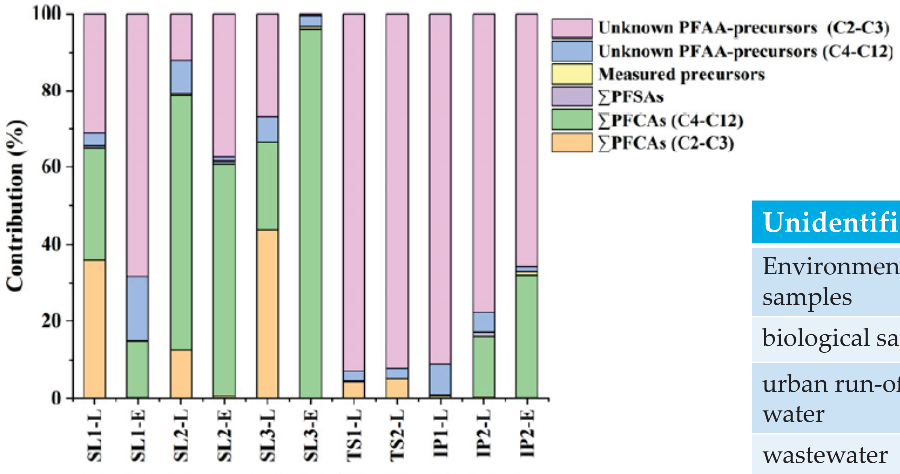
- TOP Assay (Total Oxidizable Precursor) for oxidizable PFAS (precursors)

  It does not "represent" the transformation in environment



[1]Houtz, E.F. and Sedlak, D.L. (2012). Environ. Sci. Technol., 46, 9342-9349.

Metodi chimici aspecifici: PFAS



PFAS in landfill leachates (target analysis + TOP assay)

(Wang et al., Sci Total Environ 2020)

Unidentified OF	TOF Assay	TOP Assay
Environmental samples	50-99%	
biological samples	15-99%	
urban run-off water		23%
wastewater		3-18%
AFFF-impacted groundwater		60%
river water		18-82%

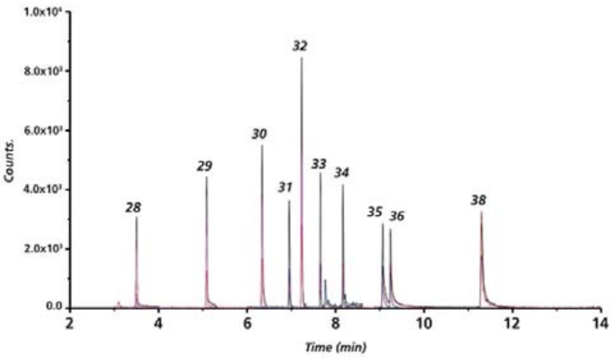
Target analysis: GC-MS (volatili, neutri)

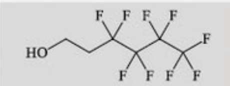
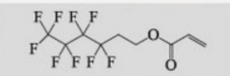
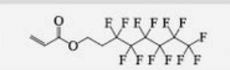
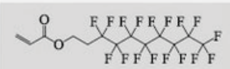
The Determination of Trace Per- and Polyfluoroalkyl Substances and Their Precursors Migrated into Food Simulants from Food Contact Materials by LC–MS/MS and GC–MS/MS

July 1, 2019

Dan Li, Lei Zhu, Jing-jing Pan, Huai-ning Zhong, Zi-hao Zhang, Qin-bao Lin, Jian-guo Zheng, Hui Liu

LCGC North America

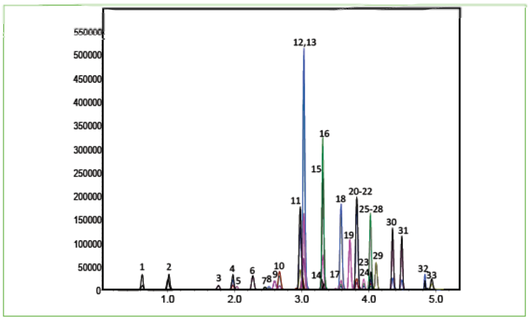


28	1H,1H,2H,2H-Perfluoro-1-hexanol (4:2FTOH)	2043-47-2	
29	1H,1H,2H,2H-Perfluoro-1-octanol (6:2FTOH)	647-42-7	
30	1H,1H,2H,2H-Perfluoro-1-decanol (8:2FTOH)	678-39-7	
31	1H,1H,2H,2H-Perfluoro-1-dodecanol (10:2FTOH)	865-86-1	
32	1H,1H,2H,2H-Perfluorooctylacrylate (6:2FTA)	27619-97-2	
33	1H,1H,2H,2H-Perfluorodecylacrylate (8:2FTA)	17527-29-6	
34	1H,1H,2H,2H-Perfluorodecylacrylate (10:2 FTA)	27905-45-9	
35	N-Methyl-Perfluorooctanesulfonamidoethanol (N-MeFOSE)	24448-09-7	
36	N-Ethyl-Perfluorooctanesulfonamidoethanol (N-EtFOSE)	1691-99-2	

Target analysis: LC-MS/MS (ESI-)

ionisation	ESI-negative mode
Column	C18
eluent	fase mobile acquosa e metanolo/acetonitrile contenente 5-50 mM di acetato di ammonio

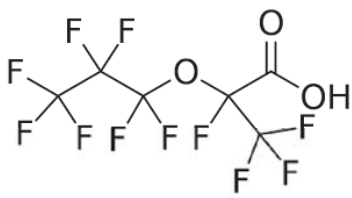
class		Fragment
PFCA	Decarbossilazione in sorgente	$[M-CO_2-H]^-$ $[C_2F_5]^-$ $[C_3F_7]^-$
PFSA		[FSO3]- [SO3]-
FASA		[M-HF-H]-
PFECA	Frammentazione in sorgente Dimeri/trimeri	[M-CF2CO2-H]- $[C_2F_5O]^-$ $[C_3F_7O]^-$



Peak #	Compound	Transition	t_R (min)
1	PFBA	213.0000>169.0000	0.755
2	4:2FTS	229.0000>85.0000	1.031
3	PFPeA	263.0000>219.0000	1.762
4	PFBS	299.0000>80.0000	1.979
5	PFHpS	279.0000>85.0000	2.035
6	PFPeS	315.0000>135.0000	2.273
7	PFMPA	327.0000>307.0000	2.454
8	PFHxA	313.0000>269.0000	2.514
9	PFEESA	349.0000>80.0000	2.599
10	HFPO-DA	285.0000>169.0000	2.670
11	PFHxS	399.0000>80.0000	3.013
12	NaDONA	377.0000>251.0000	3.033
13	ADONA	377.0000>250.9000	3.034
14	FOSA	427.0000>407.0000	3.299
15	PFOA	413.0000>369.0000	3.316
16	PFMBA	449.0000>80.0000	3.328
17	PFHpA	363.0000>319.0000	3.388

Peak #	Compound	Transition	t_R (min)
18	PFOS	499.0000>80.0000	3.588
19	9Cl-PF3ONS	530.9000>351.0000	3.719
20	8:2FTS	549.0000>80.0000	3.816
21	PFNS	527.0000>507.0000	3.820
22	PFDA	513.0000>469.0000	3.822
23	N-MeFOSAA	570.0000>419.0000	3.925
24	PFNA	463.0000>419.0000	3.942
25	NFDHA	599.0000>80.0000	4.015
26	PFUnA	563.0000>519.0000	4.025
27	N-EtFOSAA	584.0000>419.0000	4.029
28	6:2FTS	498.0000>78.0000	4.033
29	11Cl-PF3OUdS	630.7000>451.0000	4.110
30	PFTrDA	663.0000>619.0000	4.355
31	PFDoA	613.0000>569.0000	4.496
32	PFTeDA	713.0000>669.0000	4.745
33	PFDS	295.0000>201.0000	4.921

PFECA: GenX



L. Mullin et al. / Trends in Analytical Chemistry 118 (2019) 828–839

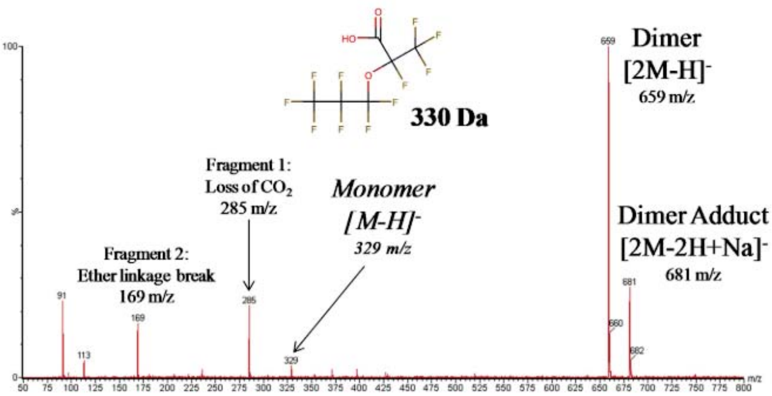
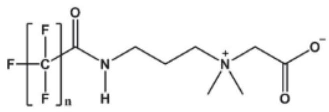


Fig. 2. Spectrum of HFPO-DA derived from full scan acquisition on a Xevo TQ-D (Waters Corporation) following injection using 2 mM ammonium acetate (pH 5.0) in approx. 50/50 water/methanol and separation on an Acquity BEH C_{18} UPLC column. Under these typical method conditions, minimal $[M-H]^-$ formation is apparent.

Target analysis: Zwitterionic and cationic PFAS (ESI+)

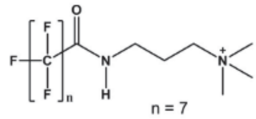
G. Munoz et al. / Talanta 152 (2016) 447–456

PFOAB



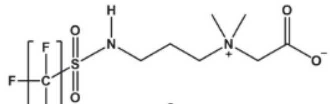
n = 7

PFOAAmS



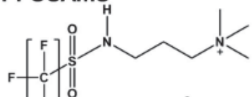
n = 7

PFOSB



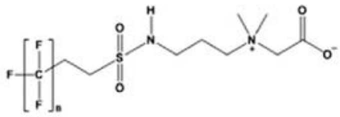
n = 8

PFOSAmS

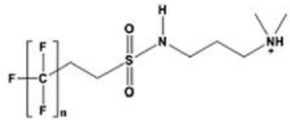


n = 8

PFOANO

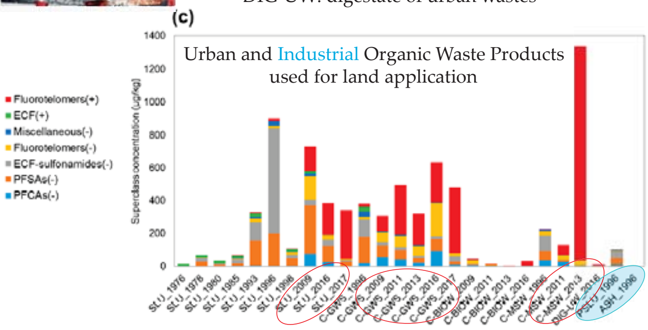


X:2 FTSA-PrB
X:2 fluorotelomer sulfonamidopropyl betaines



X:2 FTSA-PrDiMeAn
X:2 fluorotelomer sulfonamidopropyl dimethyl amines

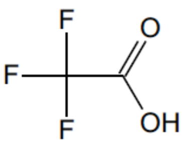
SLU: sewage sludge
C-GWS: compost of green wastes and sludge
C-MSW: compost of municipal solid waste
DIG-UW: digestate of urban wastes



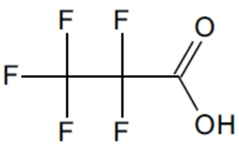
Sewage sludge composted or not, compost of municipal solid wastes and biowastes, and paper sludge and ashes (Munoz, *et al*. 2021. Environ. Sci. Technol.)

Ultra-short-chain PFAS$_{C2-C3}$

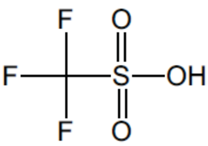
TFA



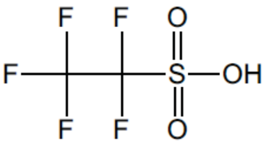
PFPrA



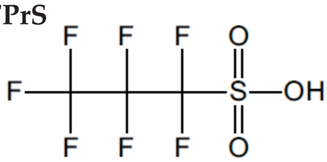
TFMS



PFEtS



PFPrS



Acid
Persistent
Low bioaccumulable + high solubility

High accumulation in environmental water bodies (drinking water)

C2-C3 accounted for >40% of the total PFAS in rain samples (Canada)
TFA is the most abundant PFAS in rainwater (Japan)
PFEtS and PFPrS are detected in AFFF
TFA detected in fluopolymer and pharmaceutical industries discharges
PFEtS detected in fluorochemical industry discharges

Target analysis:
Ultra-short-chain PFAS$_{C2-C3}$

Technique	strengths	limitations
Water concentration by rotary evaporation		Time consuming
WAX-SPE	Most common extraction technique for PFAS	affected by the pH and matrix of the samples
Direct injection	Enough sensible for environmental sample	
GC-MSD / GC-ECD		Derivation step
LC-MS/MS or HRMS (ion-exchange or mixed-mode columns)	Ultra-short + other PFAS	Matrix interferences Peak shape
SFC-MS/MS	**most time-efficient separation technique high chromatographic performance**	**SFC instrumentation**

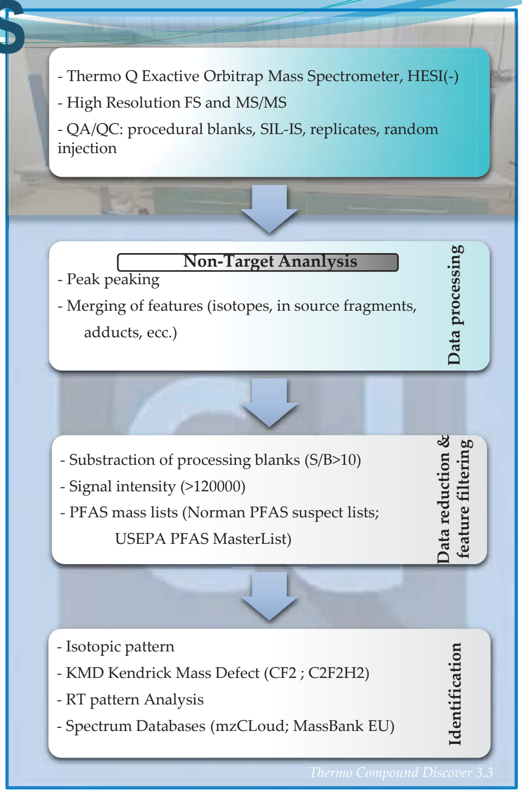
NON-TARGETED ANALYSIS

1) Producing HRMS full-scan data

- RP ≥ 60,000 for compounds composed only of C, H, O, N, F and P
- RP ≥ 100,000 for analytes that may additionally contain a sulfur atom (to differentiate between the 3.37 mDa mass split of C3 and H4S)

2) Selecting prospective PFAS feature identification

- Feature data-reduction
  - grouping by stable isotopes and adducts (e.g. dimer, trimer)
  - background signal removal
  - intensity threshold cut-off
- Feature data-filtering
  - Study design (e.g. case vs control)
  - mass defect filtering (18.9984 DA; Δm/z=-0.0016; -0.1 a +0.15 rounding) e.g. m/z 460.9267; m/z 626.9120
  - Matching to database of suspect (suspect screening)
  - Homologous series searching / RT pattern Analysis



NON-TARGETED ANALYSIS

Feature data-filtering

- Homologous series searching / RT pattern Analysis

n:3 FTCA CF3 CF2 CF2 CF2 CH2CH2COOH

KDM-$CF_2C_2H_2$=0.034 (round

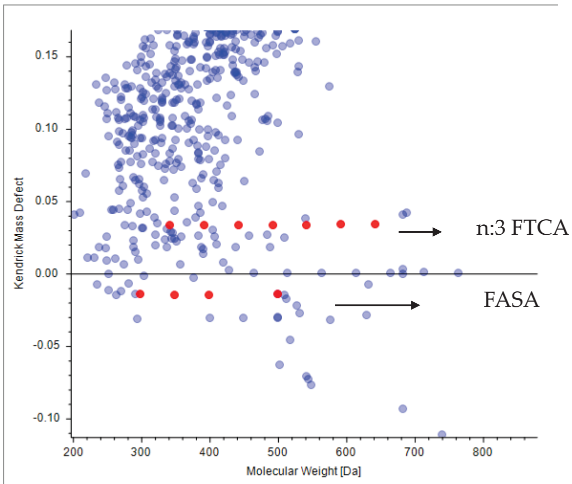
FASA CF3 CF2 CF2 CF2 CF2 CF2 SO3NH2

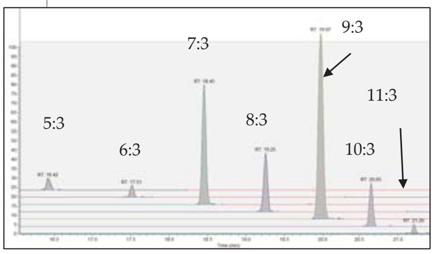
KDM-CF_2= -0.014 (round)

$$\text{exactKendrickMass} = m/z^* \frac{\text{nominal mass (repeating unit)}}{\text{exact mass (repeating unit)}}$$

KendrickMassDefect (KMD)=nominal KM-exactKM

PFAS homologue m/z spacing
CF2 (49.997)
CF2CF2 (99.994)
CH2CF2 (64.012)
CF2O (65.991)….





NON-TARGETED ANALYSIS

2) prospective PFAS feature identification

Feature data-filtering

- PFAS retention time flagging through diagnostic fragments (e.g. $C_2F_5^-$) or neutral losses (e.g. HF) in addition to FS
    - DDA (Data-Dependent Acquisition)
    - DIA pre-defined m/z windows (Data-Independent Acquisition)
    - AIF (all ion fragmentation), "All Ions MS/MS", MS^E
    - ISF (in-source- fragmentation)

Class coverage (CD): capturing MS1 precursors from designated MS2 fragments

- $[C_2F_5]^-$ (m/z 118.992)
- $[C_3F_7]^-$ (m/z 168.988)
- $[SO_4H]^-$ (m/z 96.959)……

Y. Liu et al. / Trends in Analytical Chemistry 121 (2019) 115420

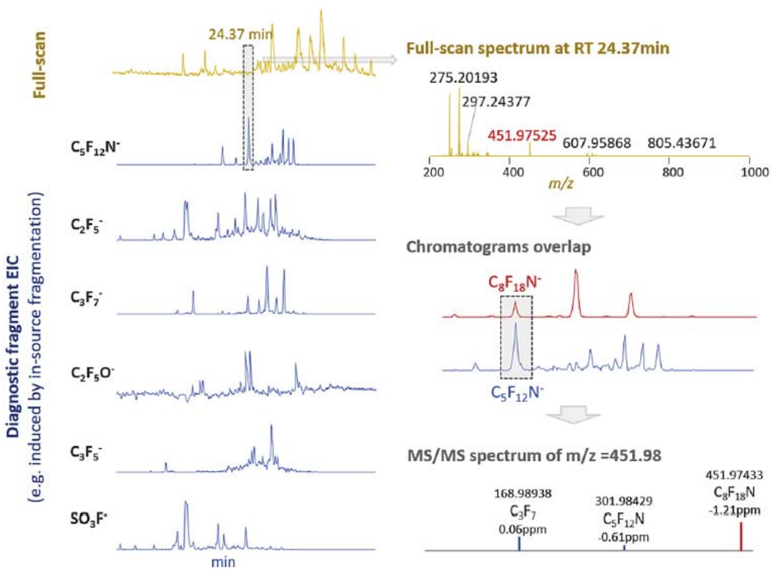


Fig. 3. Example workflow showing PFAS identification by diagnostic F-containing fragments and subsequent confirmation steps.

NON-TARGETED ANALYSIS

3) Molecular formula assignment

- Matching to database of suspects (suspect screening)

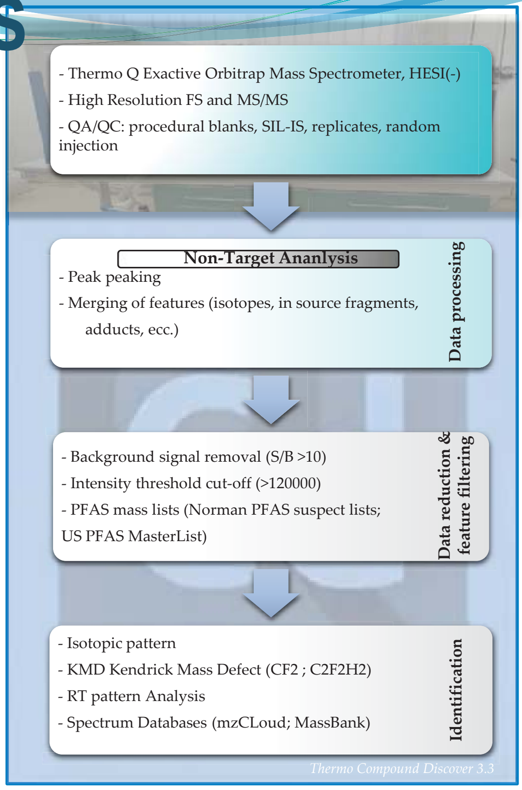
4) Structural characterization by MS/MS^n (n≥2)

- DDA additional acquisition avoidable
- DIA, AIF or ISF additional acquisition needful

5) Structural proposal (Confidence level) and confirmation

- Based on MS^n profile
  - MS/MS database (mzCLoud; MassBank)
  - in-silico fragmentation (Suspect screening)
- Matching to database of suspects (EPA PFAS master list; NormanSLE)
- RT pattern Analysis
- Standard comparison

Charbonnet, et al. Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry. Environ Sci Technol Lett. 2022



PFAS: Suspect Screening via Mass Lists

norman

NORMAN Suspect List Exchange – NORMAN SLE

Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances

MassBank

High Quality Mass Spectral Database

NIST PUBLIC DATA REPOSITORY 1.8.0

Public Data Resource

Suspect List of Possible Per- and Polyfluoroalkyl Substances (PFAS)

EPA United States Environmental Protection Agency

Environmental Topics | Laws & Regulations

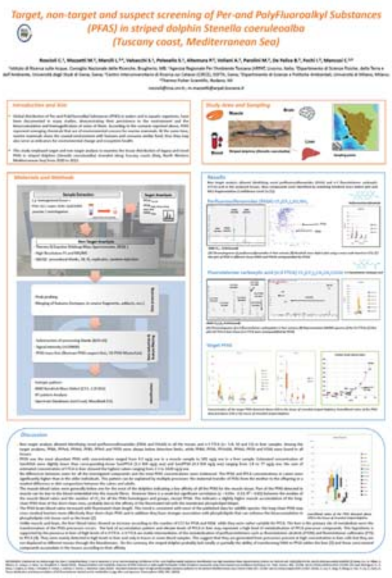
CompTox Chemicals Dashboard | Home

m/z CLOUD™

Advanced Mass Spectral Database

Target, non-target and suspect screening of Per- and PolyFluoroalkyl Substances (PFAS) in striped dolphin Stenella coeruleoalba (Tuscany coast, Mediterranean Sea)

Roscioli C.[1], Mazzetti M.[2], Marsili L.[3,4], Valsecchi S.[1], Polesello S.[1], Altemura P.[2], Voliani A.[2], Parolini M.[5], De Felice B.[5], Fochi I.[6], Mancusi C.[2,3]



GRAZIE PER L'ATTENZIONE

Italian Mass Spectrometry Society

IMaSS Emerging Contaminants Day

Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

Special focus: Novel Strategies for the Analysis of New Generation PFAS

Bologna, September 15, 2022
Aula Magna U.E.1 - Polo Didattico Navile
Via della Beverara 123/1

Claudio CARRER – Loretta GALLOCCHIO

L'importanza dei PTP dinamici per la valutazione delle prestazioni dei test valutativi di laboratorio utilizzando matrici naturali - PFAS

IMaSS Emerging Contaminants Days Traditional and "Emerging" PFAS: a Common Concern for Environment and Food Science, Connected by Food Packaging Issues

The importance of dynamic PTPs for performance evaluation of laboratory proficiency testing using natural matrices - PFAS

Claudio Carrer, Loretta Gallocchio
InterCind-Lab Service Analytica

InterCinD started in 2009 by collaboration between INTERCAL and CIND.

This collaboration, for instance, was intended to offer to participant laboratories a larger flexibility in the number of samples and in the sample type by using similar study design and statistics. The improvements and benefits of this collaboration are now fully integrated in the InterCinD.

Essence of InterCinD are the materials used, all REAL MATRIX available in NATURE, prepared and characterized according to the international guidelines for the production of reference materials.

WHY PARTICIPATE IN PTS ?

UNI CEI EN ISO/IEC 17025:2018

- Assess and monitor performance
- Identify problems and implement actions for risk mitigation and improvement
- Define the effectiveness and comparability of test methods
- Ensure greater reliability for laboratory customers
- Validate uncertainty statements

What criteria should be used when choosing the right PTP and PT?

4 Things to consider when choosing a PT provider:

1. Accreditation ISO17043
2. Type of sample
3. Parameter selection
4. Comunication

Accreditation UNI CEI EN ISO/IEC 17043

The PTP that has achieved accreditation according to UNI CEI EN ISO/IEC 17043:2010 **demonstrates competence** in designing, preparing, managing and organising the tests specified in the scope of accreditation.

An accredited PTP demonstrates, with the Accredia mark, the achievement of conformity to the standard, specific competence and fuels consumer confidence in the service provided.

InterCIND accredited ACCREDIA Nr. PTP0007 P since February 2015 is a globally recognised PTP for POP schemes.

Type of sample

The accredited Proficiency Testing scheme in which a laboratory participates must be as similar as possible to the activities and operations that the laboratory itself routinely performs, in terms of measurement standards, test materials and type of measurement.

PT suppliers should provide naturally contaminated materials (NCMs). In these 'real samples', the analyte is naturally incorporated into the matrix and allows a **more realistic evaluation than in artificially fortified samples.**

INTERCIND offers evaluation tests for various environmental parameters and matrices, using only naturally contaminated materials and homogenous samples.

Parameter selection

The matrices, analytes and **concentrations** used should fit in with routine laboratory practices.
On natural matrices, it is not always possible to guarantee uniformity across all parameters under investigation.

Comunication

The necessary information must then all be readily available and understandable.
The results must be provided in a timely manner.

INTERCIND PROGRAM 2023

FEBRUARY	IC2023 PFAS	PFAS	LEACHATE
MARCH	IC2023SSE/1	PCDD/F	WATER
		PCB	WATER
APRIL/MAY	IC2023SSE/2	PCDD/F	SEDIMENT
			FLY-ASH
			FEED-FOOD
		PCB	SEDIMENT
			FLY-ASH
			FEED-FOOD
SEPTEMBER	IC2023SSE/3	PCDD/F	SOIL
			FLY-ASH
		PCB	SOIL
			FLY-ASH
		METALS	SOIL
OCTOBER	IC2023 ODOR	2-BUTHANOL	GAS
		THT	GAS

DRAFT

https://www.intercind.eu

How to create a proficiency test

- Matrix identification
- Characterisation
- The technical director decides whether the matrix is interesting or not.
- If positively evaluated, the matrix is appropriately processed, checked and sent to the homogeneity verification phase.

Homogeneity

The homogeneity of the matrices is assessed before distribution to the participants to ensure that it does not affect the performance evaluation.
The homogeneity valutation is done in conformity to UNI ISO 13528:2022 annex B.
The results of the homogeneity tests are available in the final report by provider.

The matrix is considered to be adequately homogeneous if:

$$S_s \leq 0.3\ \sigma PT$$

where:
Ss: is standard deviation between samples
σPT is PT deviation standard

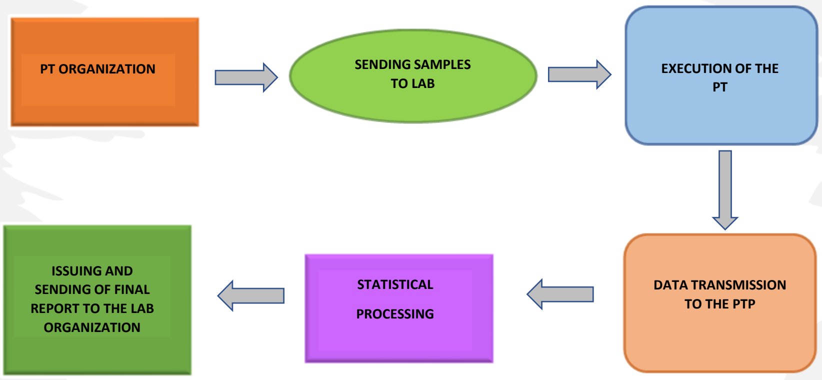
Stability

The same matrices are tested for stability in conformity UNI ISO 13528:2022 annex B
Usually, the PTP tests the matrix immediately before and after the PT or, in special cases, during the proficiency test to determine any deviation.
Stability is verified for each analyte to be determined in the PT.

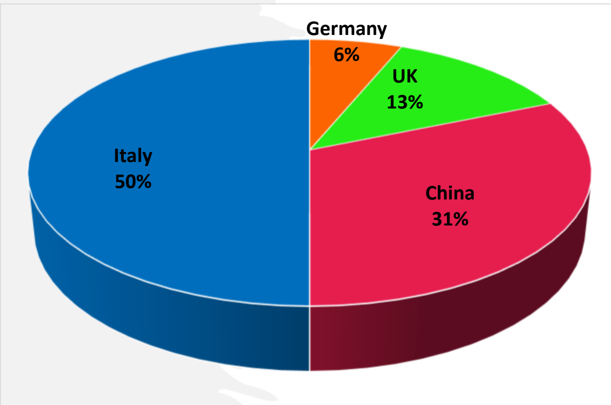
$$|y_i - y| \leq 0,3 \times \sigma_{PT}$$

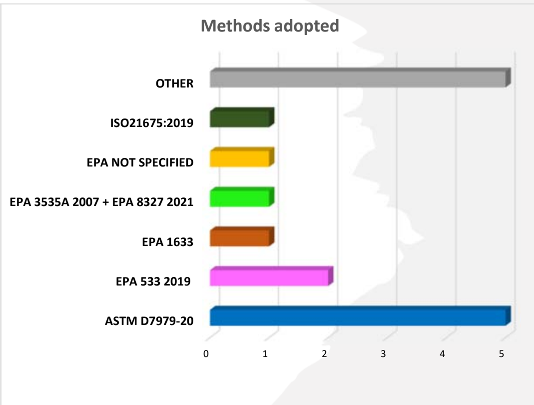
where:
yi is the average value of the results of the last phase of the stability test
y is the average value of the results of the first phase of the stability or homogeneity test
σ_{PT} is the standard deviation of the PT

PT SCHEME

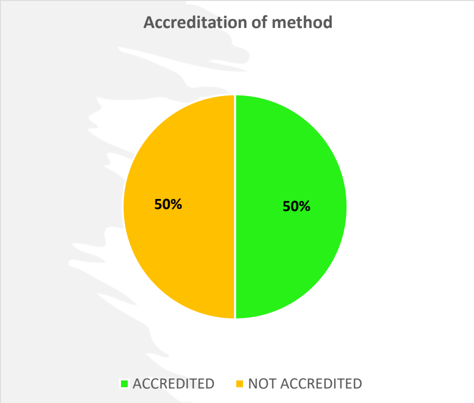


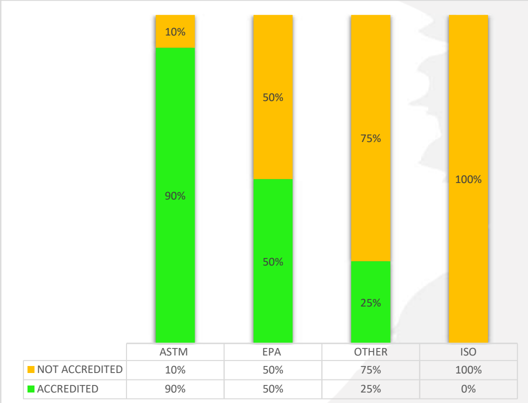
Country of provenience of the laboratories participating to the InterCinD IC-PFASWA 2022 edition.





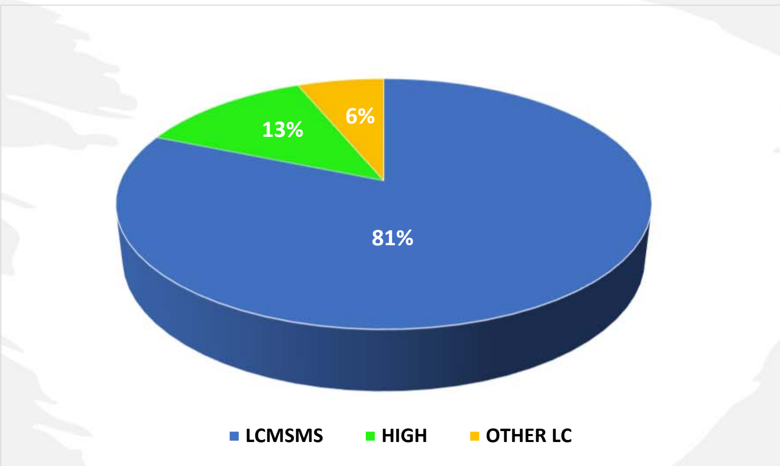
MORE INFO





	ASTM	EPA	OTHER	ISO
NOT ACCREDITED	10%	50%	75%	100%
ACCREDITED	90%	50%	25%	0%

System adopted



CONSENSUS VALUE OR ASSIGNED VALUE AND UNCERTAINTY

UNI ISO 13528:2022, requires that the consensus value
the assigned value is calculated as a robust mean, median or arithmetic mean
The uncertainty of the assigned values u (xPT) is calculated according to ISO 13528: 2022 as:

$$u\,(x\mathrm{PT}) = 1{,}25 \times s^{*}/\sqrt{p}$$

where :
s *: robust standard deviation
p: the number of results

The UNI ISO 13528: 2022 standard put a limit on the value of $u\,(x\mathrm{PT})$. The following relationship must be verified:

$$u\,(x\mathrm{PT}) < 0.3\ \sigma_{PT}$$

Target standard deviation of a PT determines the performance limits in a proficiency test

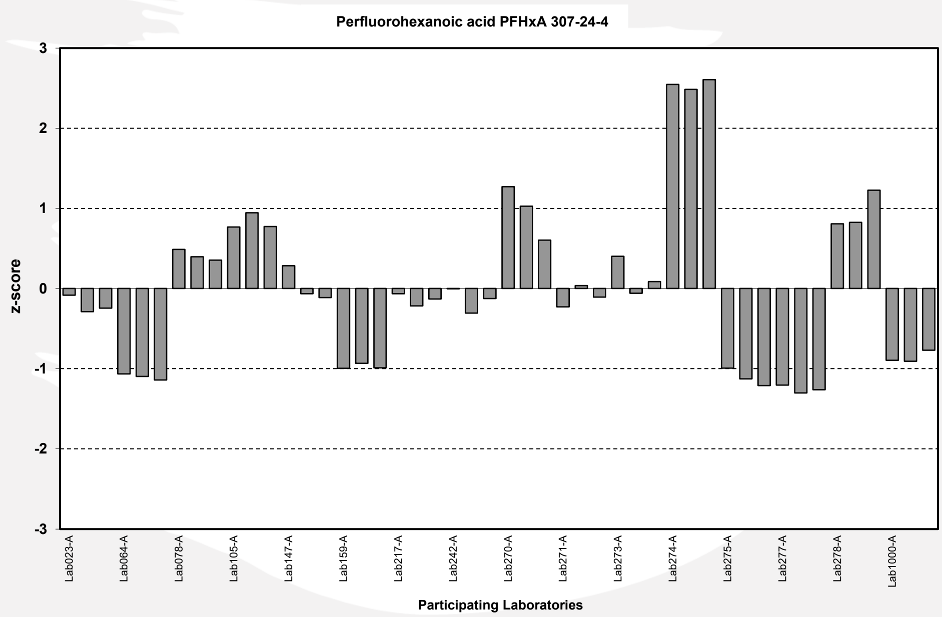
WASTEWATER - Table 6.1	Summary statistics of dataset																Consensus	
	ND	NA	O	E	Valid data	Labs	average	median	S	u	Q25	Q75	Min	Max	U(Q75-Q25)	RSD%	X	σ_p
PFPAS [ppb]																		
Perfluorooctanoic acid PFOA 335-67-1	0	0	0	0	48	16	90.1939	82.9031	24.3249	6.0812	70.9242	112.6792	57.4800	132.7333	41.7550	0.2697	83	31
Perfluorooctane sulfonic acid PFOS 1763-23-1	2	0	0	0	42	14	14.9817	15.5833	5.7340	1.5325	10.3517	17.5333	7.0333	24.0000	7.1817	0.3827	15.0	5.7
Perfluorobutanoic acid PFBA 357-22-44	0	2	0	1	39	13	63.2189	61.3333	10.4536	2.8993	54.2267	69.6667	51.7067	81.6667	15.4400	0.1654	63.2	10.5
Perfluoropentanoic acid PFPeA 2706-90-3	0	0	0	0	48	16	56.5029	56.2967	19.6946	4.9236	49.2717	71.2892	13.5300	84.6667	22.0175	0.3486	57	20
Perfluorohexanoic acid PFHxA 307-24-4	0	0	0	0	48	16	73.0625	71.1133	16.4774	4.1194	58.4509	81.5792	52.3500	115.0000	23.1282	0.2255	73	16
Perfluoroheptanoic acid PFHpA 375-85-9	1	0	0	0	45	15	16.0651	16.7333	3.9235	1.0130	12.8150	18.5600	10.3000	24.9333	5.7450	0.2442	16.1	3.9
Perfluorobutane sulfonic acid PFBS 375-73-5	0	0	0	0	48	16	318.1931	315.6250	55.7658	13.9414	270.5365	362.6883	237.3933	404.6667	92.1519	0.1753	318	56
Perfluorohexane sulfonic acid PFHxS 355-46-4	4	0	0	0	32	12	7.5937	7.2467	2.0293	0.5858	6.2933	8.2992	5.2000	12.2167	2.0058	0.2672	7.6	2.4
FOUEA (2H-Perfluoro-2-decenoic acid) 70887-84-2	0	16	0	0	0	0	-	-	-	-	-	-	0.0000	0.0000	-	-	-	-
Fluorotelomer sulfonic acid 6: 2 FTS	0	16	0	0	0	0	-	-	-	-	-	-	0.0000	0.0000	-	-	-	-
Perfluorononanoic acid PFNA 375-95-1	0	16	0	0	0	0	-	-	-	-	-	-	0.0000	0.0000	-	-	-	-
Perfluorodecanoic acid PFDA 335-76-2	0	16	0	0	0	0	-	-	-	-	-	-	0.0000	0.0000	-	-	-	-
Perfluoroundecanoic acid PFUnDA 2508-94-8	0	16	0	0	0	0	-	-	-	-	-	-	0.0000	0.0000	-	-	-	-
Perfluorododecanoic acid PFDoA 307-55-1	12	2	0	0	6	2	8.6950	8.6950	8.5395	6.0383	5.6758	11.7142	2.6567	14.7333	6.0383	0.9821	-	-
HFPO-DA - Hexafluoropropylene oxide-dimer acid 13252-13-6	9	6	0	0	0	1	0.5367	0.5367	-	-	0.5367	0.5367	0.5367	0.5367	0.0000	-	-	-
PFTrDA - Perfluoro-n-tridecanoic acid 72629-94-8	9	5	0	0	6	2	4.9417	4.9417	6.8707	4.8583	2.5125	7.3708	0.0833	9.8000	4.8583	1.3904	-	-

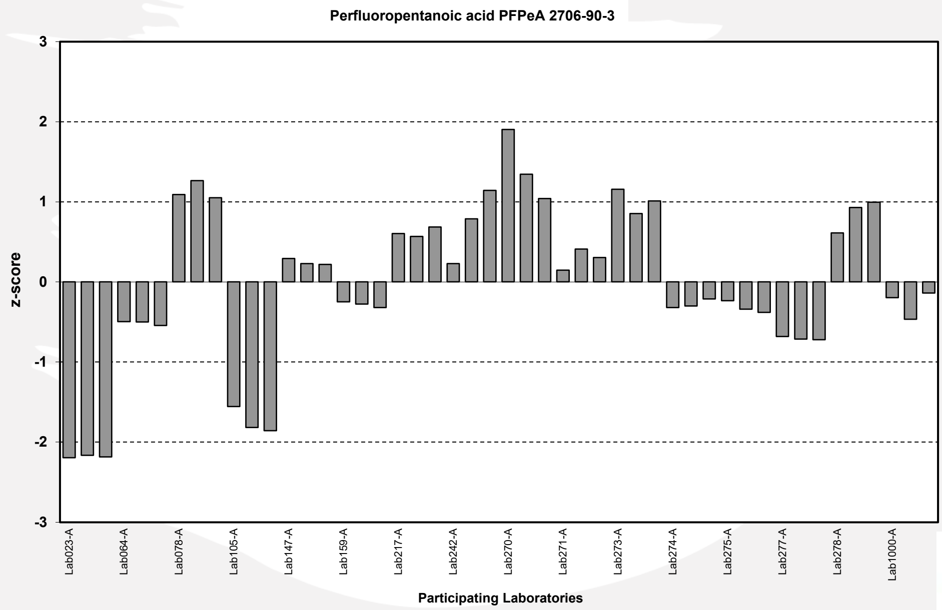
Z score

$$z_i = (x_i - x_{PT})/\sigma_{PT}(FFP)$$

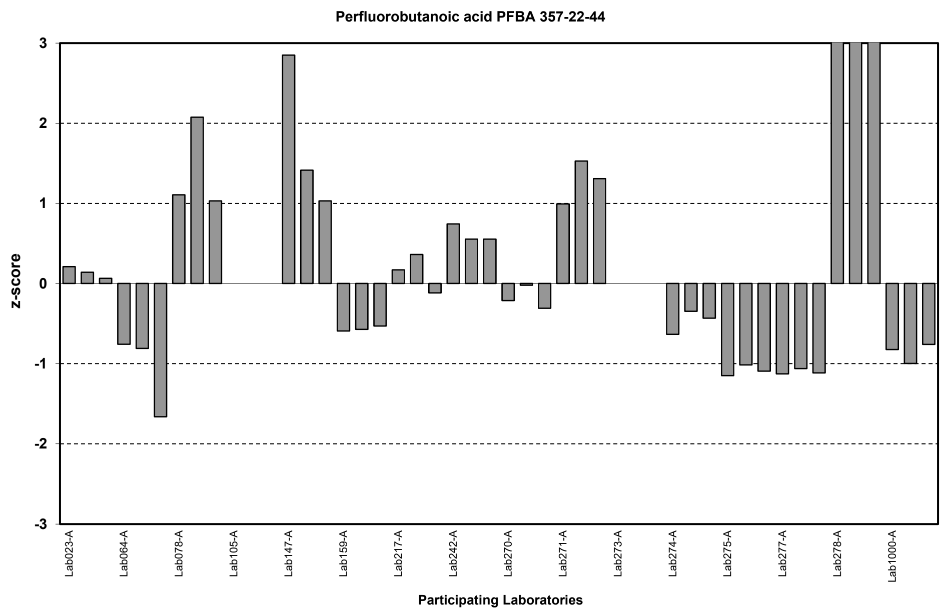
Where:

| z | ≤ 2.0 are considered still of reasonable accuracy
2.0 <| z | < 3.0 are considered of low accuracy
| z | ≥ 3.0 are considered indicative of poor accuracy

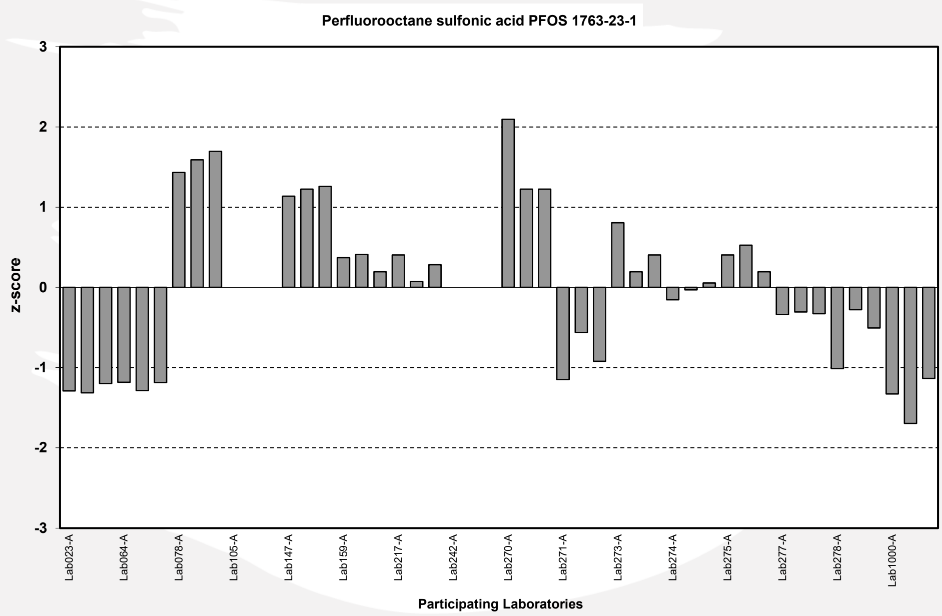




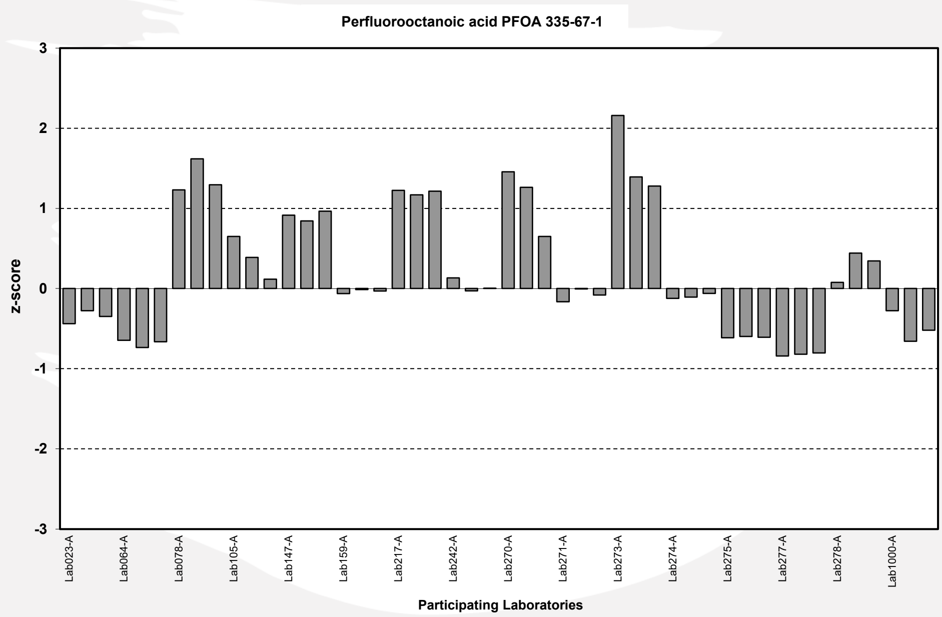
IMaSS Emerging Contaminants Days 15 September 2022



IMaSS Emerging Contaminants Days 15 September 2022



IMaSS Emerging Contaminants Days 15 settembre 2022



IMaSS Emerging Contaminants Days 15 September 2022

CONCLUSION

PTs are one of the most important tools that laboratories can must put in place to guarantee their performance.

Qualora la condizione $u(x_{PT}) < 0.3\ \sigma_{PT}$ non fosse rispettata, ossia $u(x_{PT}) > 0.3\ \sigma_{PT}$, il calcolo di z-score avviene con un'altra relazione:

$z' = (x_i - x_{PT})/\sqrt{(\sigma_{PT}^2 + u(x_{PT})^2)}$

Italian Mass Spectrometry Society

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Alessandro PELLIZZARO - Massimo FANT

Evaluation of perfluoroalkyl acids uptake by hydroponic crops and emerged macrophytes: from extraction to LC-MS/MS determination

UNIVERSITÀ DEGLI STUDI DI PADOVA

DAFNAE
Department of Agronomy Food Natural resources Animals Environment

Acque del Chiampo s.p.a.
Servizio Idrico Integrato

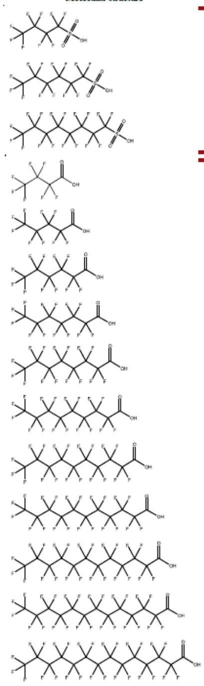
Evaluation of perfluoroalkyl acids uptake by hydroponic crops and emerged macrophytes: from extraction to LC-MS/MS determination

Pellizzaro A.[a], Dal Ferro N.[b], Fant M.[a] & Borin M.[b]

a) Acque del Chiampo S.p.A. – Servizio Idrico Integrato, Via Ferraretta 20, 36071 Arzignano, Italy
b) Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy

Intro: perfluoroalkyl acids (PFAAs)

Abbreviation	Name	CAS number	Molecular structure
PFBS	perfluorobutanesulfonic acid	59933-66-3	
PFHxS	perfluorohexanesulfonic acid	355-46-4	
PFOS	perfluorooctanesulfonic acid	754-91-6	
PFBA	perfluorobutanoic acid	375-22-4	
PFPeA	perfluoropentanoic acid	2706-90-3	
PFHxA	perfluorohexanoic acid	307-24-4	
PFHpA	perfluorohepanoic acid	375-85-9	
PFOA	perfluorooctanoic acid	335-67-1	
PFNA	perfluorononanoic acid	375-95-1	
PFDA	perfluorodecanoic acid	335-76-2	
PFUnDA	perfluoroundecanoic acid	2058-94-8	
PFDoDA	perfluorododecanoic acid	307-55-1	
PFTrDA	perfluorotridecanoic acid	72629-94-8	
PFTeDA	perfluorotetradecanoic acid	376-06-7	



Perfluoroalkane sulfonic acids (PFSAs, CnF2n+1 SO3H) and perfluoroalkyl carboxylic acids (PFCAs, CnF2n+1COOH) from Wang et al. (2011)

PFSAs

3 Perfluorosulfonic acids (C4 – C8)

PFCAs

11 Perfluorocarboxilic acids (C3 – C13)

Objectives

1. Evaluation of PFAAs model uptake by different hydroponic crops
2. Assessment of PFAAs uptake by aquatic macrophytes

Estimation of human exposure to PFAAs through food chain

Assessment of wastewater phytoremediation by selected plants

Experimental set-up

- Unheated greenhouse
- T and U% monitoring
- Two hydroponic tests sessions (leafy crops)
- Constructed wetlands mesocosm (emerged aquatic macrophytes)

IMaSS Emerging Contaminants Days – Bologna September 15, 2022

Experimental set-up

- Plants were exposed to PFAAs spiked solutions and wastewaters
- Tested waters were added of nutrients (N, P, K, Ca, Mg,…)

- Determination of PFAAs concentration in roots and leaves
- Determination of bioaccumulation factors (RCF, LCF, TF)

Experimental set-up: PFAAs determination


HARVESTING

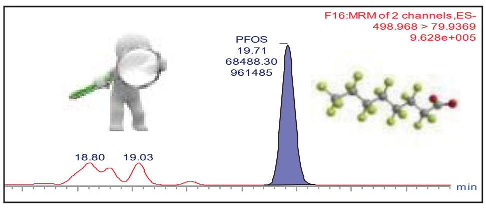

DRYING


HOMOGENIZATION


EXTRACTION


CLEAN-UP


INTRUMENTAL ANALYSIS



PFAAs QUANTITATION

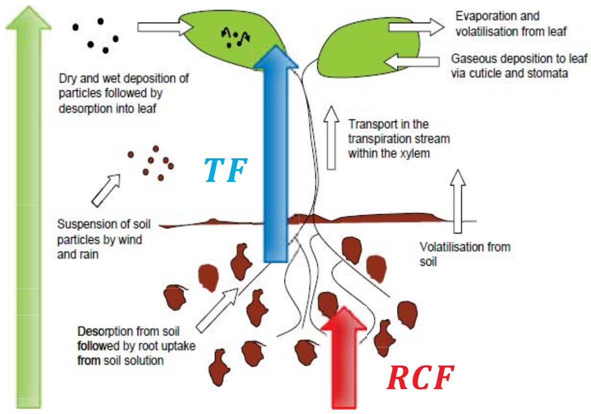
Plant uptake evaluation

- PFAAs concentration in leaves and roots
- Vegetation biomass
- Bioconcentration factors:

$$RCF = \frac{PFAA\ concentration\ in\ roots\ \ (ng\ g_{dw}^{-1})}{PFAA\ concentration\ in\ nutrient\ solution\ \ (ng\ mL^{-1})}$$

$$LCF = \frac{PFAA\ concentration\ in\ leaves\ (ng\ g_{dw}^{-1})}{PFAA\ concentration\ in\ nutrient\ solution\ (ng\ mL^{-1})}.$$

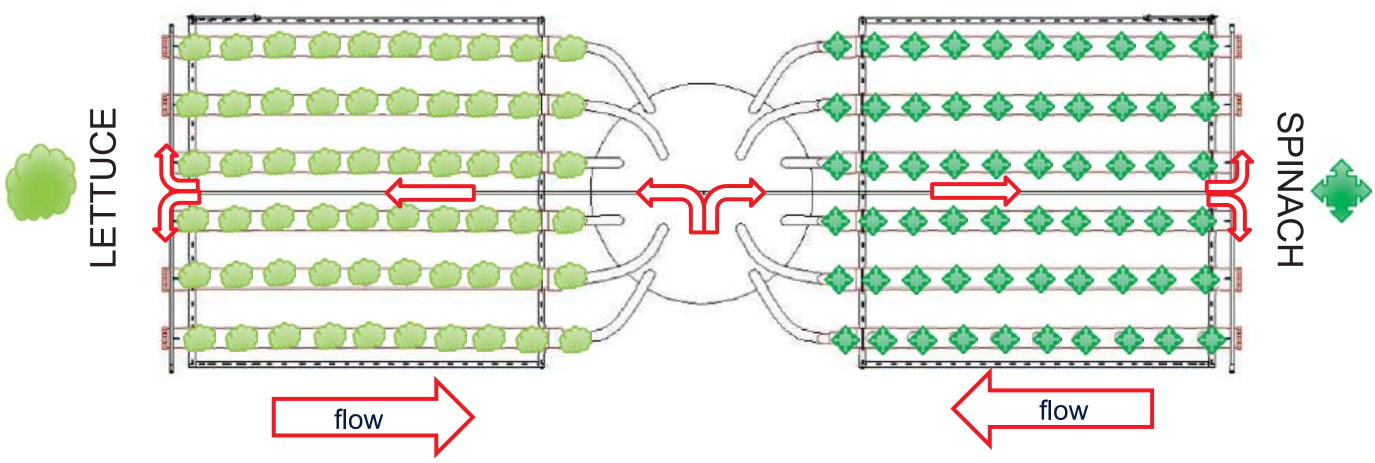
$$TF = \frac{PFAA\ concentration\ in\ leaves\ (ng\ g_{dw}^{-1})}{PFAA\ concentration\ in\ roots\ \ (ng\ g_{dw}^{-1})}$$



Principal pathways for plant uptake of organic chemicals from «Evaluation of models for predicting plant uptake of chemicals from soil» (Collins et al., 2006)

- PFAA concentration in water (nutrient solution) and substrate
- No loss of PFAAs degradation or volatilization was considered

Hydroponics: scheme

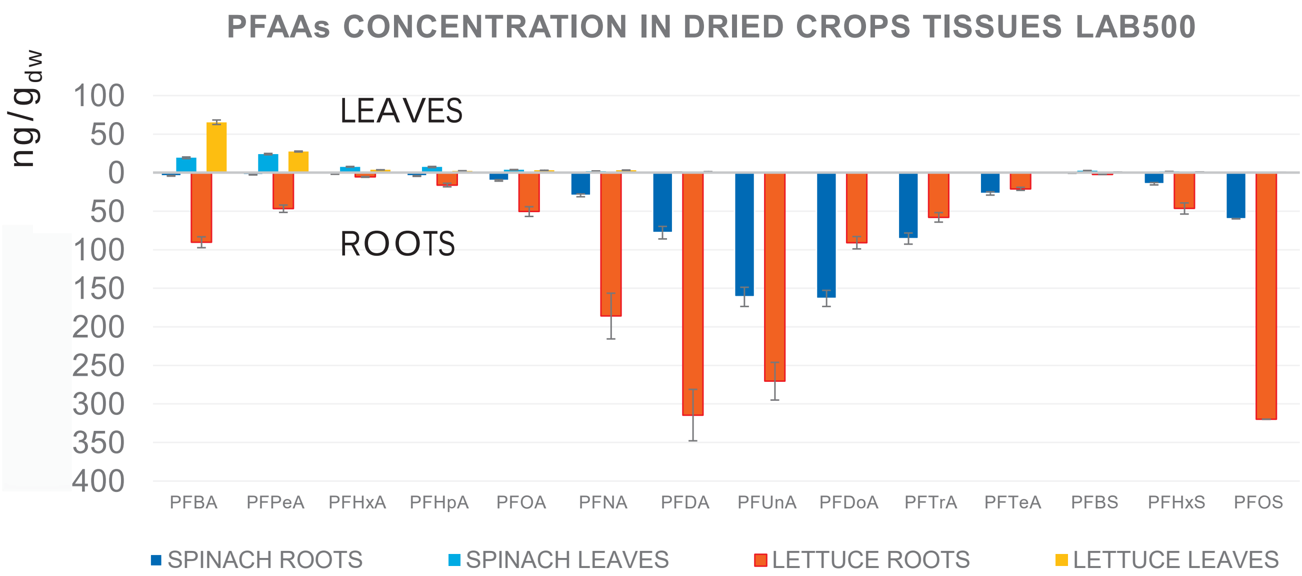


- Two test sessions (oct – dec 2019, feb – apr 2020)
- All batches hosted 120 plants, 60 plants per horticoltural species
- 12 plants per crop species and per batch were analyzed

Hydroponics: tested waters

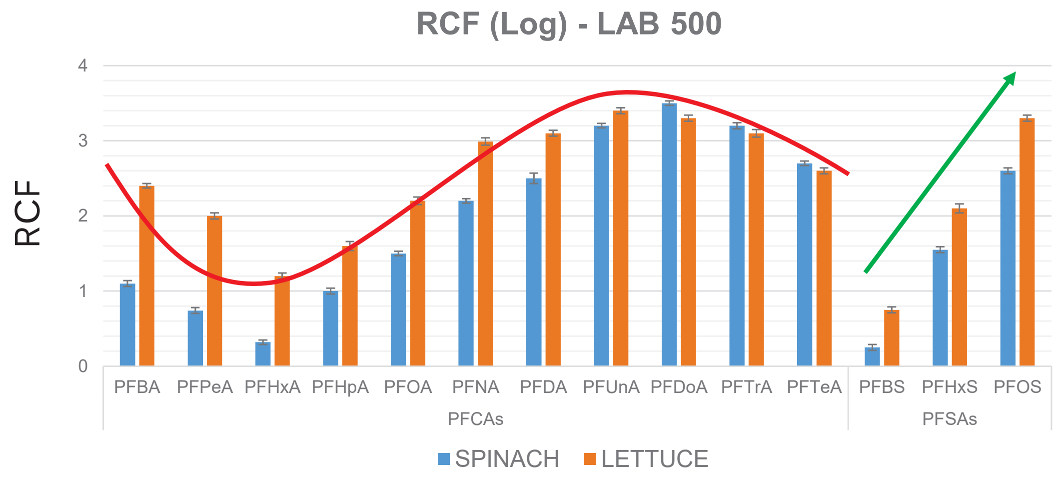
PFAA (ng L^{-1})	Perfluorinated C-chain length	1st session (Oct – Dec 2019)			2nd session (Feb – Mar 2020)	
		LAB500	$WWTP_{Lo}$	$WWTP_{Mo}$	LAB5000	$WWTP_{Arz}$
PFBA	3	500	82	15	5000	41
PFPeA	4	500	187	13	5000	52
PFBS	4	500	49	12	5000	681
PFHxA	5	500	321	11	5000	33
PFHpA	6	500	32	< 10	5000	< 10
PFHxS	6	500	< 10	< 10	5000	< 10
PFOA	7	500	220	17	5000	40
PFOS	8	500	8	8	5000	10
PFNA	8	500	< 10	< 10	5000	< 10
PFDA	9	500	< 10	< 10	5000	< 10
PFUnA	10	500	< 10	< 10	5000	< 10
PFDoA	11	500	< 10	< 10	5000	< 10
PFTrA	12	500	< 10	< 10	5000	< 10
PFTeA	13	500	< 10	< 10	5000	< 10
∑PFAA		7000	899	76	70000	812

Hydroponics: results



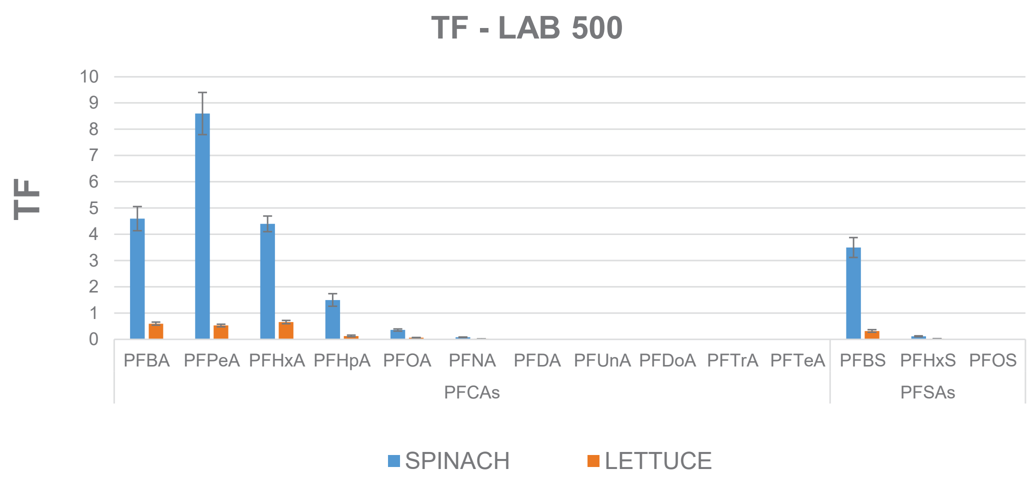
- ➢ Most of PFAAs concentrations in roots were higher than leaves for both crops
- ➢ Most of PFAAs concentrations in lettuce biomasses were higher than spinach

Hydroponics: results



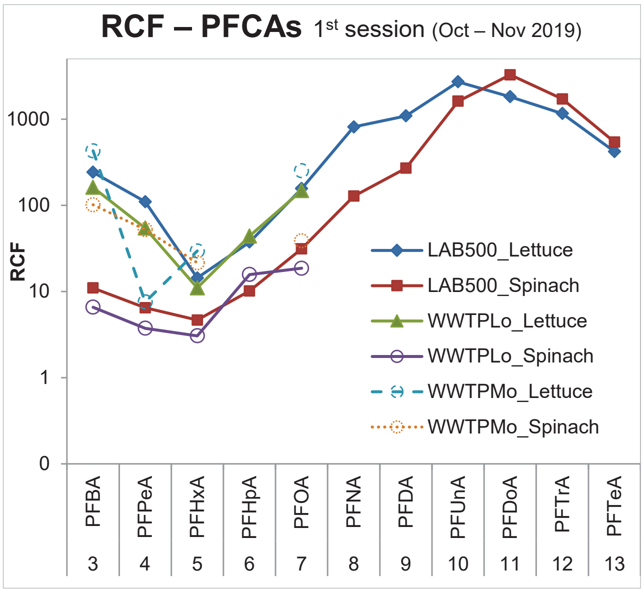
- PFCAs RCFs: sigmoidal pattern
- PFSAs RCFs increased with increasing of perfluorinated chain length
- RCF lettuce > RCF spinach except from PFDoA to PFTeA

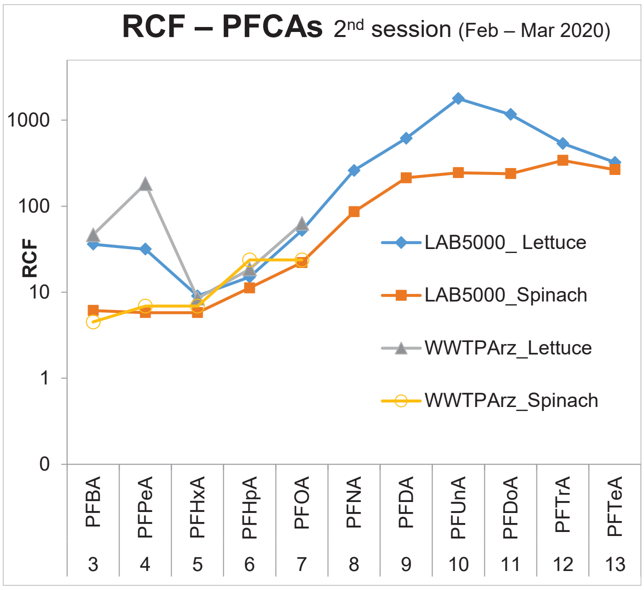
Hydroponics: results



- TF value decreased with increasing perfluorinated C-chain length
- TF of spinach resulted always higher than lettuce (up to 15 times)

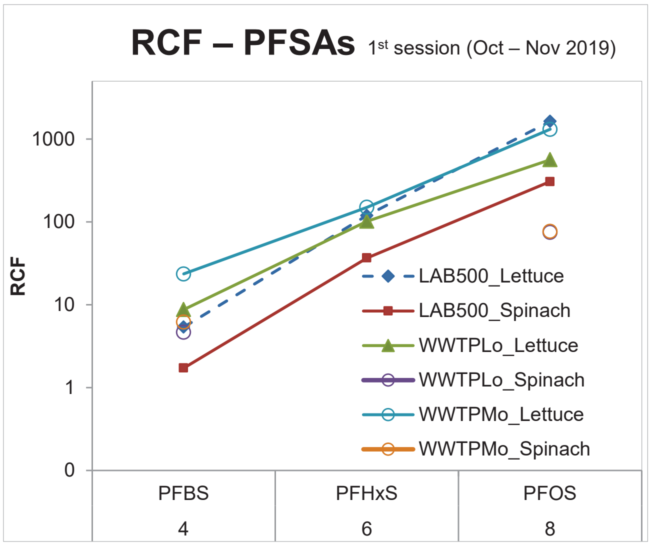
Hydroponics: results

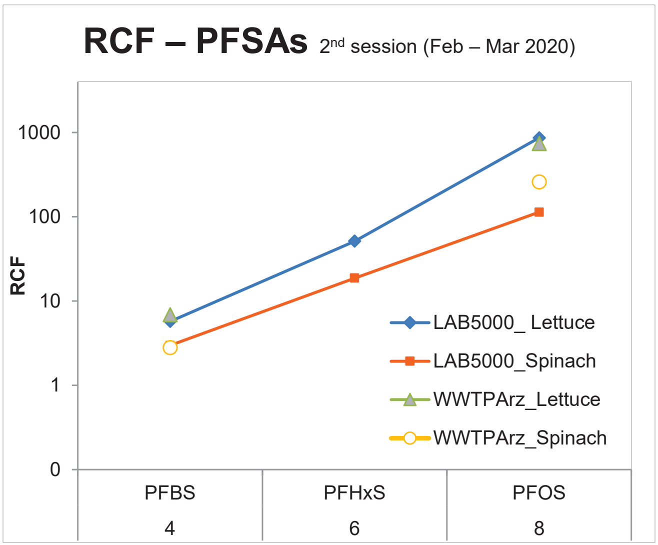




- RCF of PFCAs in WWTP showed the same pattern of LAB solutions
- PFCAs RCF values were similar among the test sessions

Hydroponics: results





- RCF of PFSAs in WWTP showed the same pattern of LAB solutions
- PFSAs RCF values were similar among the test sessions

Hydroponics: conclusions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Short Communication

Uptake and translocation of perfluoroalkyl acids by hydroponically grown lettuce and spinach exposed to spiked solution and treated wastewaters

Nicola Dal Ferro [a], Alessandro Pellizzaro [a,b,*], Massimo Fant [b], Mirco Zerlottin [b], Maurizio Borin [a]

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[b] Acque del Chiampo S.p.A. – Servizio Idrico Integrato, Via Ferraretta 20, 36071 Arzignano, Italy

- RCF of PFCAs showed a sigmoidal relationship with C-chain length
- PFSAs RCF increased with increasing of perfluorinated chain length
- TF value of all compounds was higher in spinach than lettuce
- Lettuce uptake (% PFAAs mass) was higher than spinach
- Future perspectives: fruiting crops (tomatoes, cucumbers)

CW mesocosm: experimental set-up

- Unheated greenhouse
- Mesocosm systems → 0.56 × 0.35 × 0.31 m
- 4 plants per tank
- 6 kg expanded clay per tank
- Zero discharge system
- 55 L tot PFAA synthetic solution per tank + nutrient solution volume on request
- 248 L wastewater (WWTP) per tank

- 4 tested waters
- 3 plant species
- 3 replicates

CW mesocosm: treatments

PLANT SPECIES

Iris pseudacorus L.

Typha latifolia L.

Phragmites australis (Cav.) Trin. Ex Steud

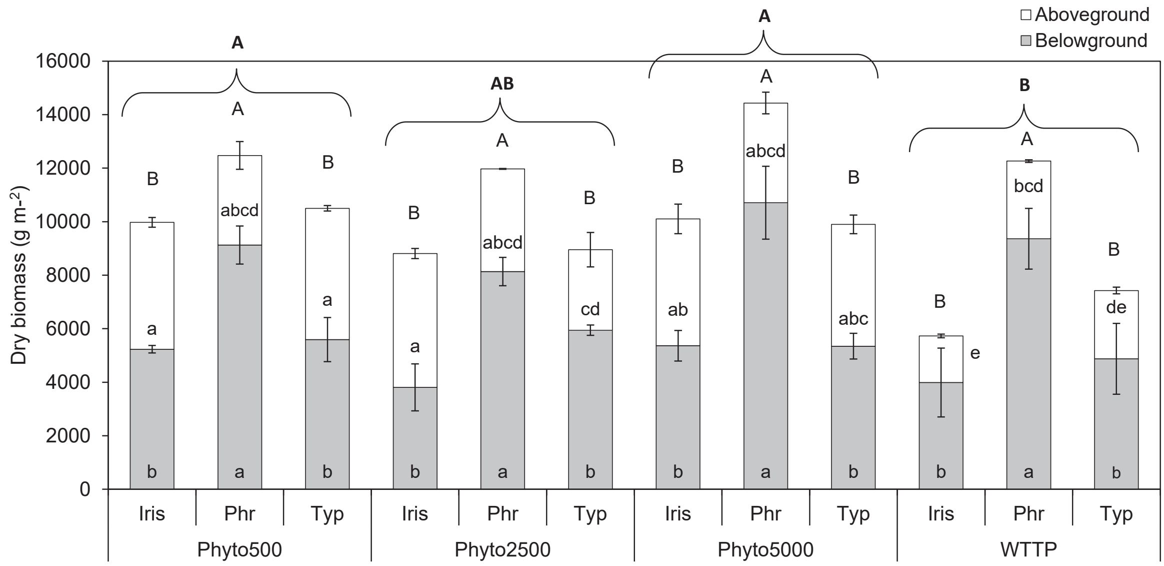
TESTED WATERS

PFAA (ng L-1)	C-chain length	PHYTO500	PHYTO2500	PHYTO5000	WWTP
PFBA	3	500	2500	5000	59
PFPeA	4	500	2500	5000	60
PFBS	4	500	2500	5000	804
PFHxA	5	500	2500	5000	45
PFHpA	6	500	2500	5000	14
PFHxS	6	500	2500	5000	< 10
PFOA	7	500	2500	5000	58
PFOS	8	500	2500	5000	7
PFNA	8	500	2500	5000	< 10
PFDA	9	500	2500	5000	< 10
PFUnA	10	500	2500	5000	< 10
PFDoA	11	500	2500	5000	< 10
PFTrA	12	500	2500	5000	< 10
PFTeA	13	500	2500	5000	< 10
∑PFAA		7000	35000	70000	1047

CW mesocosm: experimental monitoring

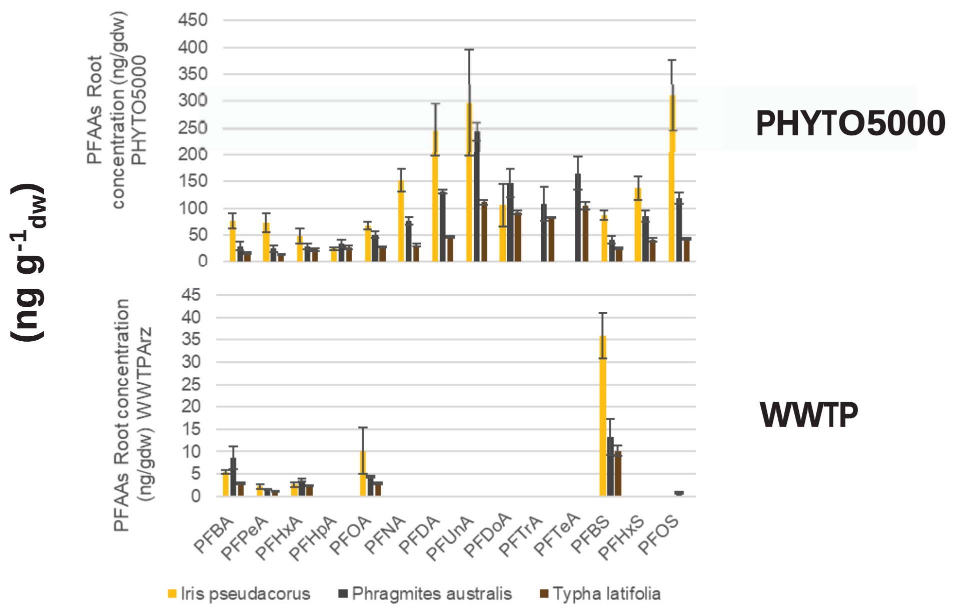
- Exposure period → 26 feb – 5 Jul 2020
- T and U% monitoring
- Water monitoring:
  - Main nutrients (N, P, K, Ca, Mg,…) → added whether required to maintain equal concentrations
  - Physicochemical properties (weekly)
  - PFAAs concentration (weekly)
- Vegetation (end of experiment):
  - Aboveground and belowground biomass
  - PFAAs concentration
- PFAAs in porous media (end of experiment)
- PFAAs in surface plastic tank (end of experiment, 10 cm^2 swab)

CW mesocosm: plant biomass



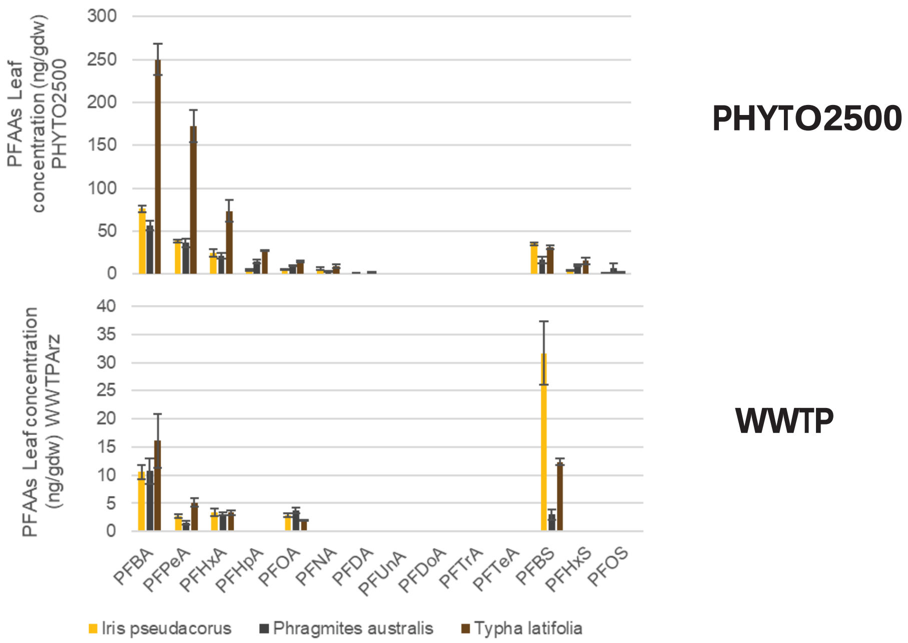
- Dry biomass didn't decrease with increasing PFAAs concentration
- Dry biomass of *Iris* and *Typha* significantly decrease in WWTP treatment

CW mesocosm: belowground PFAAs



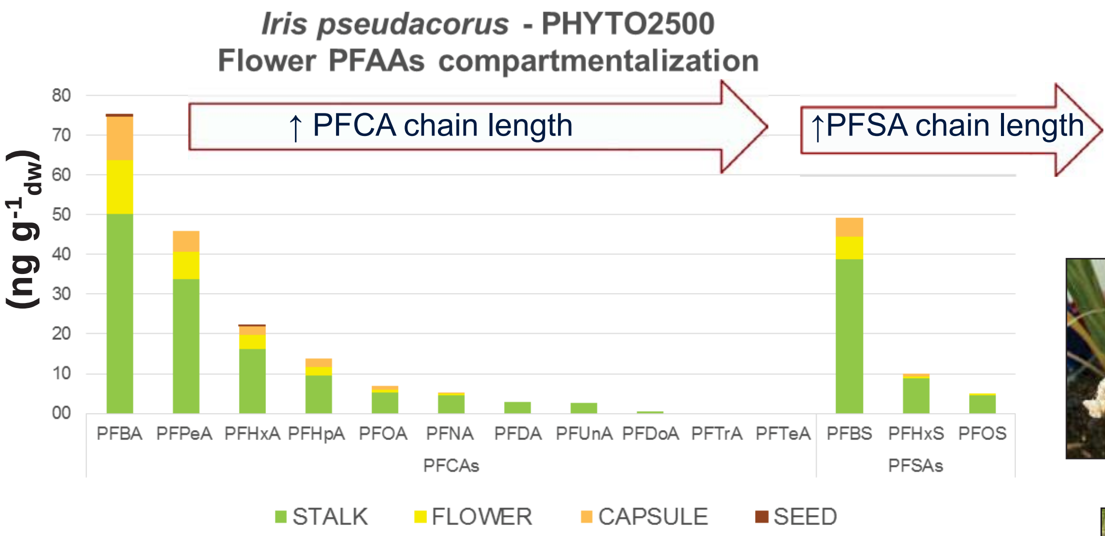
- PFAAs roots concentration *Iris* > *Phragmites* > *Typha* (C < 11)

CW mesocosm: aboveground PFAAs



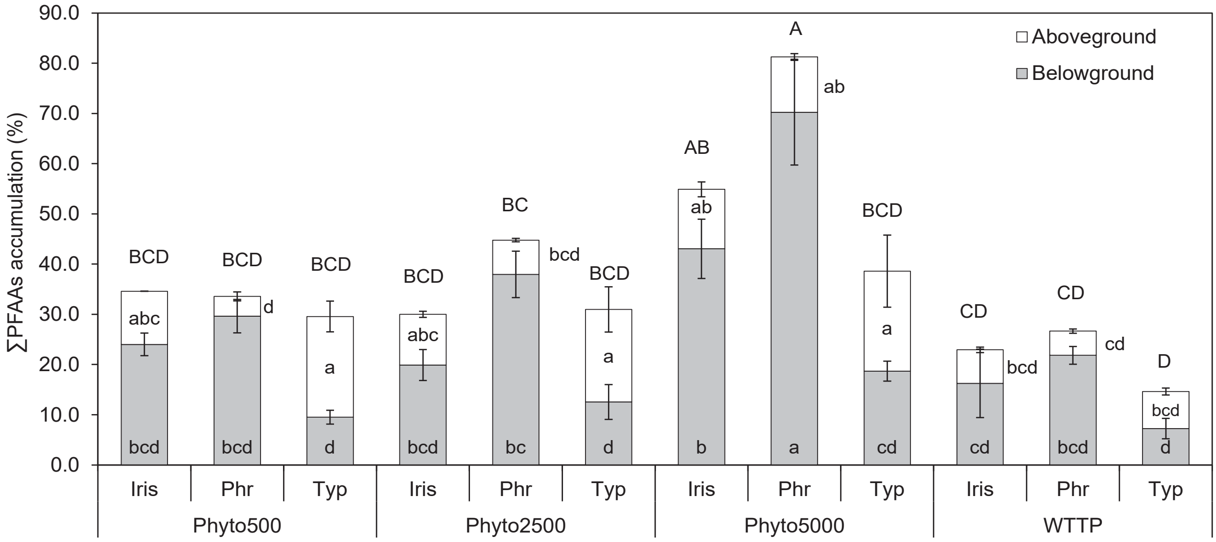
- PFAAs leaves conc. decreased with increasing C-chain length
- PFAAs leaves conc. *Typha* > *Phragmites* > *Iris* (PFCAs ≤ 5)
- *Typha* showed the lowest PFSAs concentration in leaves

CW mesocosm: PFAAs in *Iris* tissues



- PFAAs conc. stalk > flower > capsule > seeds
- PFAAs conc. decreased with increasing C-chain length

% PFAAs mass removal by plants



- Removal efficiency grew with increasing total PFAAs concentration
- % Removal generally resulted in this order: *Phragmites* > *Iris* > *Typha*
- *Typha* translocated from roots to leaves up to 67% of the total PFAAs removed
- *Phragmites* accumulated in roots up to 88% of the total PFAAs removed

CW mesocosm: conclusions

- Vegetation did not suffer the increasing PFAAs concentration in spiked solutions
- Growth limitations especially occurred in *Typha* and *Iris* when they were exposed to wastewater → high total salt concentration?
- *Phragmites* had the highest total removal efficiency (from 27% to 81%) → mostly in belowground tissues
- *Typha* had the highest PFAAs removal in aboveground tissues → mainly short perfluorinated C-chain PFBA, PFPeA & PFHxA.
- Future perspectives: investigation of further matrices (sludge and leachate) and of long-term exposure to plant treatments (PFAAs re-allocation to roots?)

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Emerged macrophytes to the rescue: Perfluoroalkyl acid removal from wastewater and spiked solutions

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…thanks for your attention! Questions?

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