NTNU – Trondheim
Norwegian University of
Science and Technology

Navigating PFAS Chemistry in complicated samples: Analysis in the Modern Laboratory and Future Aspects

By Alexandros G. Asimakopoulos (PhD),
Professor

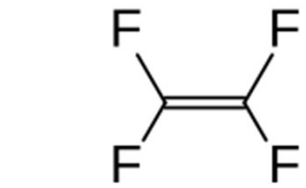
- **EnviroChemistry Lab**

EnviroChemistry Lab

Infographics ('A picture is worth a thousand words') presenting the research out-put of the *EnviroChemisty lab* (NTNU) during 2024; from *www.scopus.com*.

- **How did it all start?**

-1938: The chemistry for PFAS (Per- and polyfluoroalkyl substances) was initially discovered by Roy J Plunkett at Dupont's Jackson Laboratory in Deepwater, New Jersey.



Tetrafluoroethylene (TFE)

Teflon
Polytetrafluoroethylene
PTFE

$(C_2F_4)_n$

Solid waxy powder

-1945: DuPont commercialized PTFE as Teflon
-PTFE was resistant to corrosion, had low surface friction, and high heat resistance.
-TFE can cyclize **with a wide variety of compounds which led to the creation of a range of organofluorine compounds**.

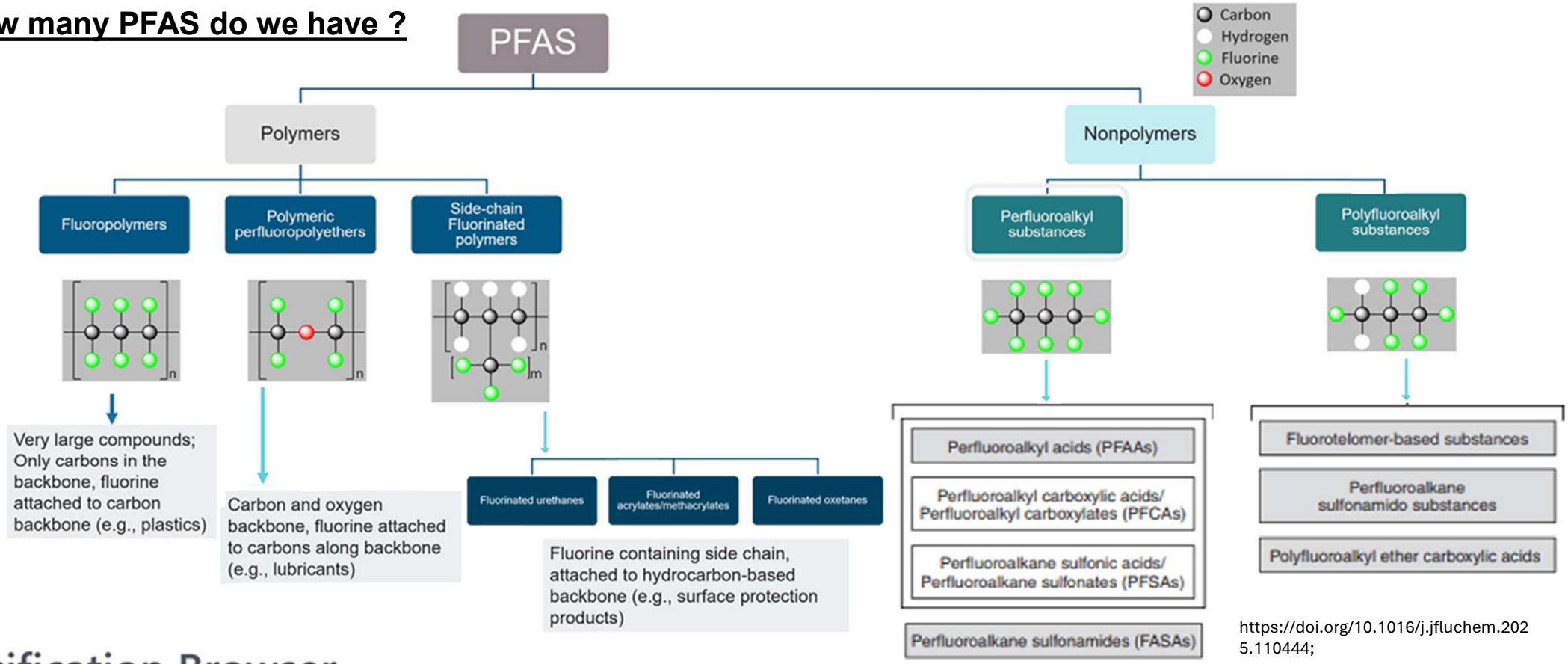
-1950s: First large-scale release of waste by 3M is documented in Lake Elmo, Minnesota, USA

-1954: DuPont, received an inquiry about **C8** (PFOA) "possible toxicity."

-1961: PFOA low dose exposure was linked to the enlargement of rats' testes, adrenal glands, and kidneys.

So the first attempts for measuring **fluorinated organic compounds/classes can be documented back to the late 1940s**.

- **How many PFAS do we have ?**



https://doi.org/10.1016/j.jfluchem.2025.110444;

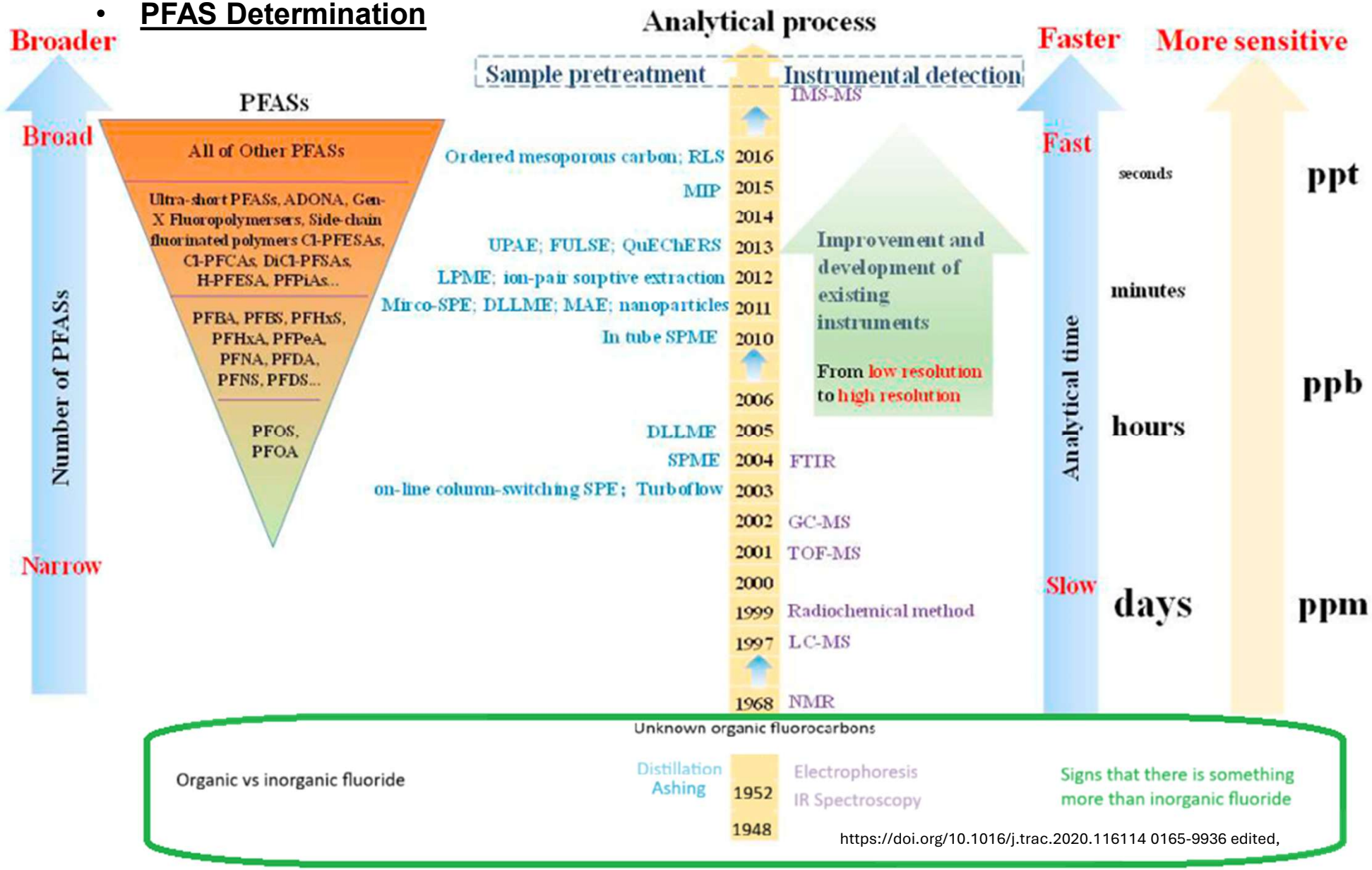
Classification Browser

Classification Description (from PubChem)
The collection of per- and polyfluoroalkyl substances (PFAS) and other fluorinated compounds in PubChem as described in https://doi.org/10.1021/acs.est.3c04855. The 'OECD PFAS definition' (no salts) and 'PFAS breakdowns by chemistry' (with salts/mixtures) nodes contain PubChem content matching the OECD PFAS definition of saturated CF2 (Report ENV/CBC/MONO(2021)25). Other nodes explore 'organofluorine compounds'; 'other diverse fluorinated compounds'; 'PFAS and fluorinated compound collections' (including CompTox; NORMAN-SLE; NIST; OntoChem PFAS lists) and 'regulatory PFAS collections' (including Stockholm Convention/EU regulations for PFOS; PFOA; PFHxS; LC-PFCAs). Expand each node for more detailed content. Read more about this tree here: https://gitlab.com/uniluxembourg/lcsb/eci/pubchem-docs/-/raw/main/pfas-tree/PFAS_Tree.pdf?inline=false

- PFAS and Fluorinated Compounds in PubChem 23,165,505
  - OECD PFAS definition 7,193,873

PubChem

- **PFAS Determination**

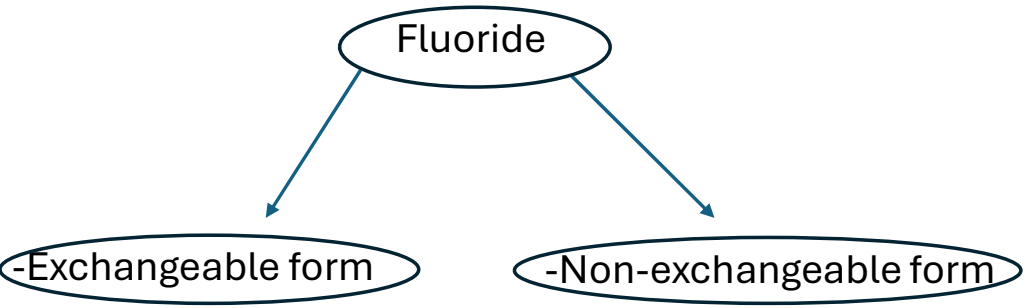


https://doi.org/10.1016/j.trac.2020.116114 0165-9936 edited,

- **Organic Fluorocompounds vs. Inorganic fluorine**

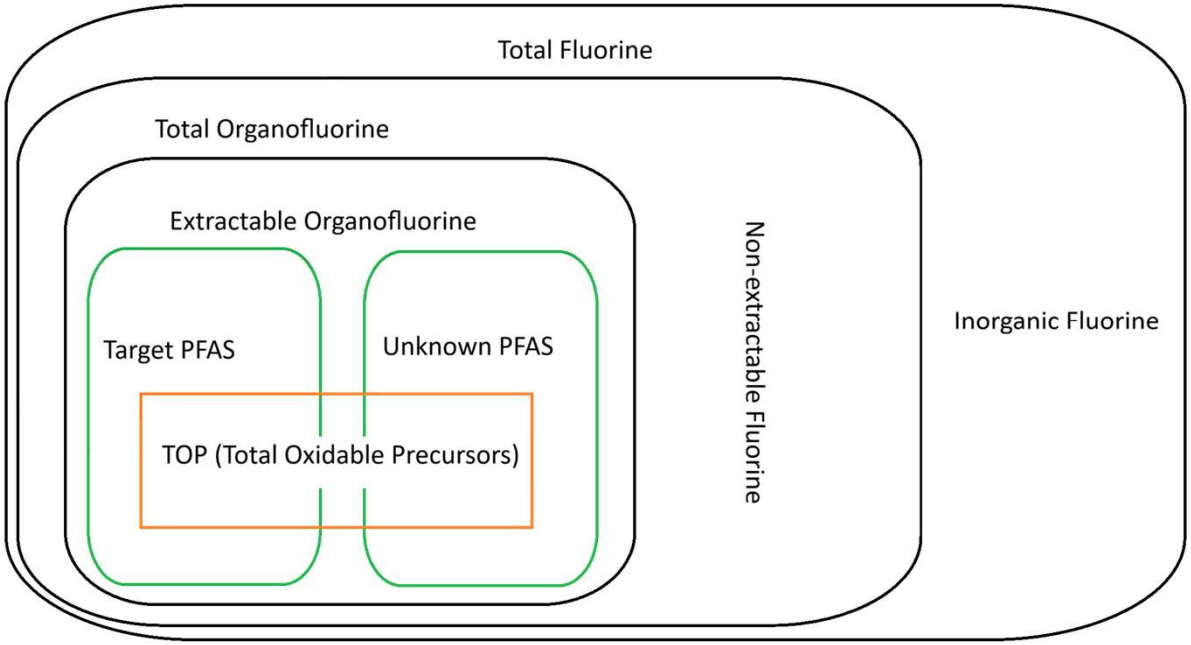
1. Taves, D.R., Nature (1966), 211, 192.
2. Taves, D.R., Nature (1968), 217, 1050.
3. Taves, D.R., Nature (1968), 200, 582.
4. Taves, D.R., Talanta (1968), 15, 1015.

- Discovered that samples of his own blood serum contained two distinct forms of fluoride.
- Only one of these was exchangeable with radioactive fluoride. The other, non-exchangeable form was detectable as fluoride only when sample preparation included ashing.



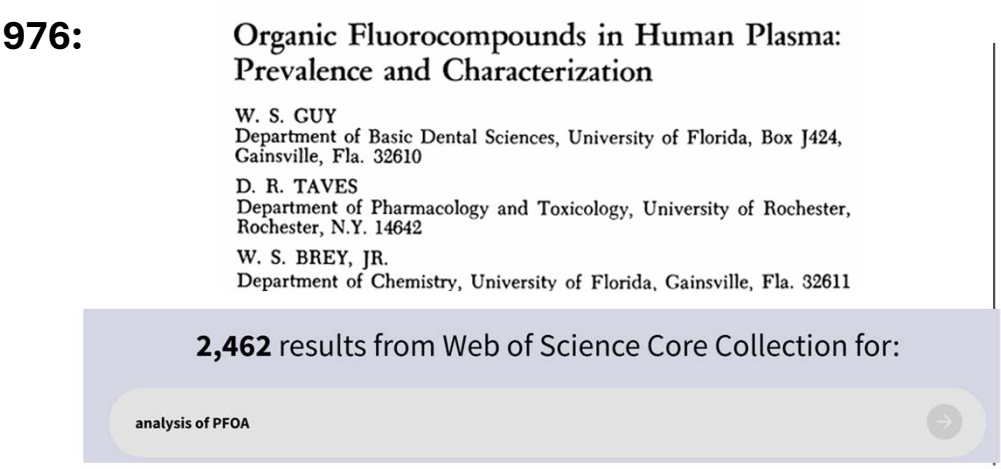
The inorganic fluorine was exchangeable with radioactive fluoride.

The organic fluorine was detectable as fluoride only when sample preparation included ashing!



- **Intensified research on Organic Fluorocompounds**

1976:



Organic Fluorocompounds in Human Plasma: Prevalence and Characterization

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Department of Basic Dental Sciences, University of Florida, Box J424, Gainsville, Fla. 32610
D. R. TAVES
Department of Pharmacology and Toxicology, University of Rochester, Rochester, N.Y. 14642
W. S. BREY, JR.
Department of Chemistry, University of Florida, Gainsville, Fla. 32611

Little has been published about the metabolic handling and toxicology of perfluorinated fatty acid derivatives. Computer assisted literature searches using Medline, Toxline and Chemcon developed no information on these subjects. This was surprising with respect to the widespread commercial use of such compounds. It would appear from information presented here that rapid excretion of such compounds into urine is unlikely since they are bound to albumin in the blood.

These findings suggest that there is widespread contamination of human tissues with trace amounts of organic fluorocompounds derived from commercial products. All available information on this subject is in accordance with this interpretation. A series of compounds having a structure consistent with that found here for the predominant form of organic fluorine in human plasma is widely used commercially for their potent surfactant properties.

2,462 results from Web of Science Core Collection for:

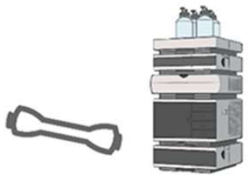
analysis of PFOA

2026	1	2013	70	2000	6
2025	342	2012	63	1999	3
2024	334	2011	49	1998	3
2023	295	2010	48	1997	2
2022	258	2009	45	1996	4
2021	211	2008	33	1995	2
2020	152	2007	23	1994	5
2019	115	2006	9	1993	1
2018	77	2005	10	1991	1
2017	76	2004	7	1985	1
2016	81	2003	7	1983	1
2015	57	2002	5		
2014	64	2001	1		

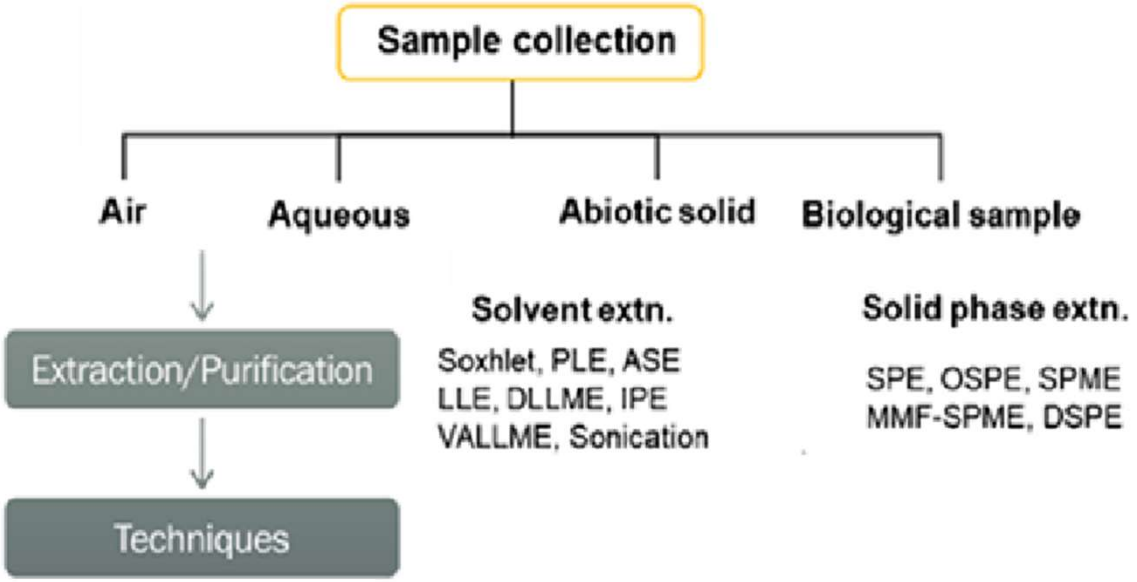
- **Navigating common sample preparation pitfalls**

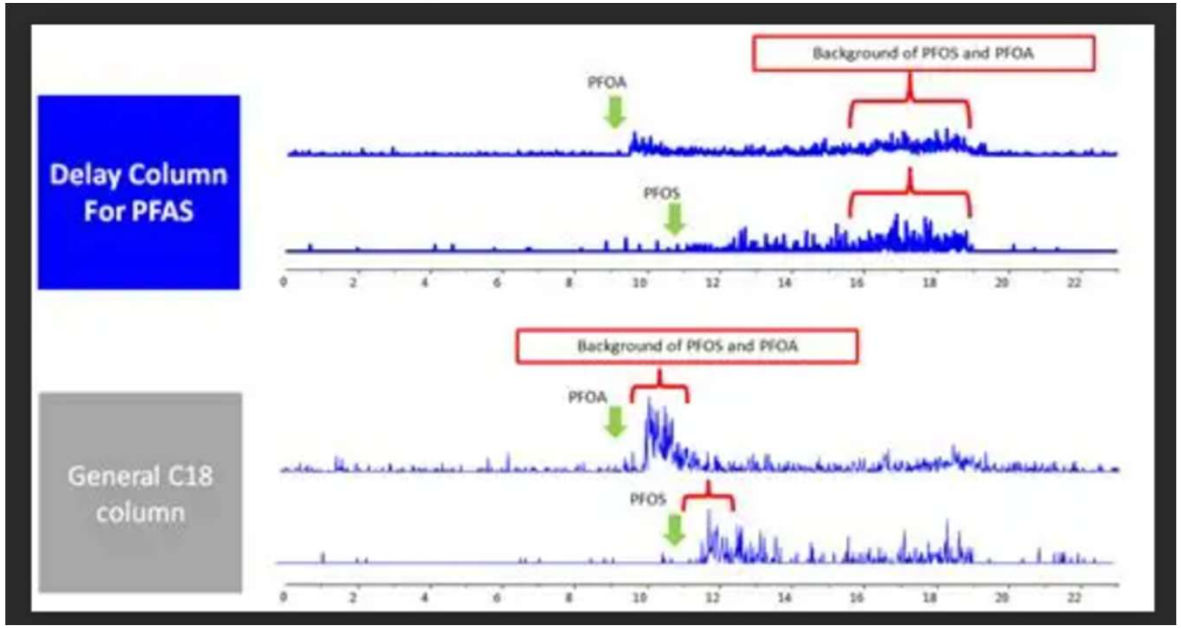
AVOID	USE
Teflon™ or other materials containing fluoropolymers	high-density polyethylene (HDPE), polyetheretherketone (PEEK), polypropylene (PP) silicone material
low-density polyethylene (LDPE) and glass bottles, Teflon™ –lined caps, chemical ice packs (i.e. Blue ice®)	HDPE or PP containers and screw caps, regular ice, Zip-lock bags
clothes and shoes made of Gore-Tex® or other synthetic water- and stain-resistant materials, Tyvek materials, fabric softeners	cotton, polyurethane, wax-coated clothing; PVC or polyurethane shoes
cosmetics, moisturizers, hand creams, sunscreen, insect repellents	

Background Control



- Install delay column between pump and injection in LC instrument
- Use alternative blank matrices, such as FBS devoid of target PFAS, for preparing calibration and QC samples
- Evaluate and monitor potential background contamination





DELAY COLUMN EXPLANATION; https://doi.org/10.1007/s00216-022-03905-y; https://doi.org/10.1016/j.trac.2023.117062

- **Tackling Background concentrations**

  - If instrumental background levels were found, they were eliminated before analyses by injecting sufficient blanks to cleanse the system (8 to 10 blanks were required).

  - Additionally, to minimize build-up of PFCs during mobile phase equilibration and to keep background levels constant, the time duration for which the system was kept under initial conditions was maintained as short as possible.

  - Prior to daily use, we flushed the LC column with elution solvents [MeOH/5 mM ammonium formate (70 : 30, % v/v)] before initiating a sequence.

Analytical Methods

PAPER

Cite this: *Anal. Methods*, 2014, 6, 1341

Simultaneous determination of eighteen perfluorinated compounds in dissolved and particulate phases of wastewater, and in sewage sludge by liquid chromatography-tandem mass spectrometry†

Olga S. Arvaniti,[a] Alexandros G. Asimakopoulos,[a] Marilena E. Dasenaki,[a] Elpida I. Ventouri,[a] Athanasios S. Stasinakis[b] and Nikolaos S. Thomaidis*[a]

Science of the Total Environment 463–464 (2013) 1067–1075

Contents lists available at ScienceDirect

Science of the Total Environment

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

Contribution of primary and secondary treatment on the removal of benzothiazoles, benzotriazoles, endocrine disruptors, pharmaceuticals and perfluorinated compounds in a sewage treatment plant

Athanasios S. Stasinakis [a,*], Nikolaos S. Thomaidis [b], Olga S. Arvaniti [a,b], Alexandros G. Asimakopoulos [b], Vasilios G. Samaras [a], Akinranti Ajibola [b], Daniel Mamais [c], Themistokles D. Lekkas [a]

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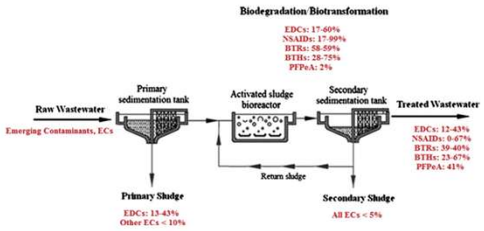
[c] Department of Water Resources, Faculty of Civil Engineering, National Technical University of Athens, Zografou, Athens 15773, Greece

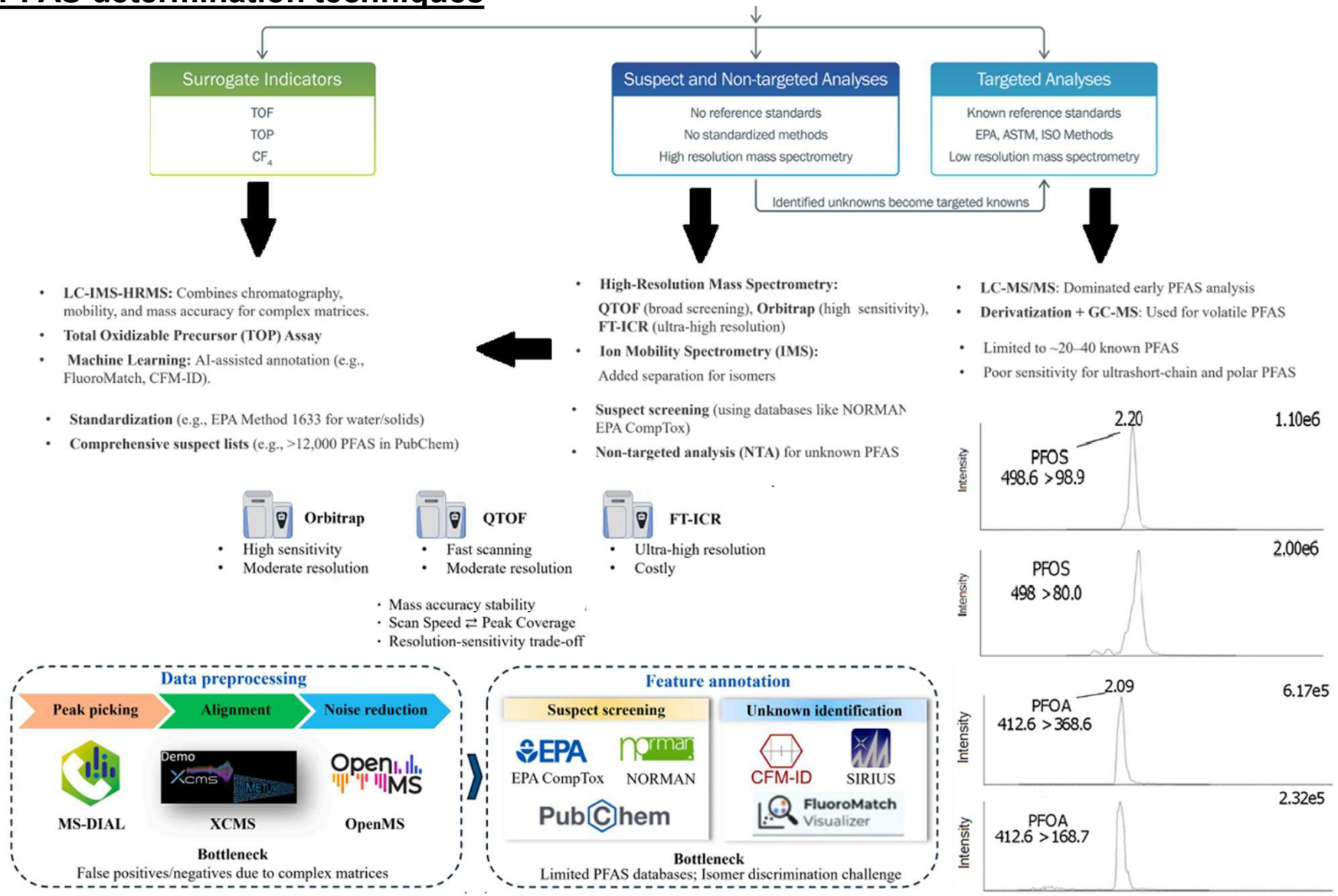
HIGHLIGHTS

- Solid-liquid distribution coefficients were calculated for various micropollutants.
- The highest concentrations were detected for TTR in raw sewage and for NP in sludge.
- Removal during wastewater treatment was >70% only for 9 over 27 detected compounds.
- 60% of BTRs and 28-75% of BTHs was removed due to biotic processes in bioreactors.
- Sorption to primary sludge was a significant mechanism for the removal of EDCs.

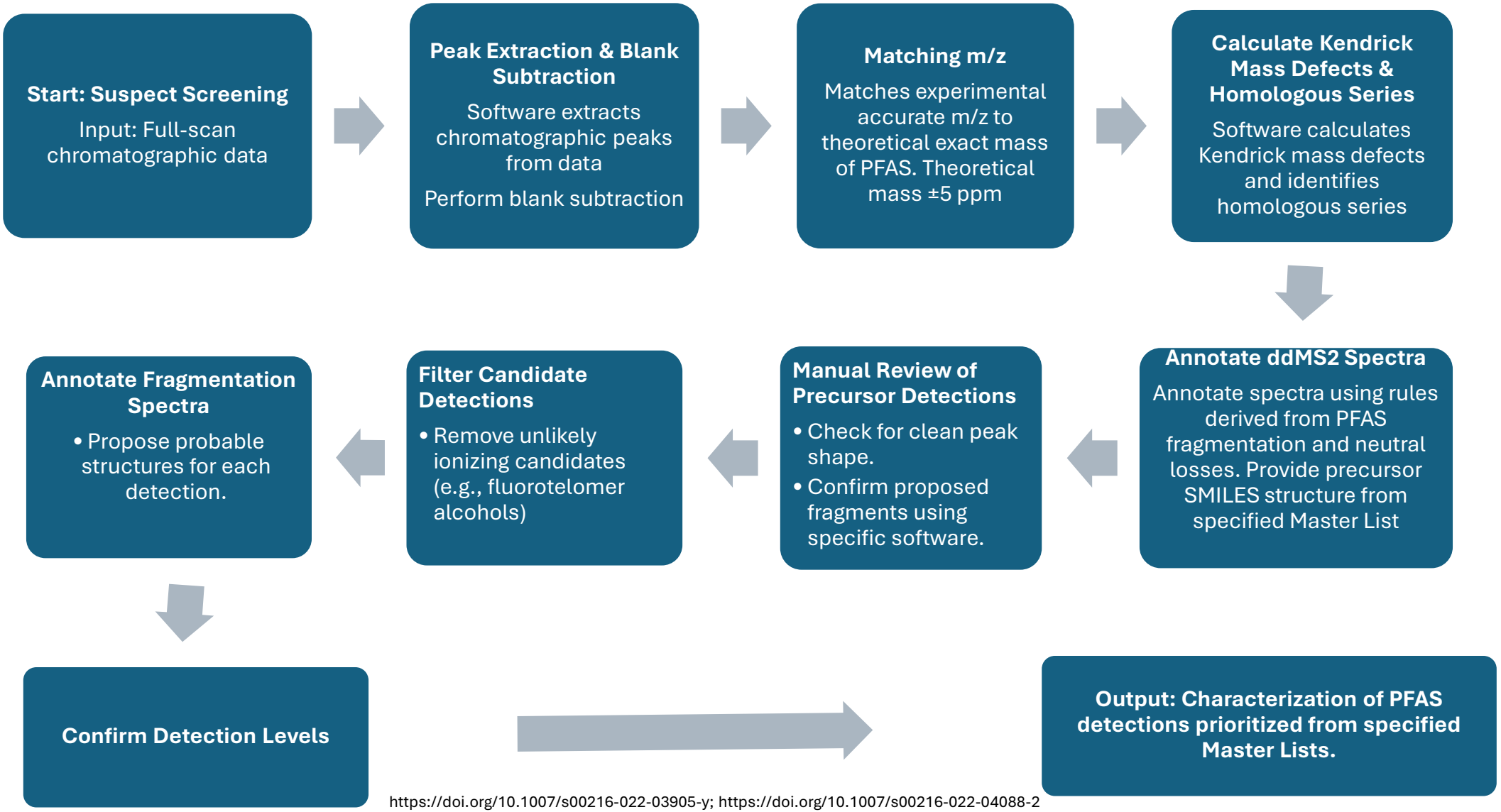
GRAPHICAL ABSTRACT



- **Mainstream PFAS determination techniques**



JCA 1760 (2025) 466261, https://doi.org/10.1007/s00216-022-03905-y; https://doi.org/10.1007/s00216-022-04088-2



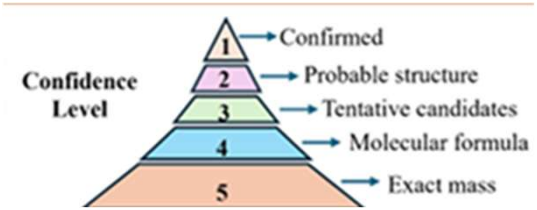
https://doi.org/10.1007/s00216-022-03905-y; https://doi.org/10.1007/s00216-022-04088-2

- **Examples for confirmation levels 1 and 3.**

Confirmed structure

→ Level 1

Fit well with the result on the reference standard



Tentative candidate

→ Level 3

MS² evidence on structure

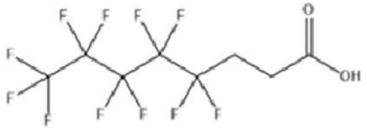
2H,2H,3H,3H-Perfluorooctanoic acid

[M-H]- : 341.0044

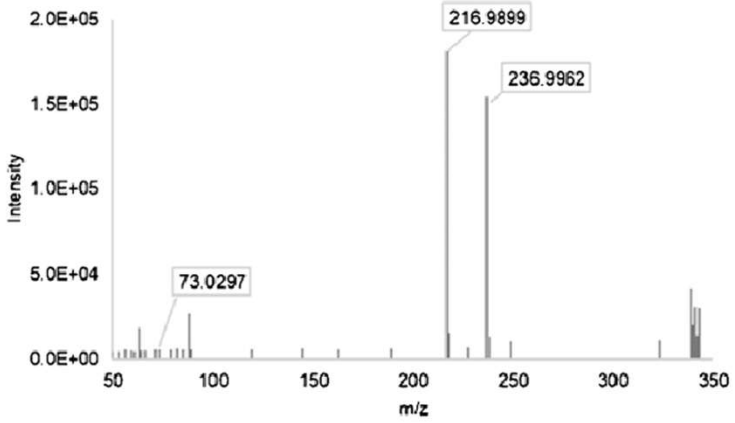
Formula: $C_8H_5F_{11}O_2$

RT: 20.4 min

Level 1 confirmation



m/z observed	Fragment Ion	Precursor loss
73.0297	$[C_3H_5O_2]^-$	M-C_5F_{11}
216.9899	$[C_7F_7]^-$	M-$CH_5F_4O_2$
236.9962	$[C_7HF_8]^-$	M-$CH_4F_3O_2$



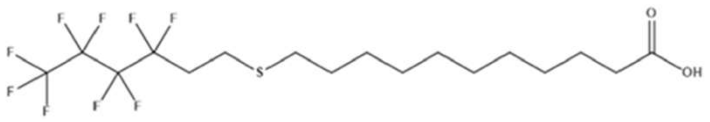
11-[(3,3,4,4,5,5,6,6,6-Nonafluorohexyl)sulfanyl]undecanoic acid

[M-H]- : 463.1337

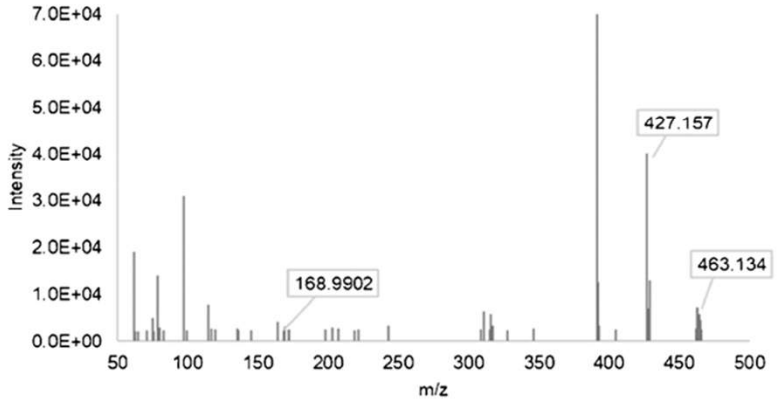
Formula: $C_{17}H_{25}F_9O_2S$

RT: 18.3 min

Level 3 detection

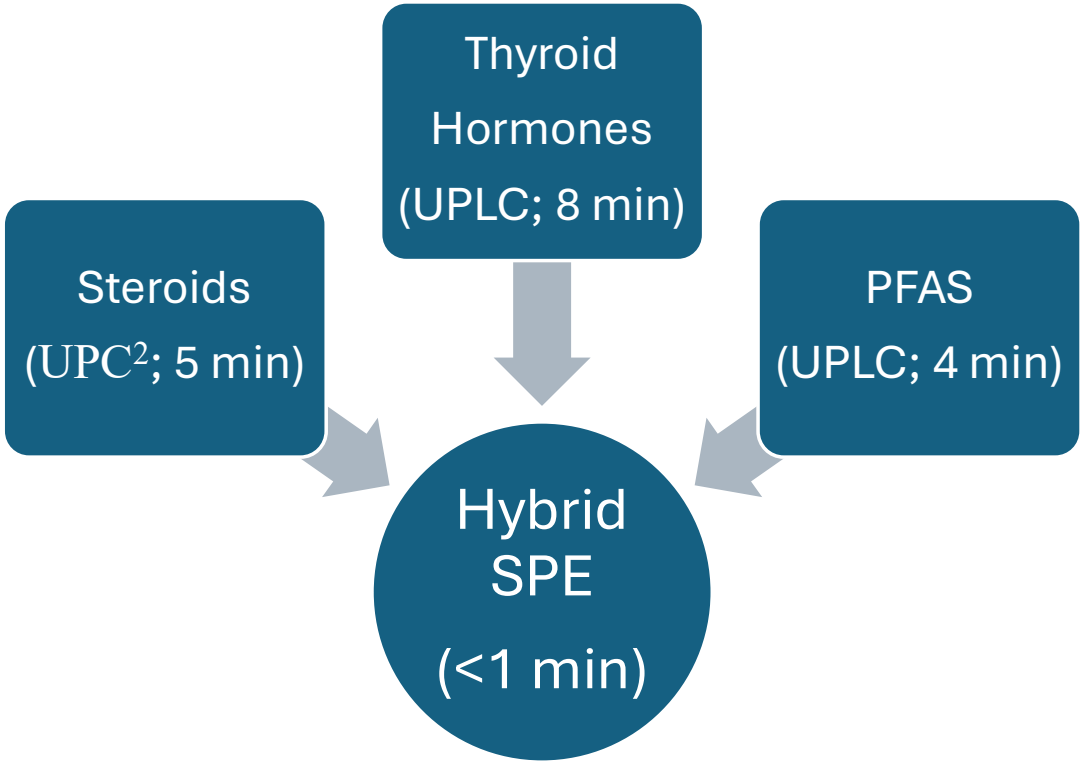


m/z observed	Fragment ion	Precursor loss
168.9902	$[C_3F_7]^-$	M- $C_{14}H_{25}F_2O_2S$
427.1570	$[C_7H_{26}F_7O_2S]^-$	[M-$C_{10}H_{25}F_2$]+H
463.1340	$[C_{17}H_{24}F_9O_2S]^-$	M-H



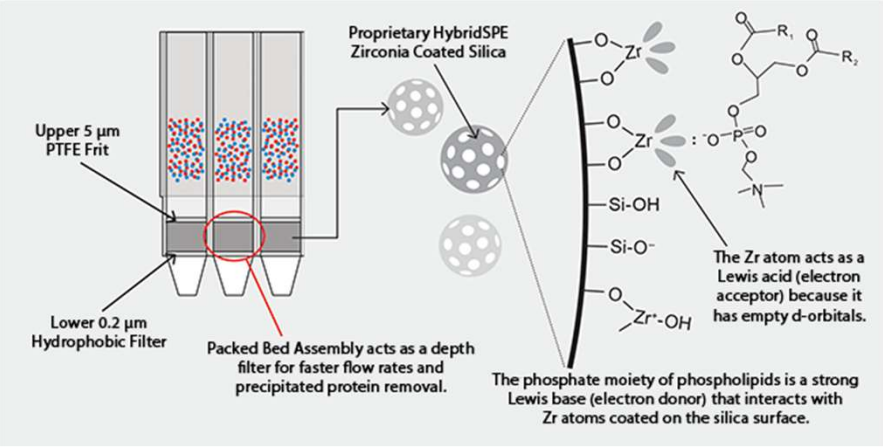
https://doi.org/10.1007/s00216-022-04088-2

- **<u>Multiple UPC^2 - & UPLC- methods tailored to a single rapid sample preparation protocol for modern (bio-) monitoring studies</u>**



Reaching the order of hundreds of samples analyzed weekly !!

- **Rapid sample preparation protocols tailored to targeted and TOP analysis.**



Journal of Hazardous Materials 487 (2025) 137189

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Shellfish and shorebirds from the East-Asian Australian flyway as bioindicators for unknown per- and polyfluoroalkyl substances using the total oxidizable precursor assay

Junjie Zhang [a,*], Lara Cioni [b], Veerle L.B. Jaspers [c], Alexandros G. Asimakopoulos [a], He-Bo Peng [d], Tobias A. Ross [e], Marcel Klaassen [e], Dorte Herzke [b,f,**]

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[b] NILU, Fram Centre, Tromsø 9296, Norway
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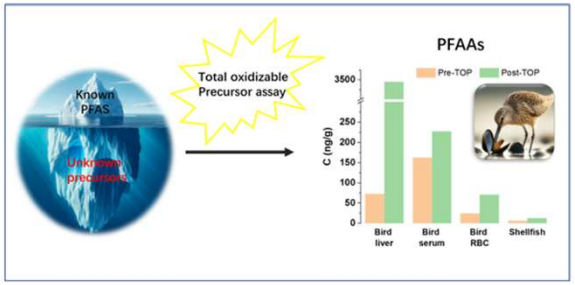
[d] Conservation Ecology Group, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen 9700 CC the Netherlands
[e] Centre for Integrative Ecology, Deakin University, Geelong 3216, Australia
[f] Norwegian Institute for Public Health, Oslo, Norway

HIGHLIGHTS

- Unknown per- and polyfluoroalkyl substances were found in biota samples.
- Perfluorosulfonic acids were produced in all samples after TOP assay.
- Shorebird serum shows higher levels of perfluorocarboxylic acids than red blood cell.
- Shorebird liver shows the highest levels of perfluoroalkyl acids after TOP assay.

GRAPHICAL ABSTRACT



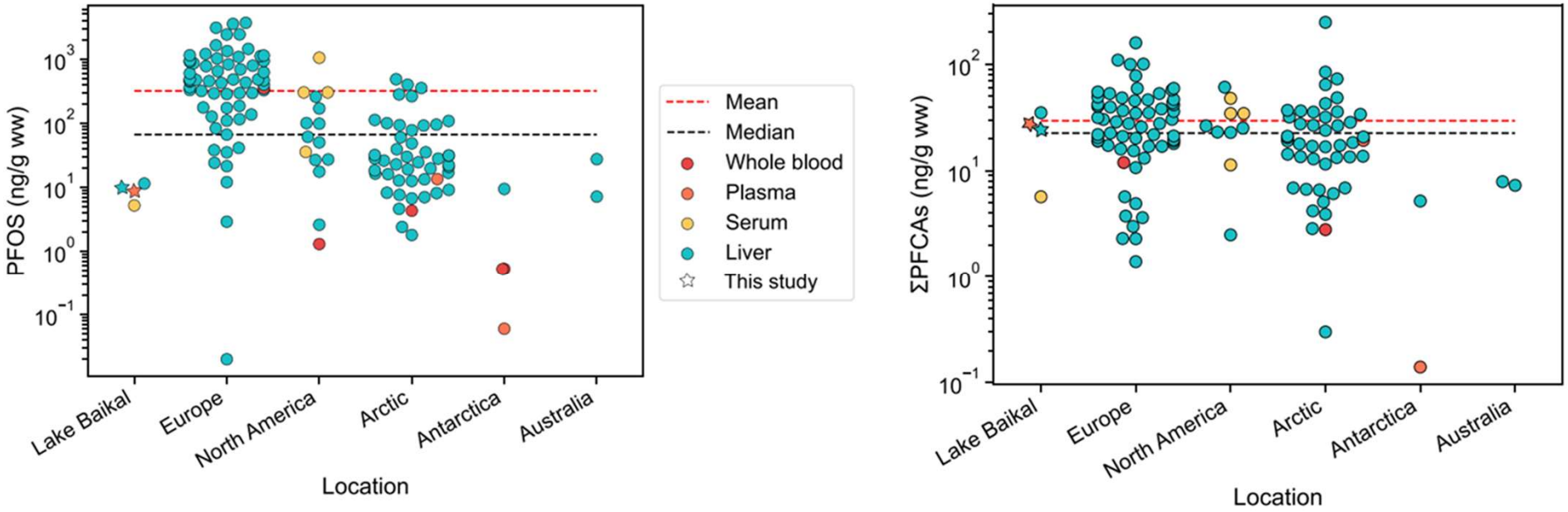
- **Global PFAS concentrations**

Science of the Total Environment

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

Occurrence and tissue distribution of 33 legacy and novel *per*- and polyfluoroalkyl substances (PFASs) in Baikal seals (*Phoca sibirica*)

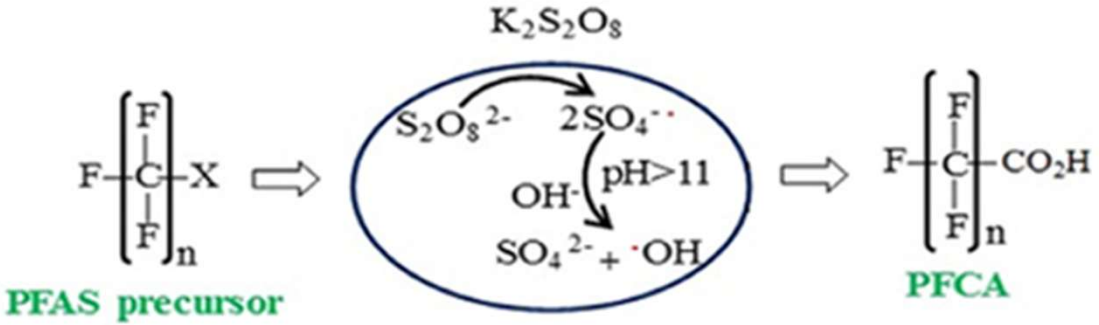
Shannen T.L. Sait [a], Silje F. Rinø [a], Susana V. Gonzalez [a], Mikhail V. Pastukhov [b], Vera I. Poletaeva [b], Julia Farkas [c], Bjørn M. Jenssen [d,e,f], Tomasz M. Ciesielski [d,e,*], Alexandros G. Asimakopoulos [a,**]

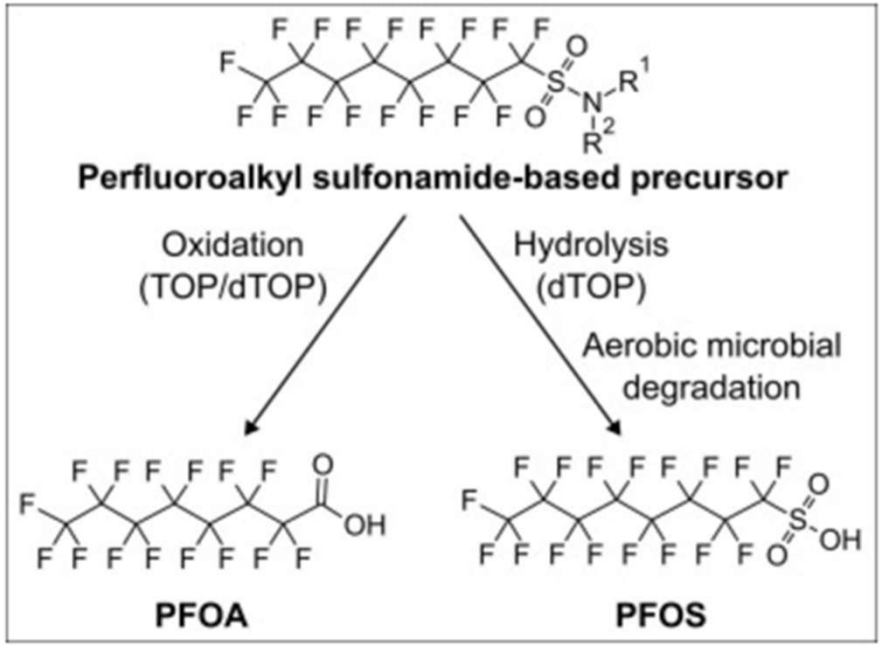


- **TOP assay and Target Analysis**

	TOP Assay	Target Analysis
Sensitivity*	*ppt*	*ppt*
Wastewater	✓	✓
Surface water	✓	✓
Groundwater	✓	✓
Tap water	✓	✓
Sediments	✓	✓
Sludge	✓	✓
Soil	✓	✓
Surface runoff	✓	✓
Biological samples	✓	✓
Dust	✓	✓
Wet Precipitation	✓	✓
Leachate	✓	✓

**ppb = µg/L or µg/kg; ppt = ng/L or ng/kg*





https://doi.org/10.1016/j.teac.2024.e00242

- **TOP assay and Target Analysis in Biological Media**

Weigh 30-50 mg biological samples in 1.5 mL PP tube and add IS

Extract PFASs uitilizing 0.3 mL of MeOH containing 1% ammonium formate under ultrasonication once

Clean up the extract by using Hybrid SPE

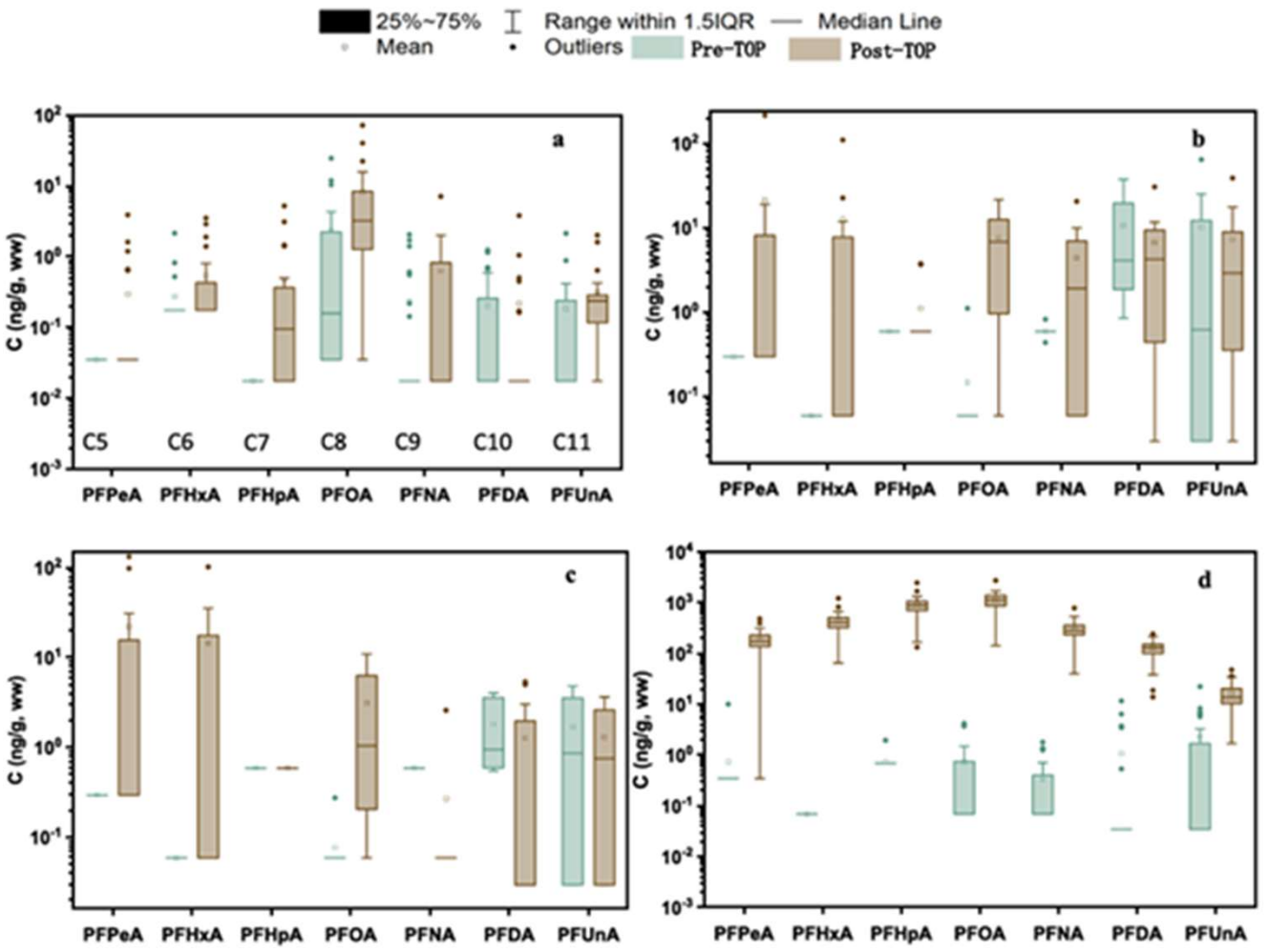
Transfer 150 µL extracts in injection vials

Transfer 60-100 µL extracts above and evaporate them to dryness

Add oxidizing agent 500 µL of sodium persulfate (0.8 M) and 120 µL of sodium hydroxide (10 M) and keep in an oven set at 85 °C for 24 h

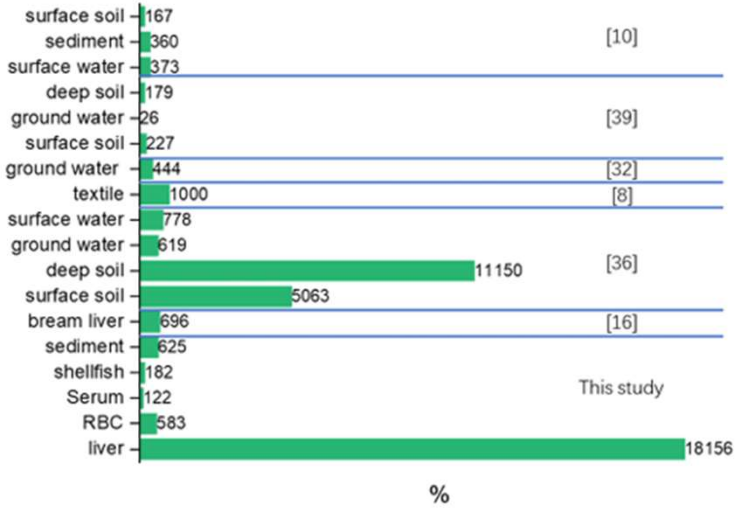
Acidify the samples with HCl to pH 1~2 and extract PFAAs by using 500 µL MTBE under ultrasonication

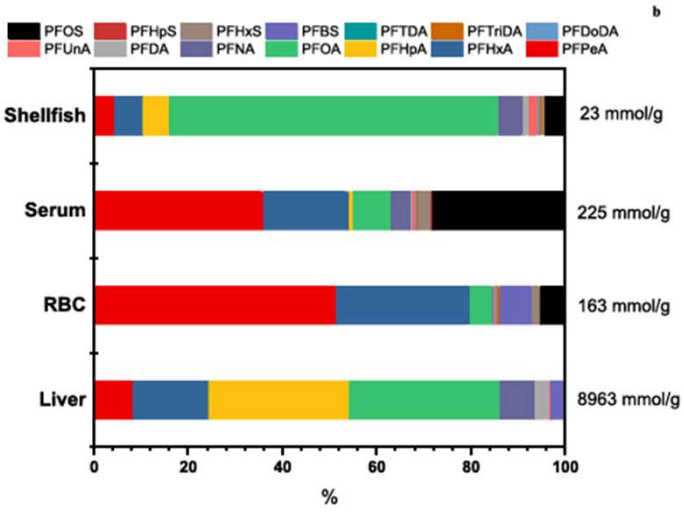
Transfer 400 µL extracts and evaporate to 50 µL, then transfer 25 µL to a new vial and add 25 µL MeOH containing 2 mM ammonium formate



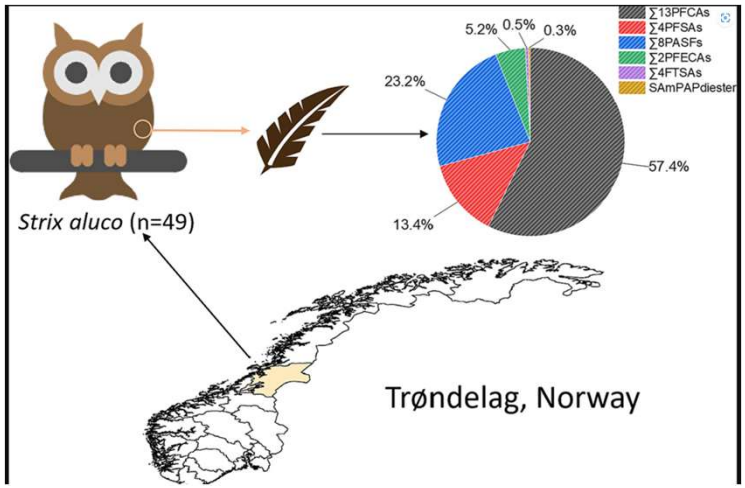
Distribution of PFCAs concentrations before and after TOP assay in shellfish (a, n=30), shorebird serum (b, n=12), shorebird red blood cell (c, n=12), and shorebird liver (d, n=25)

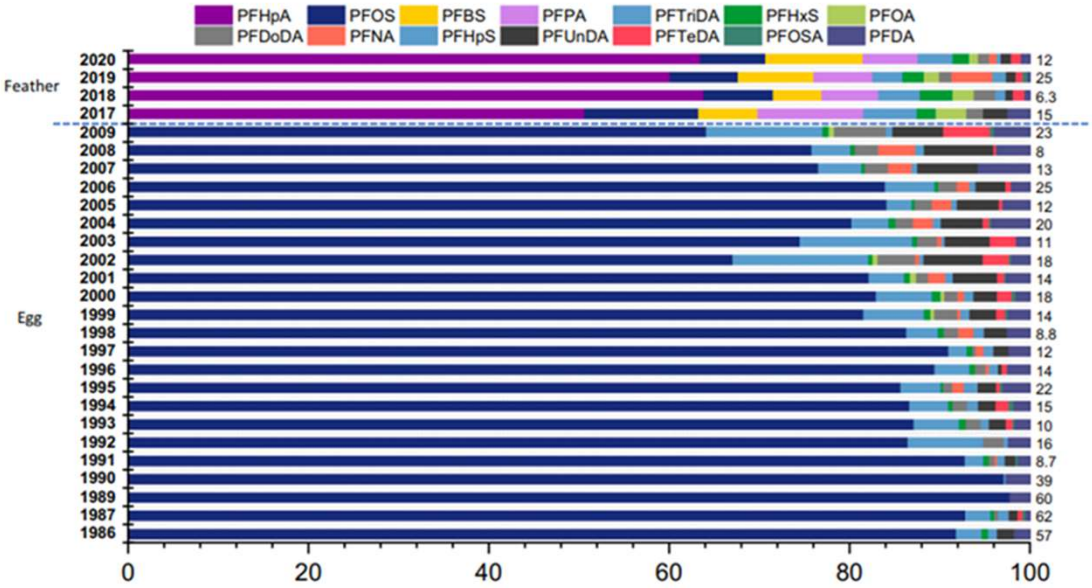
- **TOP assay and Target Analysis in Biological Media**



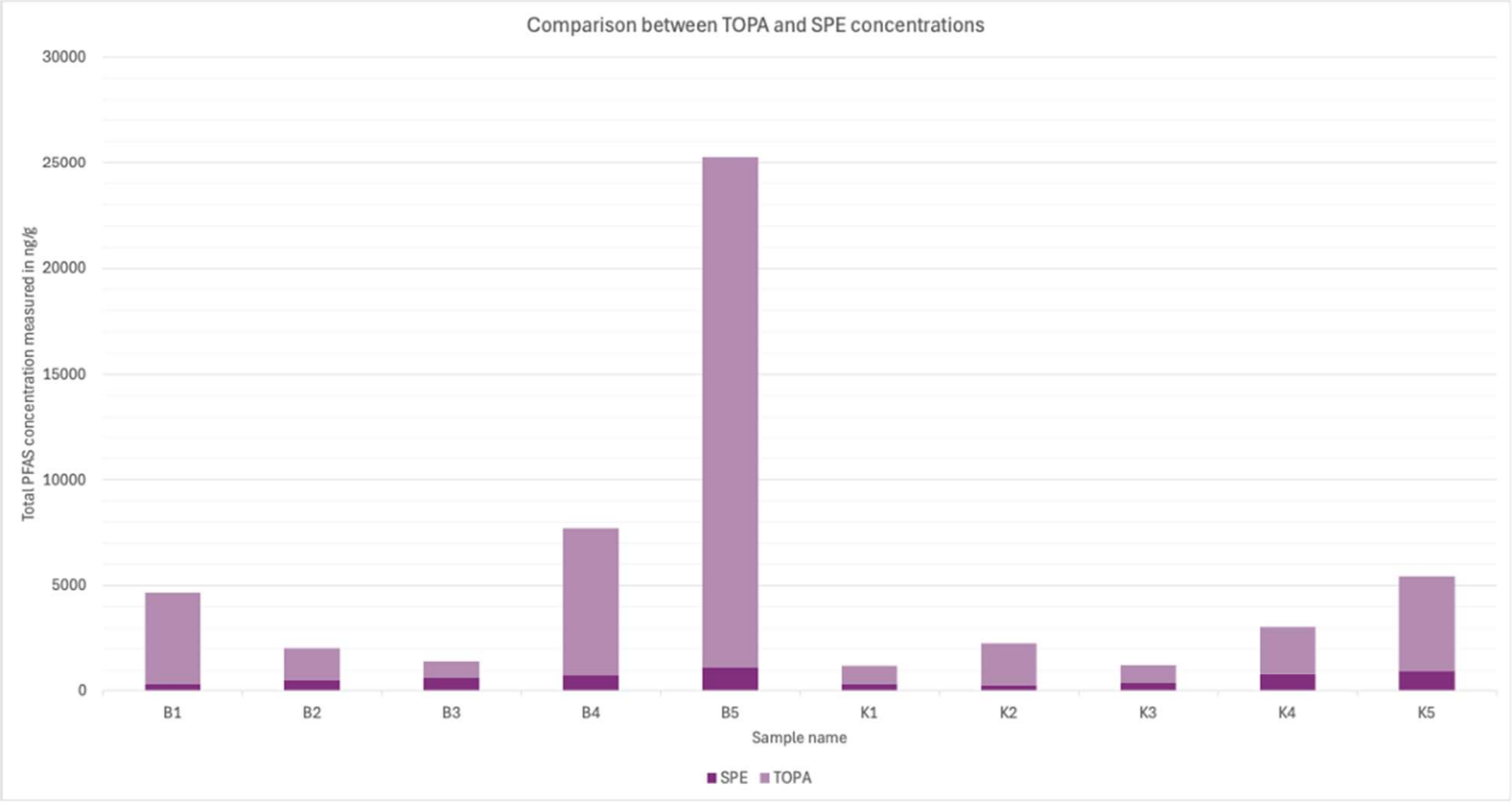


Comparison of ΔPFCAs (%) after TOP assay in this study with earlier published literature.





- **<u>TOP assay and Target Analysis in Environmental Media</u>**



Total concentrations measured from SPE and TOP assay for the ten samples given in ng/g.

- **TOP assay and Target Analysis in Environmental Media**

Enhancing Total Oxidizable Precursor Assay to Assess PFAS Pollution Across Ghana's Land-Use Areas

Liang Zhao[1†], Junjie Zhang[2†], Shivani Kubendraraj[1], Susana Villa Gonzalez[1], Murat V. Ardelan[1], K. Avarachen Mathew[1], Emmanuel Ansah[3], Millicent Kwawu[3], Christopher Gordon[3], Alexandros G. Asimakopoulos[1*], Bo Yuan[1*]

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[2]MOE Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China

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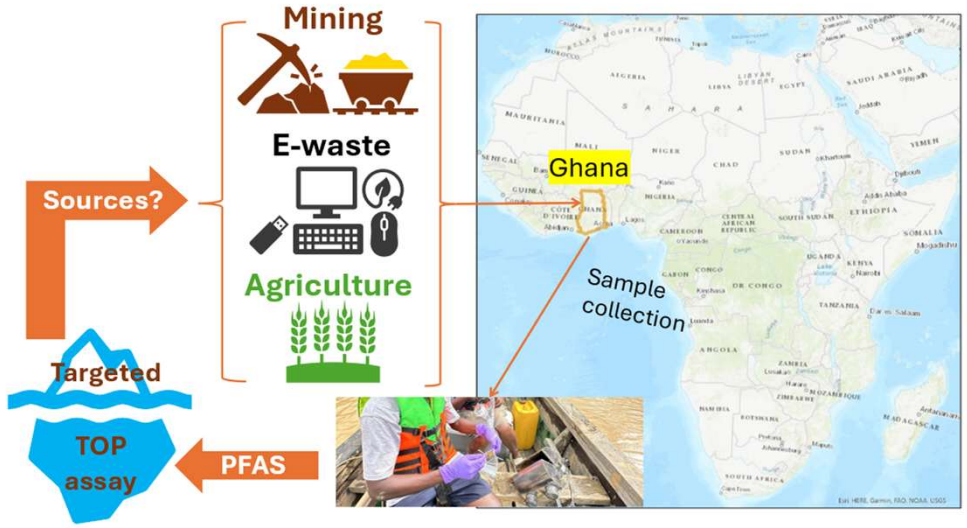

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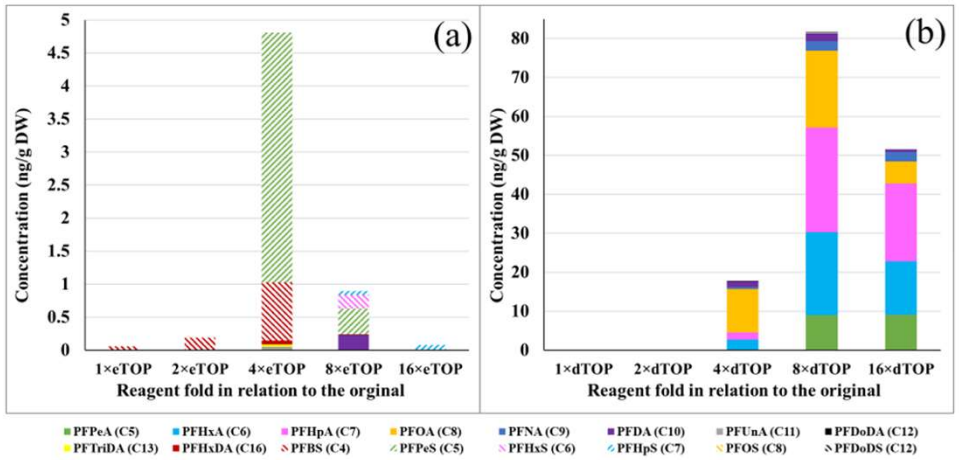
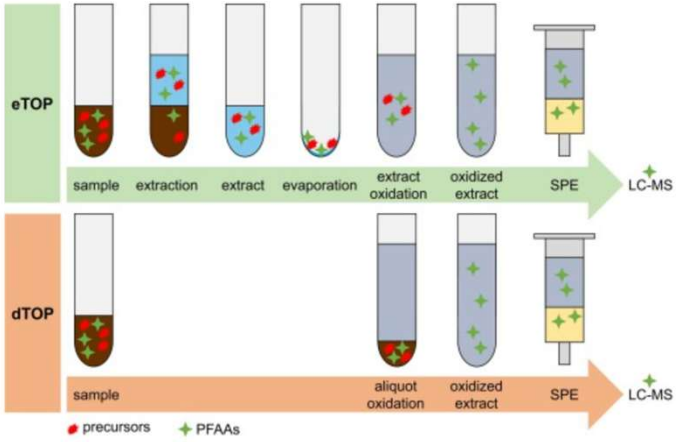
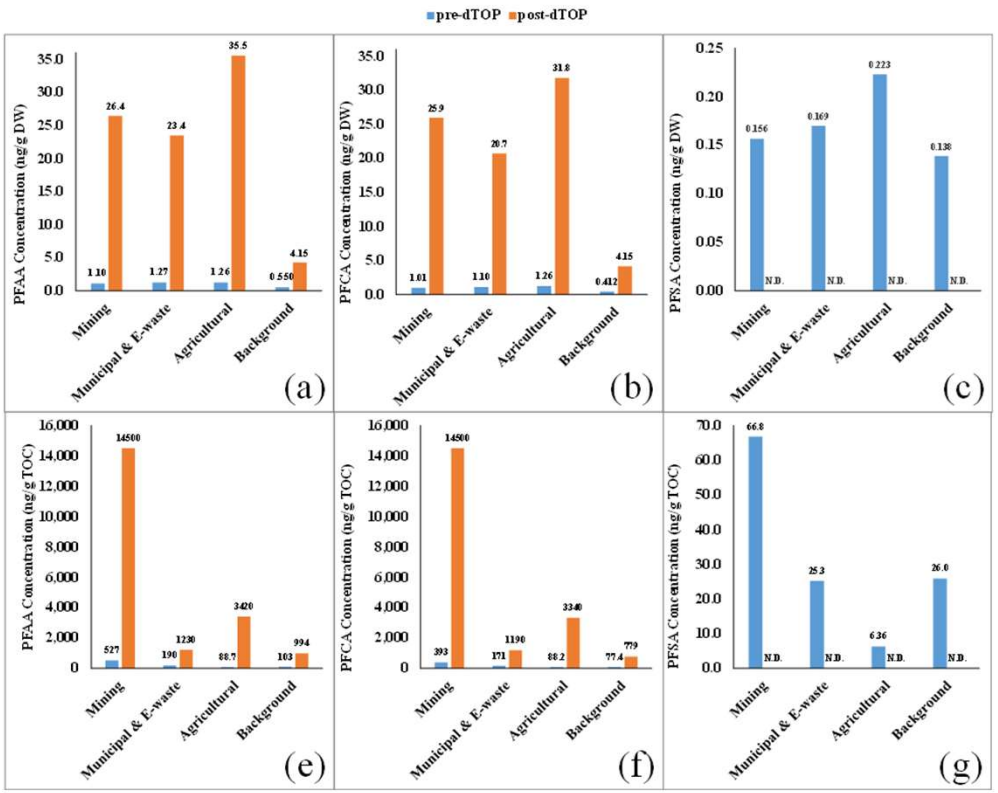
Tailoring TOP Assay for Enhanced Applicability



Comparison of the mean PFAS product yields from (a) extract-based oxidation (eTOP) versus (b) direct oxidation on sediment (dTOP), based on dry weight (DW). Solid bars represent PFCAs; striped bars represent PFSAs.





Medians of total PFAA (a), PFCA (b), and PFSA (c) and median of TOC-normalised total PFAA (e), PFCA (f), and PFSA (g)concentrations before and after dTOP assay (pre-dTOP & post-dTOP) in sediment samples from mining, municipal and e-waste, and agricultural regions in Ketа Lagoon.

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Under review

Thank you all for your attention!

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