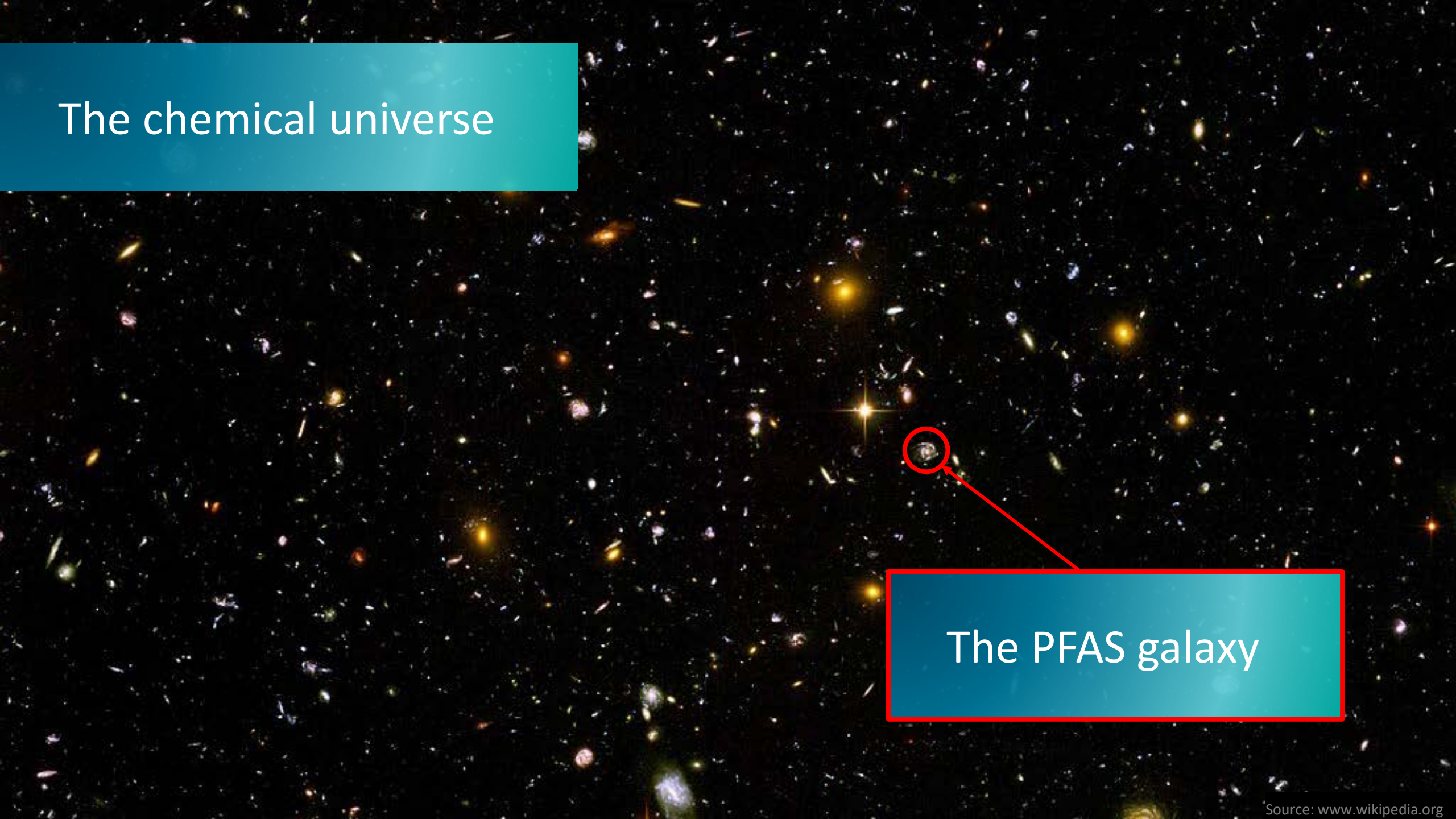
Untargeted Methods: Overview of Existing Methods

Bernd Göckener, Fraunhofer IME

PFAS – Challenges and Scientific Perspectives in Human Health Risk Assessment

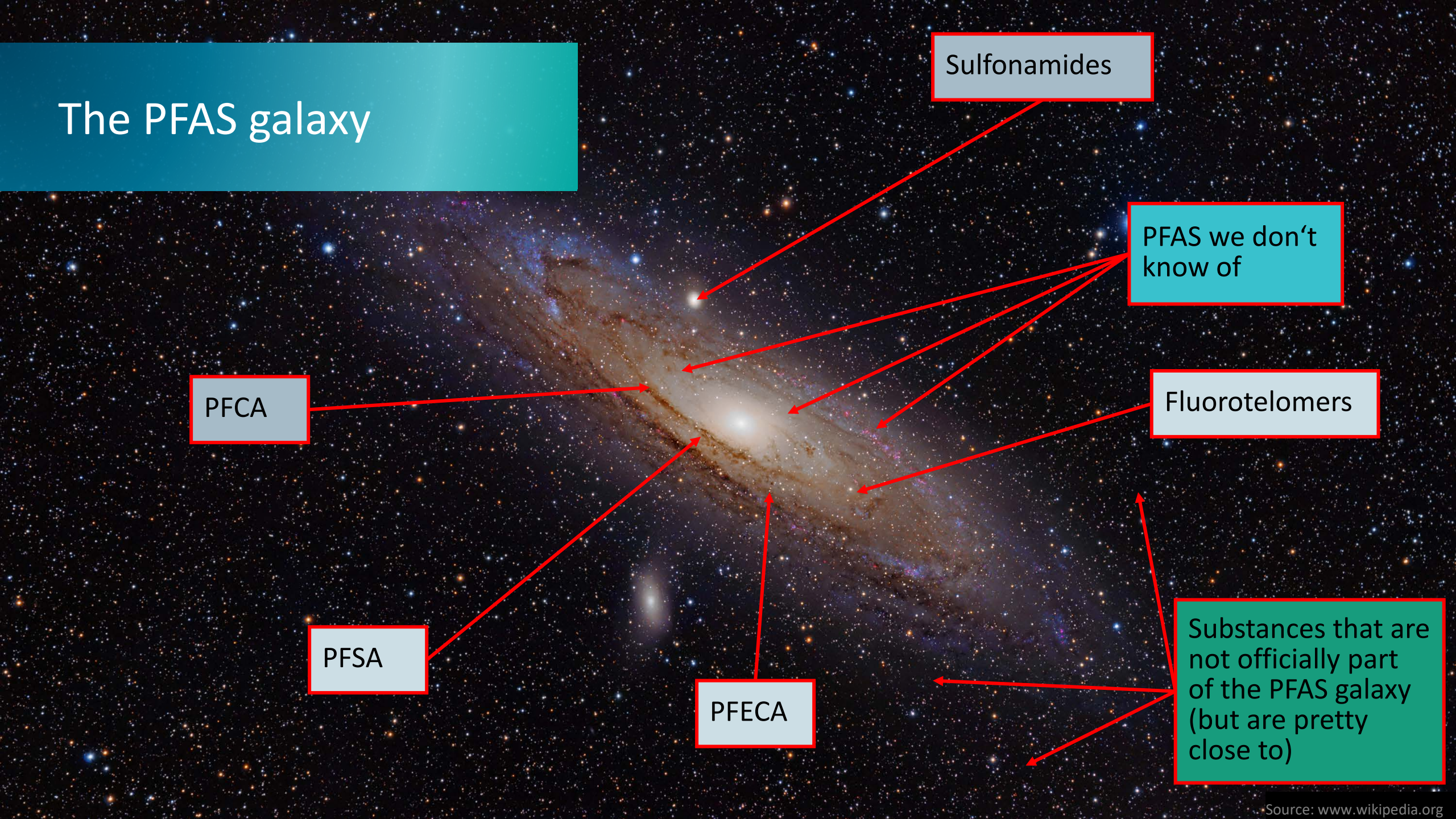
BfR, Berlin, October 8, 2025

The chemical universe



Source: www.wikipedia.org

The PFAS galaxy



Source: www.wikipedia.org

Target analysis
in the PFAS galaxy

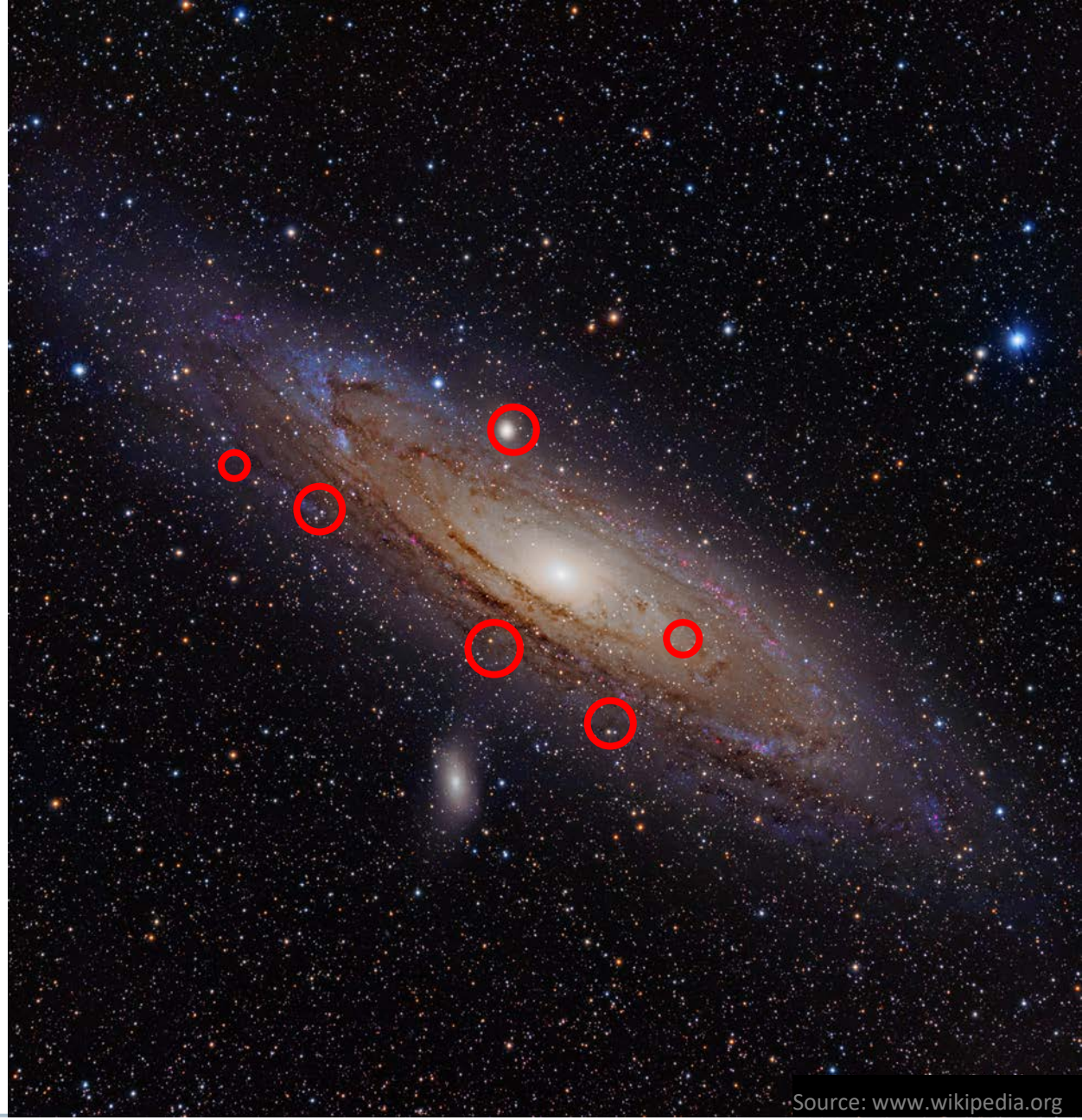
Pros

+ Quantitative
+ use of internal standards
+ highly sensitive
+ Highly specific for selected PFAS
+ Well established and often standardized

Cons

– Focus on few single PFAS (20-100 substances)
– Requires analytical standards (ideally also internal standards)
– *What you see is what you extract*

Alternatives required for a total PFAS assessment


Source: www.wikipedia.org

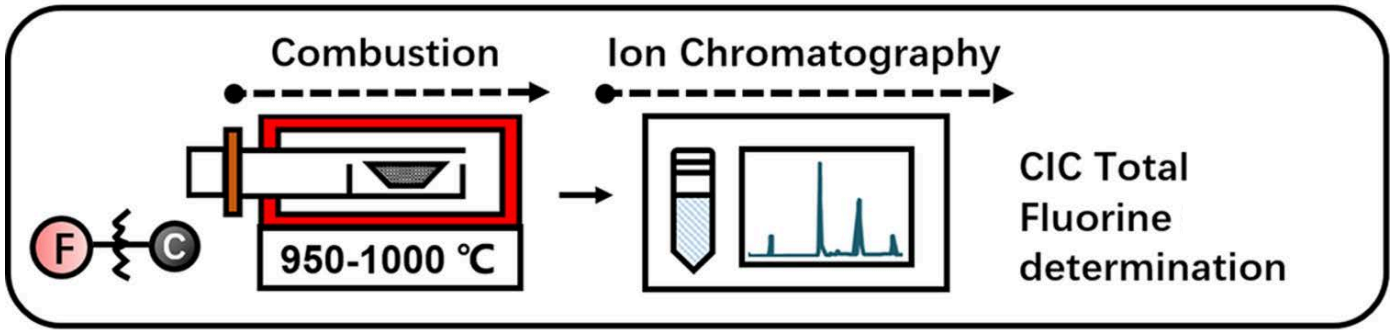
TF/EOF/AOF

Method overview (CIC)

Total Fluorine (TF)

- Combustion Ion Chromatography (CIC)
- Direct combustion of the sample and sorption of formed fluoride
- Analysis via Ion Chromatography

- Rarely used as it includes inorganic fluorine



Source: Pan & Helbling 2023: https://doi.org/10.1016/j.watres.2023.120497

TF/EOF/AOF

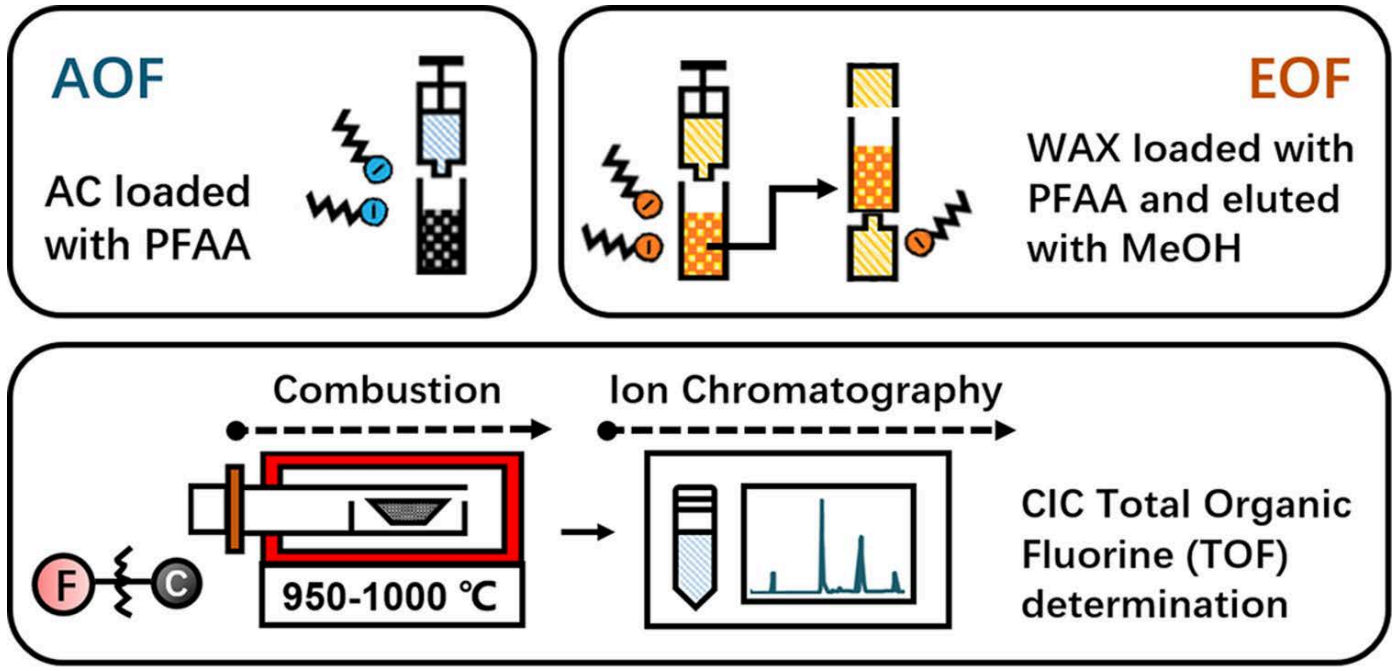
Method overview (CIC)

Adsorbable Organic Fluorine (AOF)

- Typically used for water samples
- Adsorption of fluorinated compounds on adsorbing material e.g., activated carbon
- Combustion of adsorbing material via CIC

Extractable Organic Fluorine (EOF)

- Used for more complex samples but also water samples
- Water samples: direct enrichment of PFAS via solid phase extraction (SPE)
- Complex samples: enrichment of extracts via SPE
- Followed by CIC



Source: Pan & Helbling 2023: https://doi.org/10.1016/j.watres.2023.120497

TF/EOF/AOF

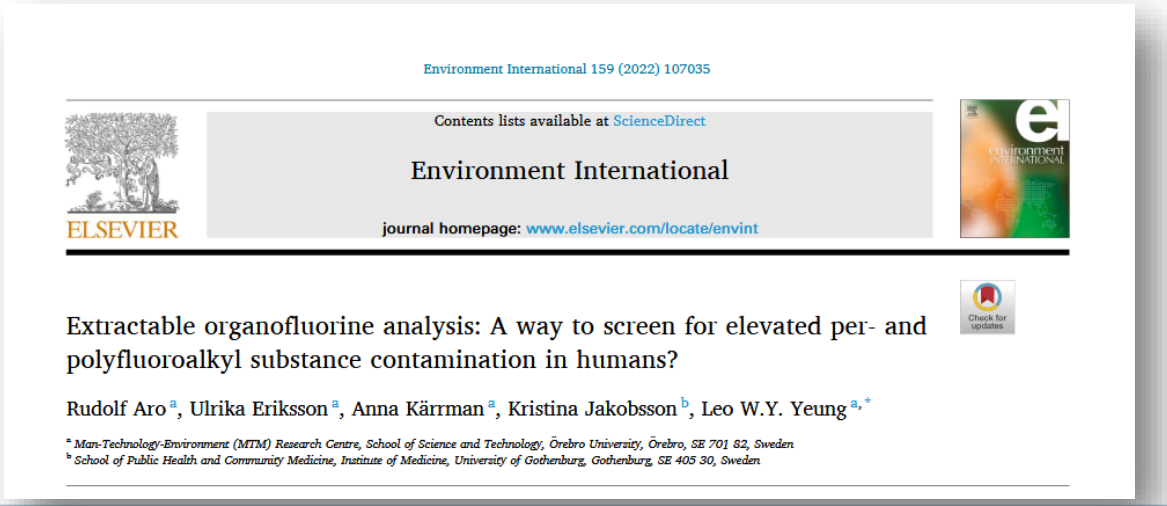
Examples

Target vs. EOF analysis in whole blood

- Blood samples from a region with known drinking water contamination
- Identified EOF: Target analysis results expressed as ng F/mL

  vs.
- Unknown Organic Fluorine (UOF)

➢ **High amount of unknown (i.e. overseen) fluorinated substances**



Environment International 159 (2022) 107035

Contents lists available at ScienceDirect

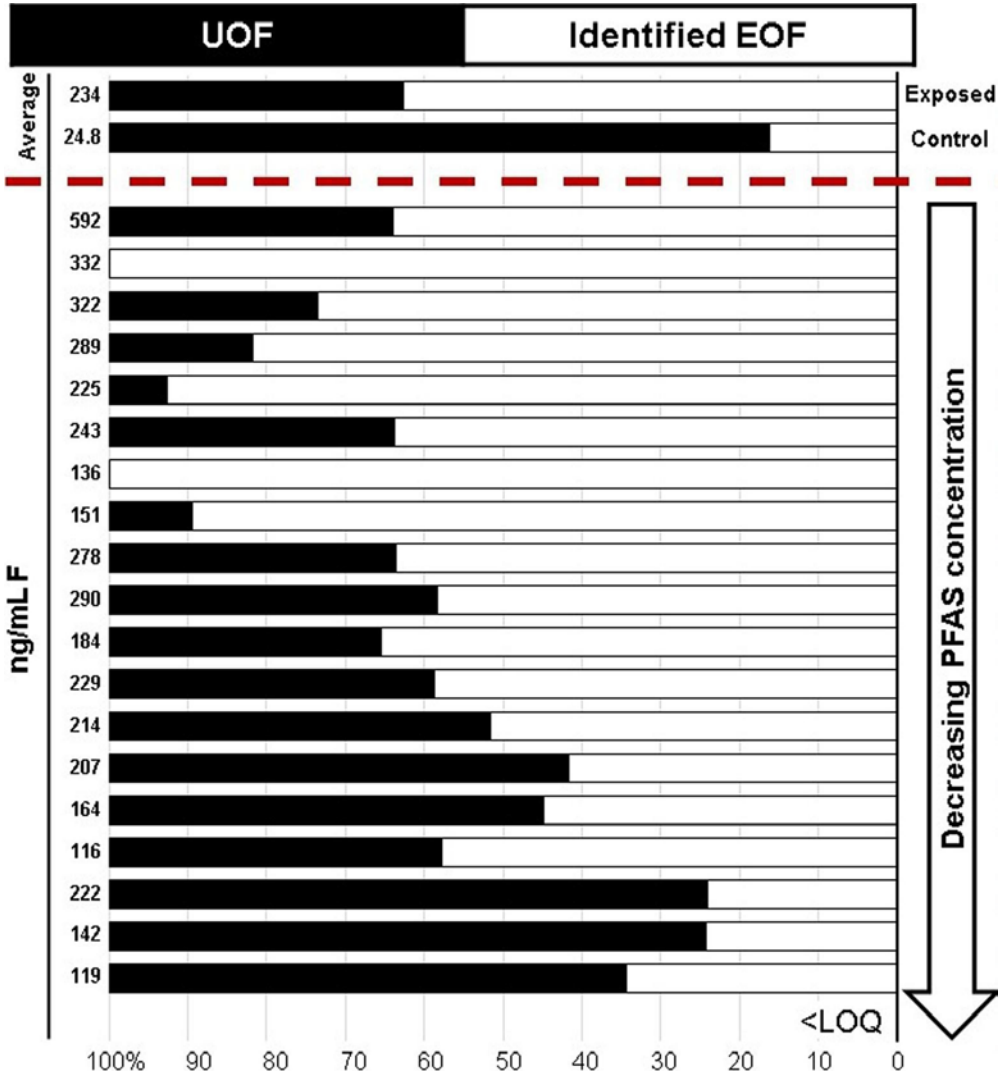
Environment International

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

Extractable organofluorine analysis: A way to screen for elevated per- and polyfluoroalkyl substance contamination in humans?

Rudolf Aro [a], Ulrika Eriksson [a], Anna Kärrman [a], Kristina Jakobsson [b], Leo W.Y. Yeung [a,*]

[a] Man-Technology-Environment (MTM) Research Centre, School of Science and Technology, Örebro University, Örebro, SE 701 82, Sweden
[b] School of Public Health and Community Medicine, Institute of Medicine, University of Gothenburg, Gothenburg, SE 405 30, Sweden



Source: Aro et al. 2022: https://doi.org/10.1016/j.envint.2021.107035

Fraunhofer IME

TF/EOF/AOF (CIC)
in the PFAS galaxy

Pros

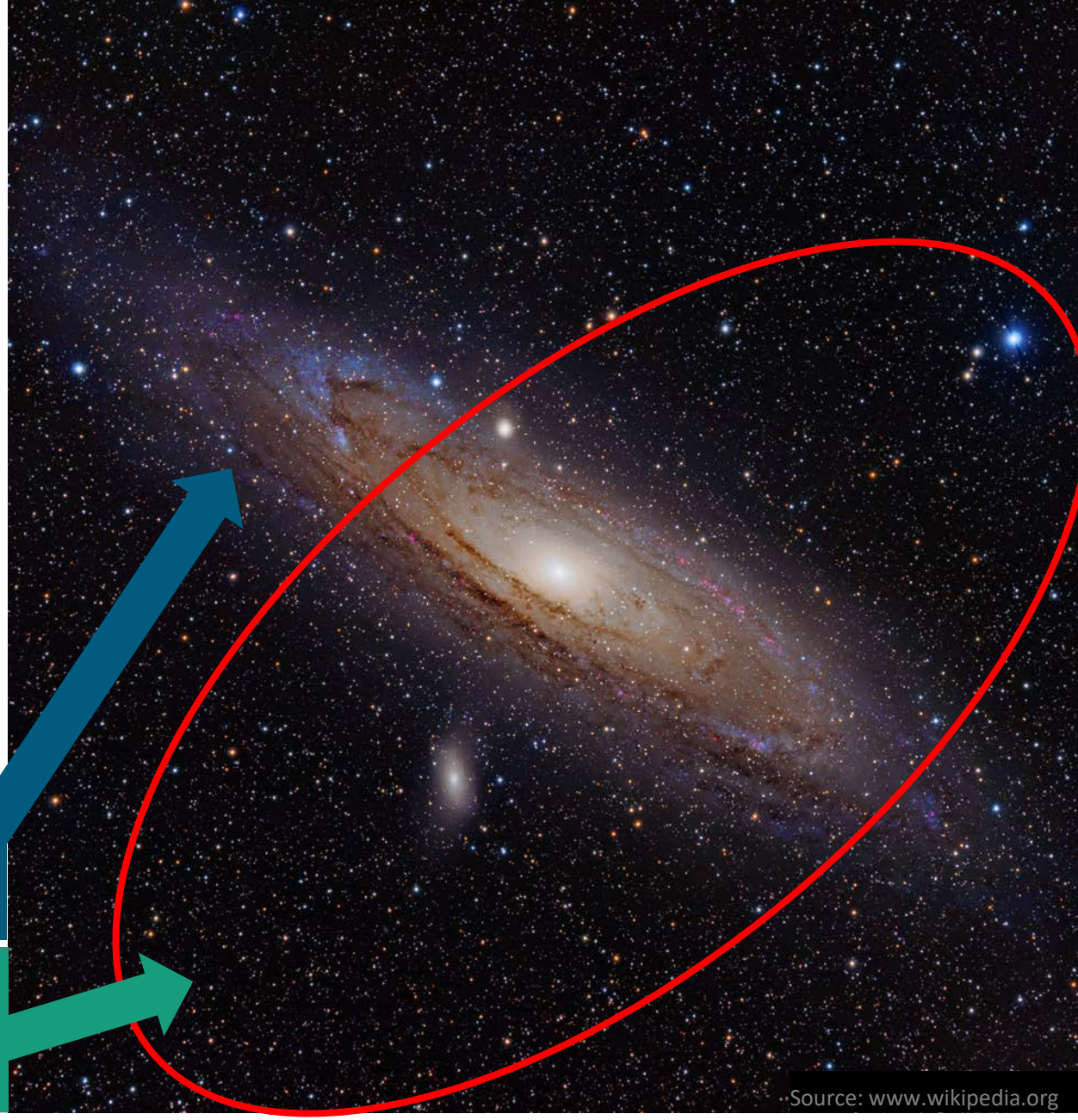
+ Covers all (extractable/adsorbable) PFAS
+ Fair sensitivity compared to other sum parameters

Cons

– Oversees non-extractable and non-adsorbable PFAS
– No structure information
– Covers Non-PFAS fluorinated substances
– CIC: potential of losses and thus underestimation

Non-extracted/non-adsorbed PFAS

Fluorochemicals, not falling under the PFAS definition

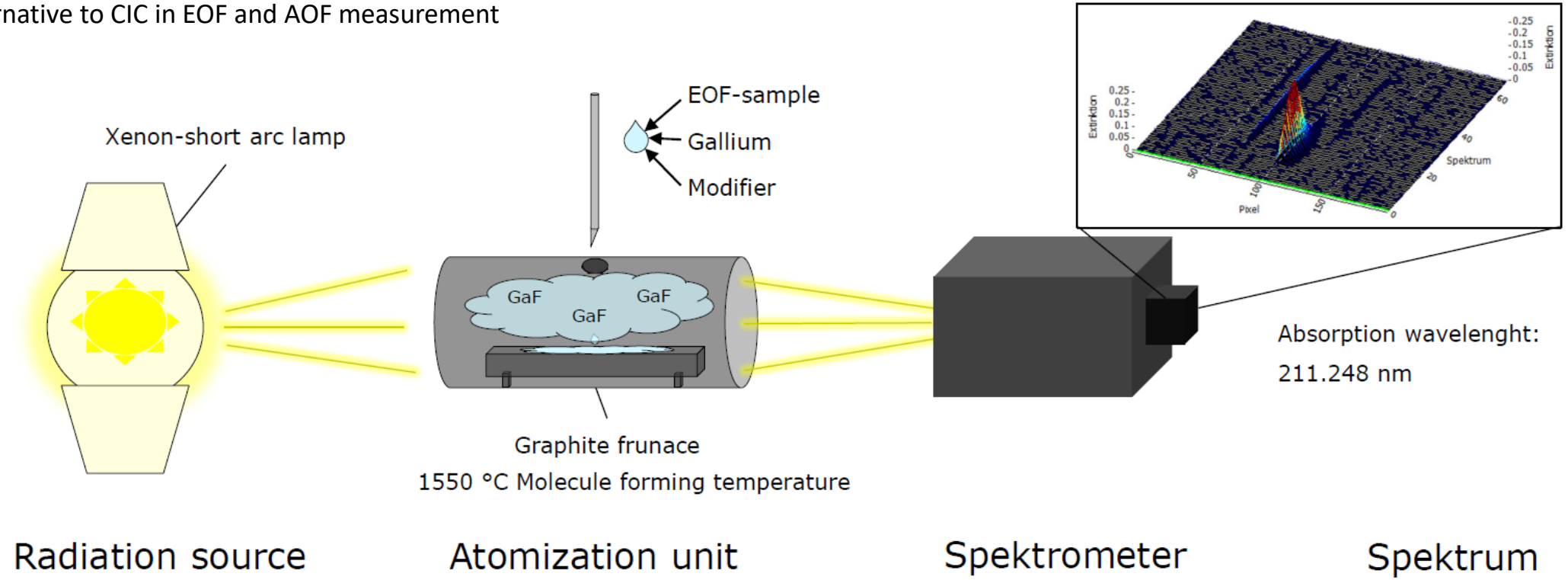


TF/EOF/AOF

Method overview (HR-GFMAS)

High-resolution graphite furnace molecular absorption spectrometry (HR-GFMAS)

- Recent alternative to CIC in EOF and AOF measurement



Meermann 2025: DAAS- Industrie Anwenderseminar, 01 July 2025

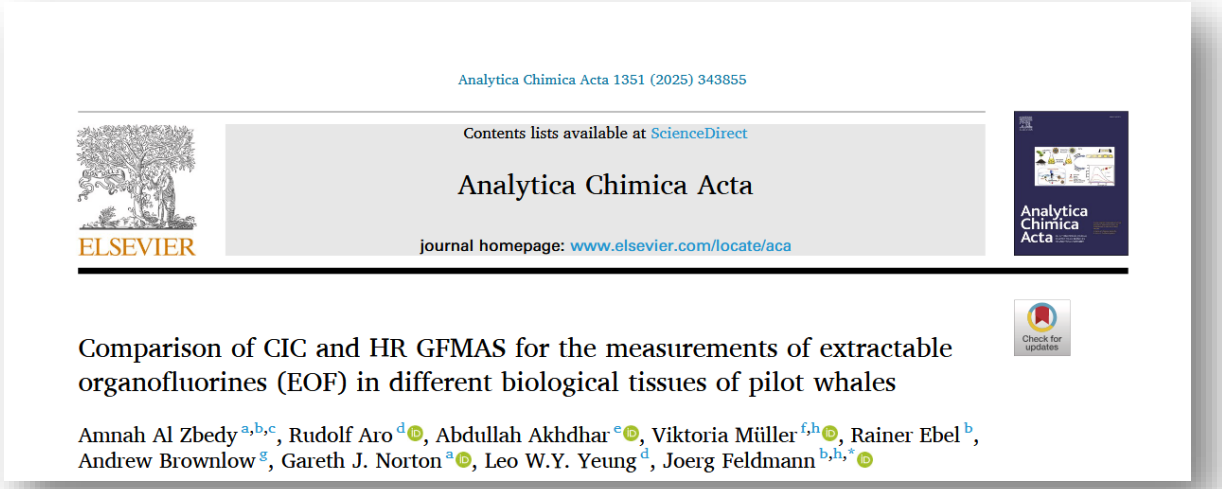
TF/EOF/AOF

Examples

CIC vs. HR-GFMAS

- EOF analysis in tissues of whales stranded in Scotland
- Identical extraction, different EOF determination methods

- HR-GFMAS showed higher levels of EOF than CIC
- Explanation: low recoveries of PFAS during the CIC lead to losses of fluorine


Analytica Chimica Acta 1351 (2025) 343855

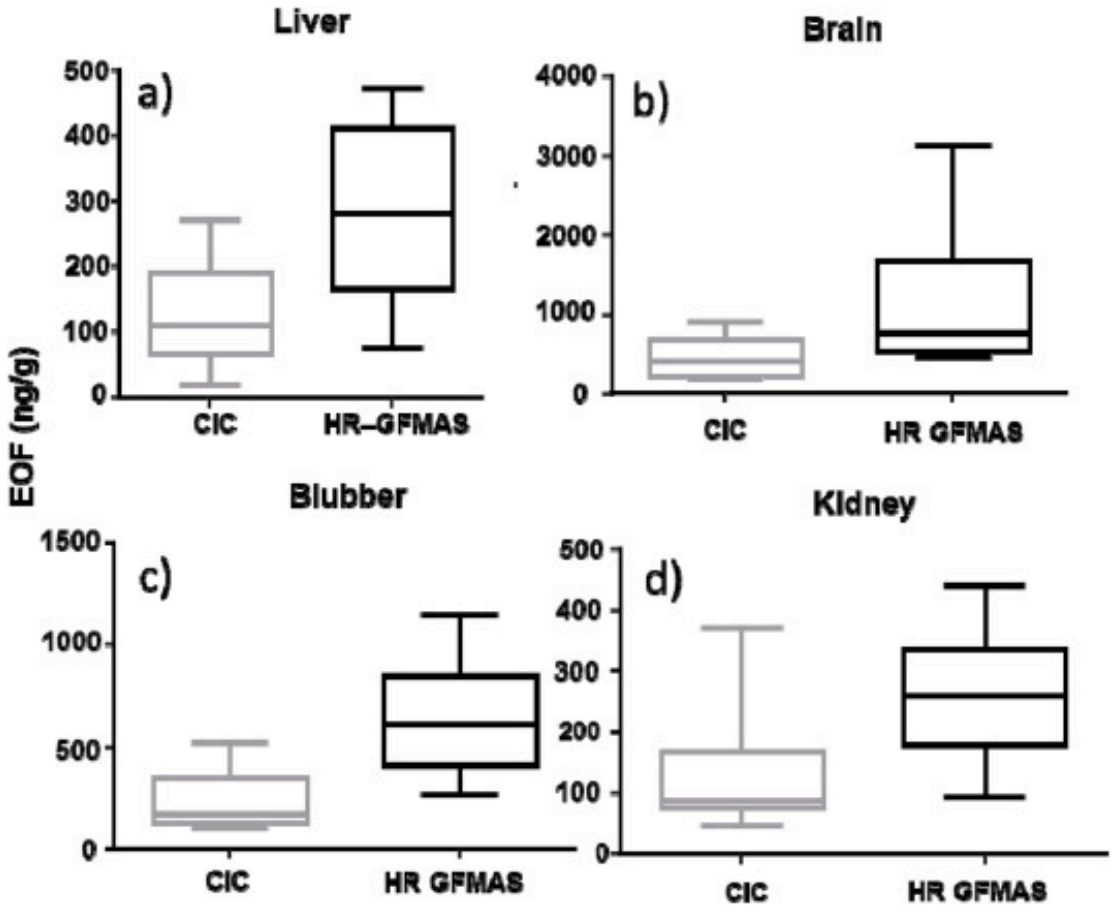
Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Comparison of CIC and HR GFMAS for the measurements of extractable organofluorines (EOF) in different biological tissues of pilot whales

Amnah Al Zbedy [a,b,c], Rudolf Aro [d], Abdullah Akhdhar [e], Viktoria Müller [f,h], Rainer Ebel [b], Andrew Brownlow [g], Gareth J. Norton [a], Leo W.Y. Yeung [d], Joerg Feldmann [b,h,*]



Source: Al Zbedy et al 2025: https://doi.org/10.1016/j.aca.2025.343855

Fraunhofer IME

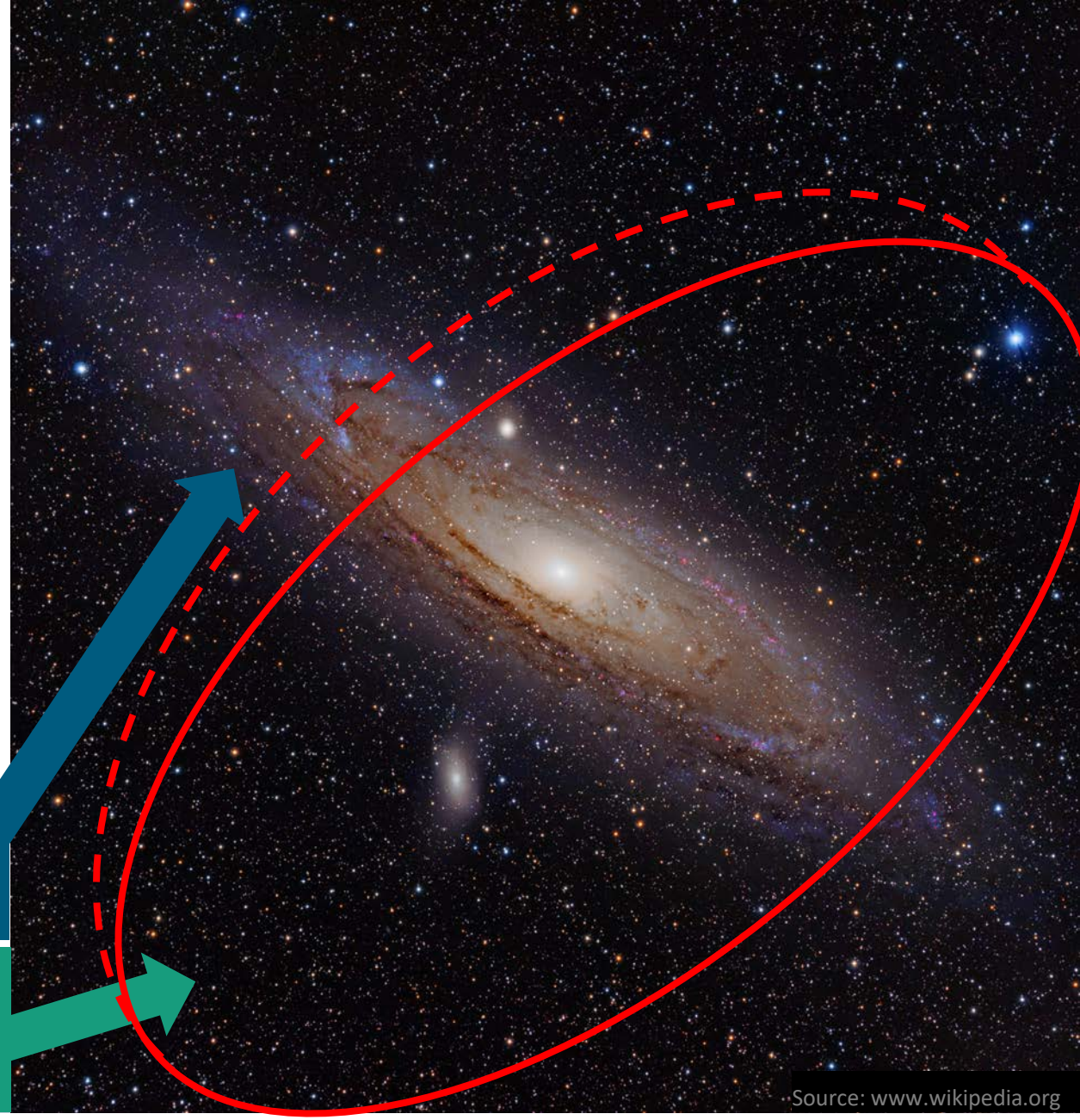
TF/EOF/AOF (HR-GFMAS)
in the PFAS galaxy

Pros

+ Covers all (extractable/absorbable) PFAS
+ Fair sensitivity compared to other sum parameters
+ Reduced losses in comparison to CIC analysis

Cons

– No structure information
– Oversees non-extractable and non-adsorbable PFAS
– Covers Non-PFAS fluorinated substances

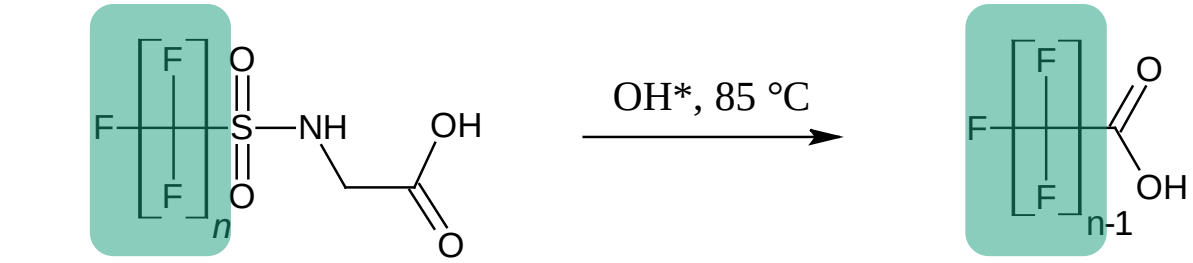


TOP Assay

Method overview

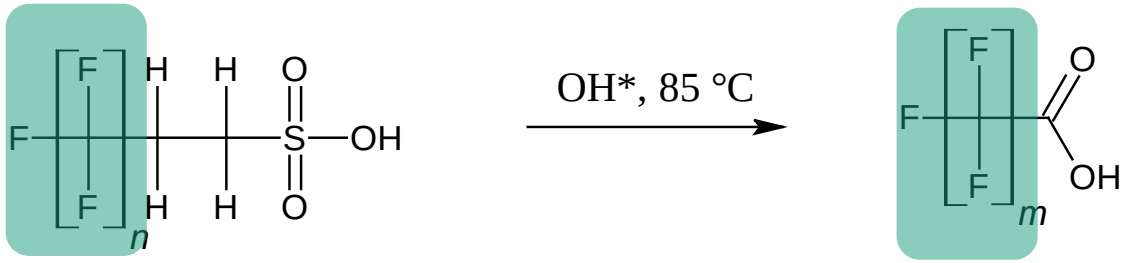
Total Oxidizable Precursor Assay

- Oxidative in-lab conversion of known and unknown precursors
- Precursors: PFAS that can be transformed to legacy PFAS in the environment and in organisms
- Formation of oxidation products (mainly PFCA) that can be measured with established methods
- Comparison of PFCA and PFSA levels before (target) and after oxidation: indicator of precursor abundance



Sulfonamidoacetic acid (FASAA)

PFCA



Fluortelomersulfonic acid (FtS)

PFCAs
m= n-2; n-1; n-3

TOP Assay

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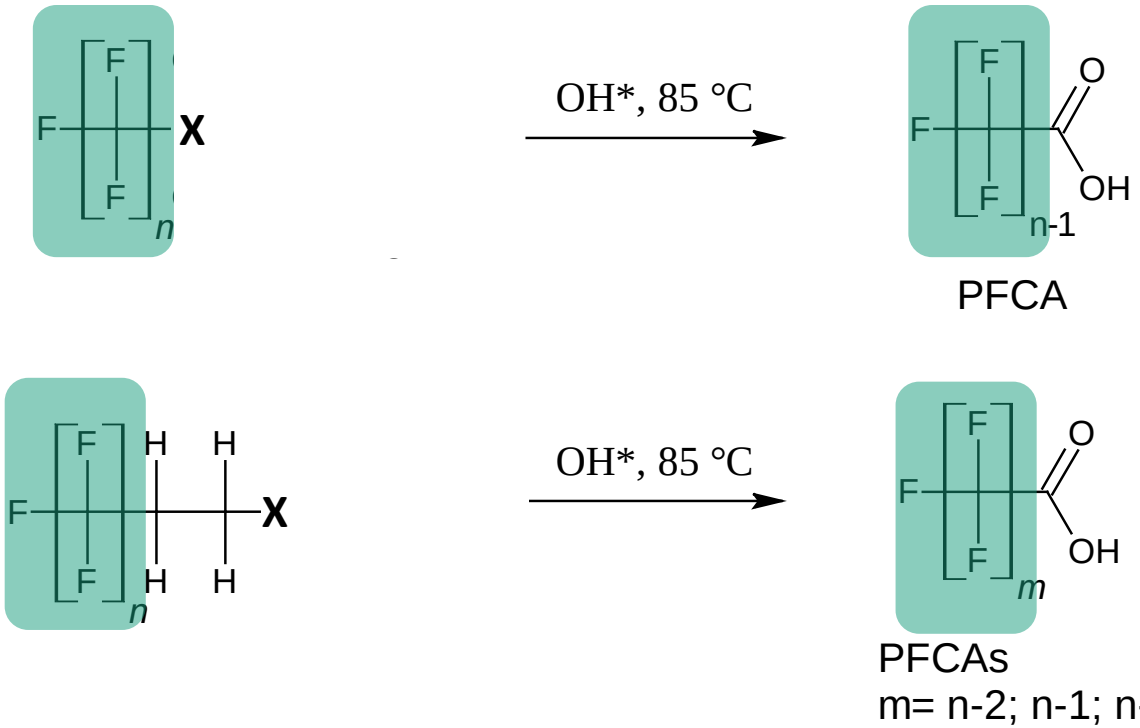

TOP Assay

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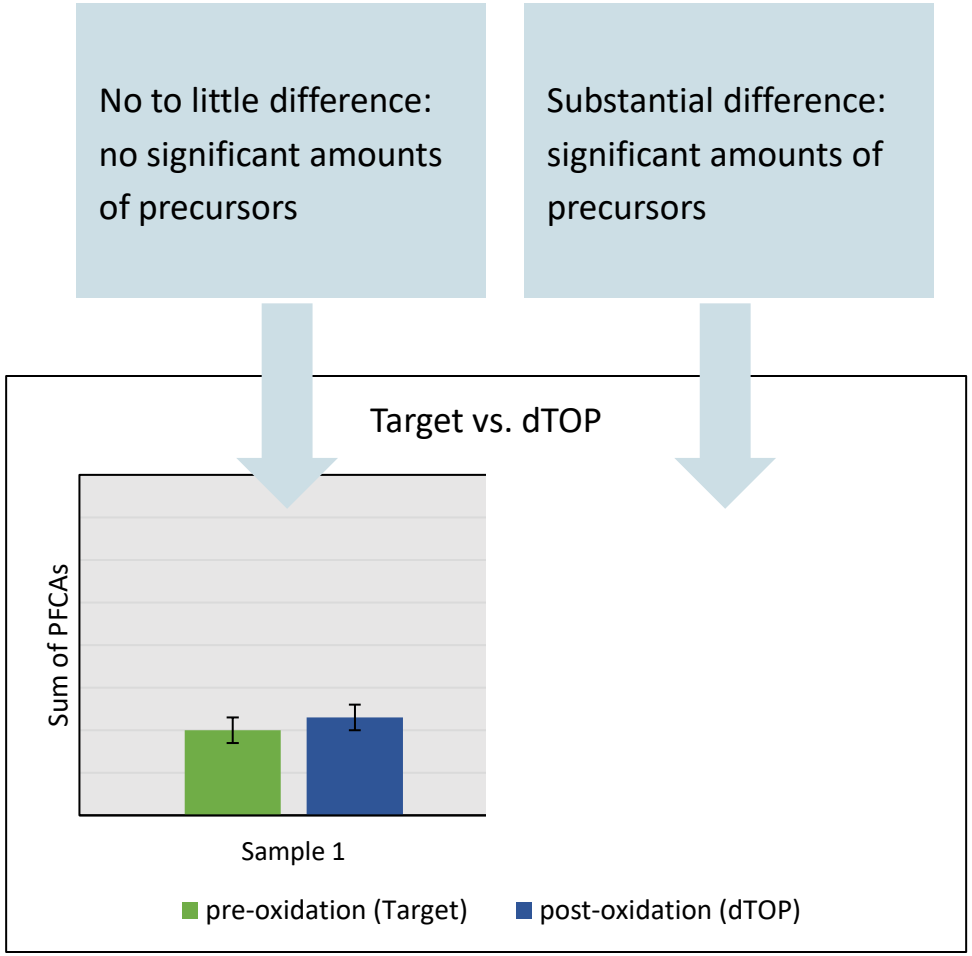
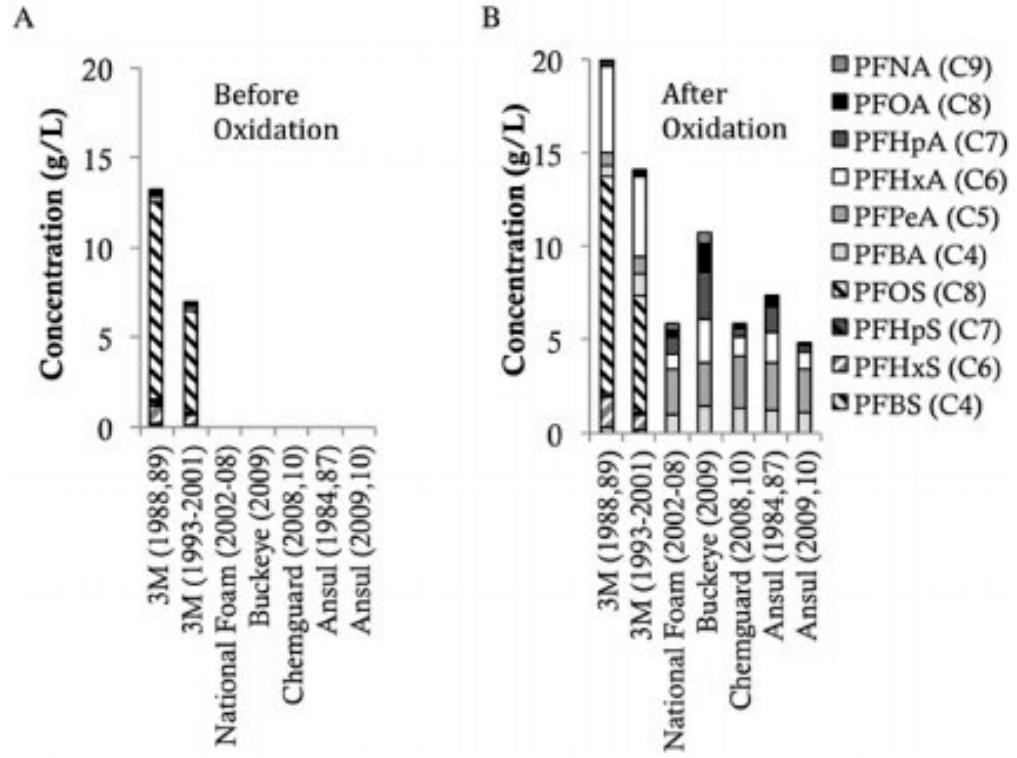


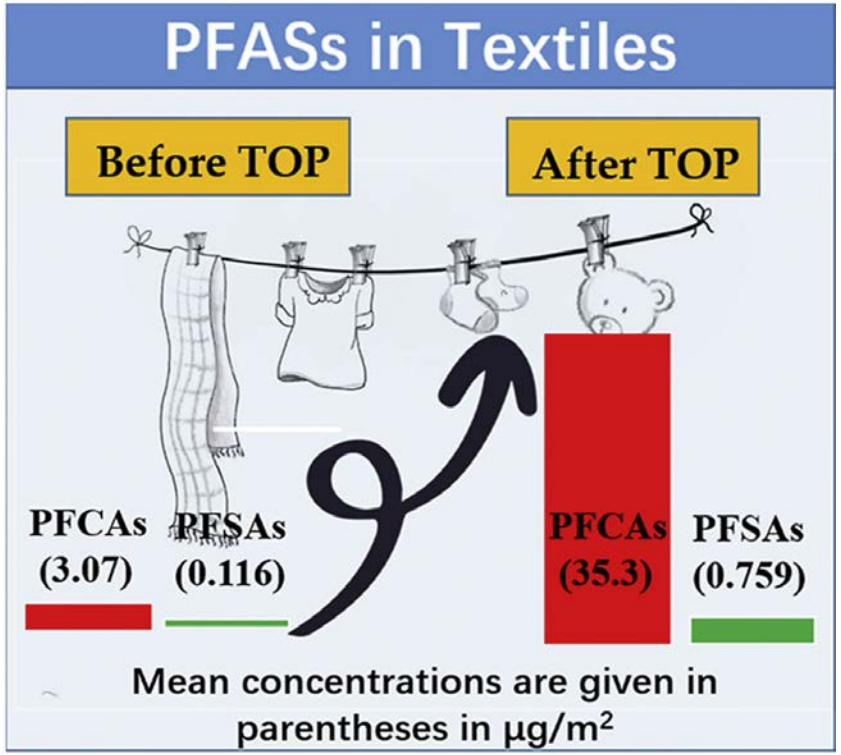
TOP Assay
Examples

TOP assay analysis in fire fighting foams



Source: Houtz et al 2013: https://doi.org/10.1021/es4018877

TOP assay analysis in textiles



Source: Zhu & Kannan 2020: https://doi.org/10.1016/j.envpol.2020.114940

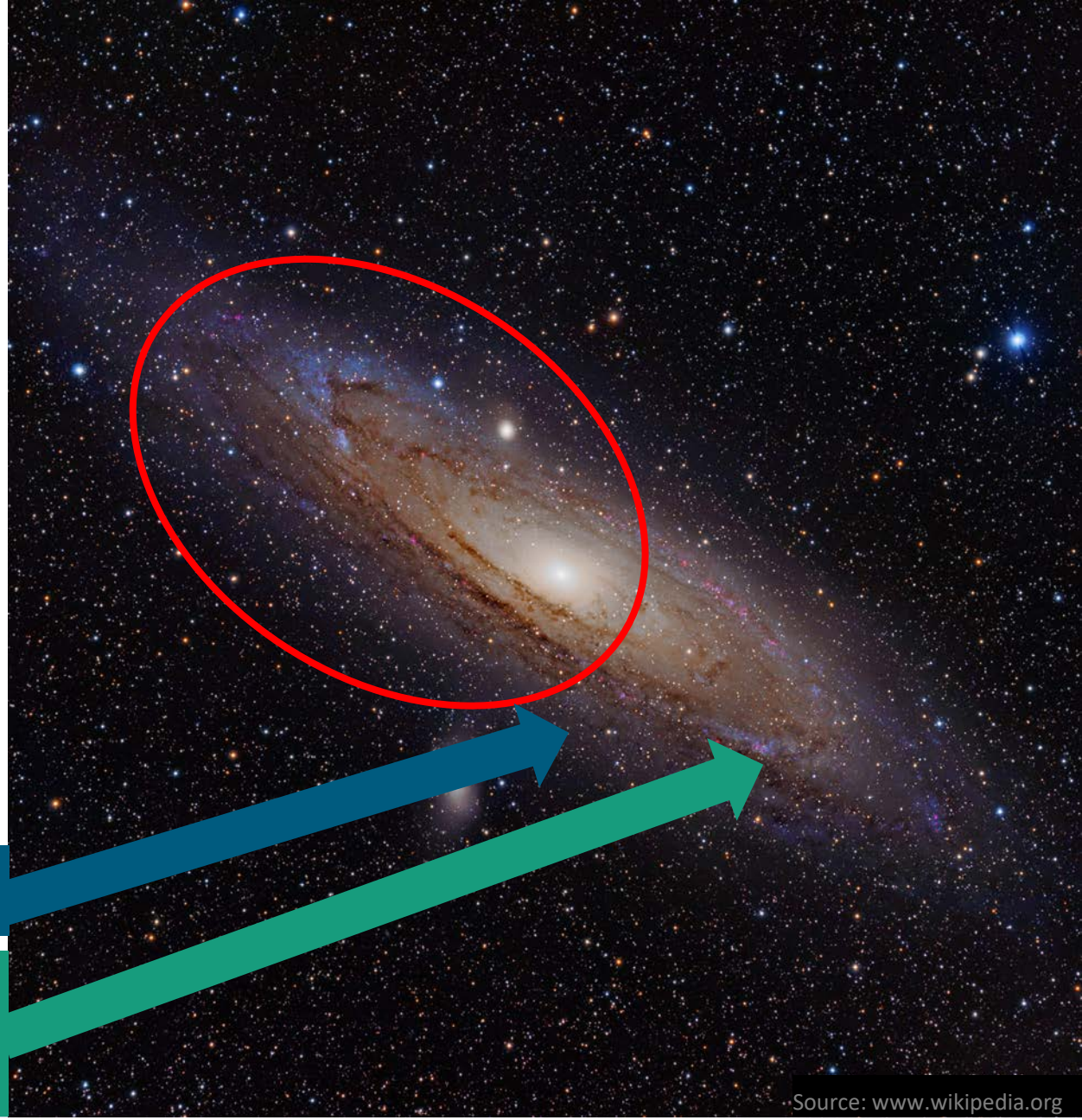
TOP Assay
in the PFAS galaxy

Pros

+ Quantitative assessment of unknown PFAS (i.e. precursors)
+ Good sensitivity
+ Highly selective for PFAS
+ Semi-destructive: Hints towards mobility/accumulation potential

Cons

– Does not include all PFAS
– Does not include non-extractable PFAS
– Limited suitability for complex samples (e.g. food/biota)

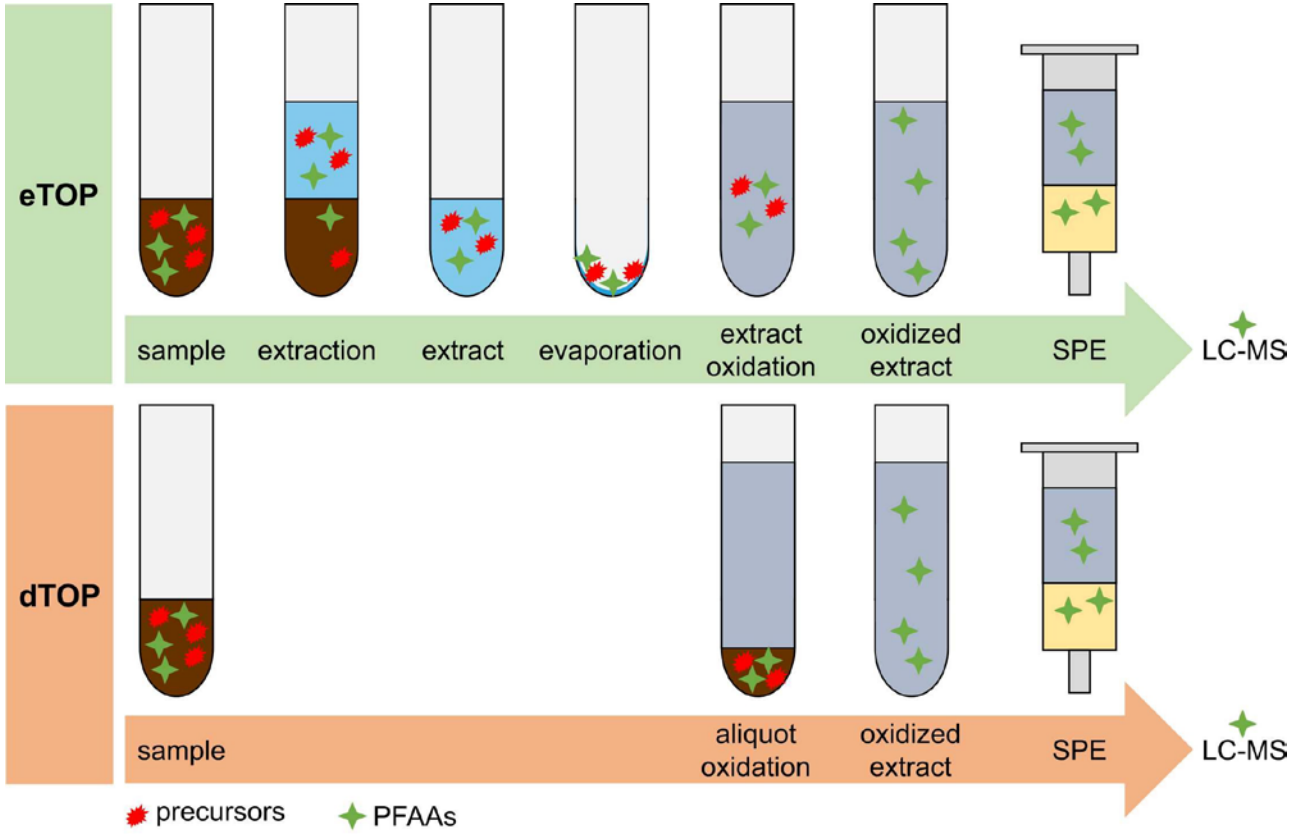


Fraunhofer IME

dTOP Assay
Method overview

Direct TOP Assay

- Normal TOP assay: challenges with complex samples. Underestimation due to
  - Non-extracted PFAS
  - Incomplete oxidation due to co-extracted organics
- dTOP Assay:
  - Full digestion and oxidation of solid samples with an excess of oxidation agent
  - No extraction losses, no incomplete oxidation



Source: Lange, Freeling, Göckener 2024: https://doi.org/10.1016/j.teac.2024.e00242

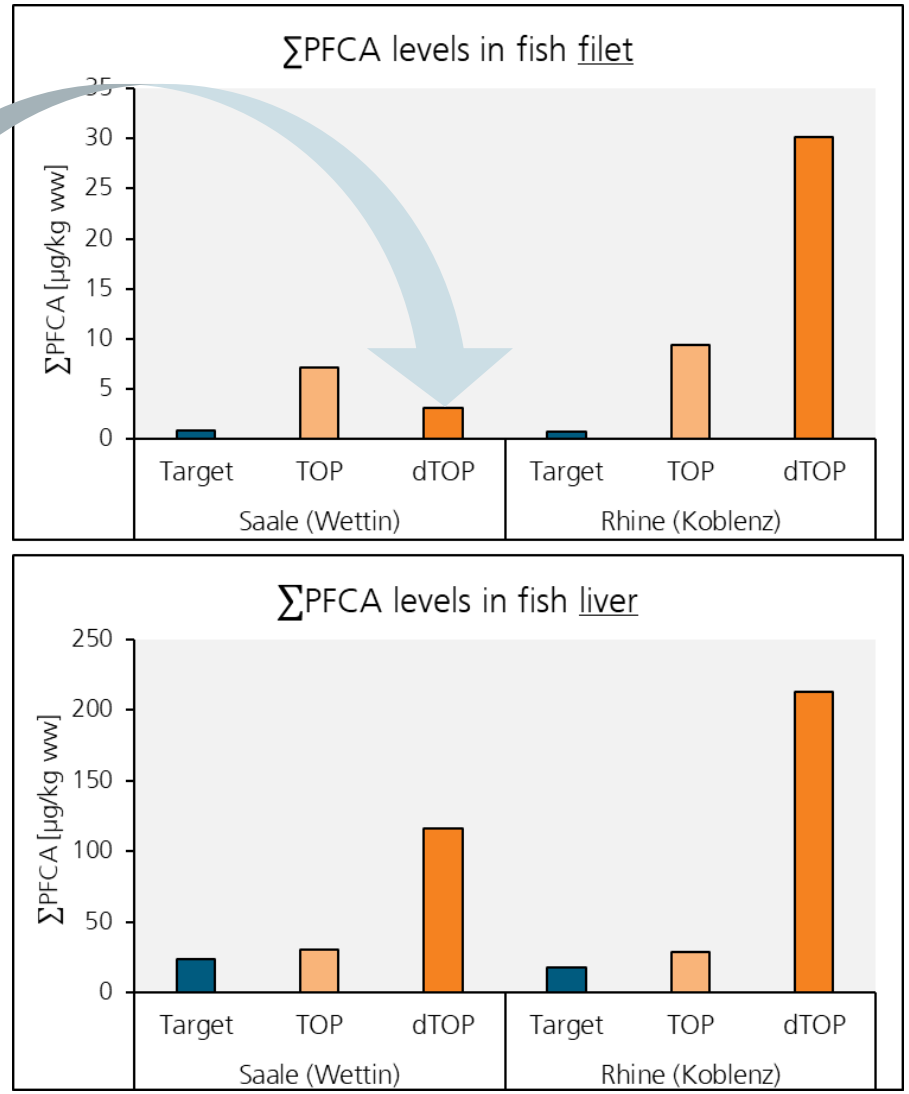
dTOP Assay

Examples

Target – TOP – dTOP comparison in fish tissue

- dTOP assay covers more PFAS than the normal TOP assay

Underestimation by the dTOP assay due to low levels
LOQ (dTOP) = 2,5 µg/kg
LOQ (TOP) << 2,5 µg/kg



Source: UBA, UFZ, Fraunhofer IME, unpublished data

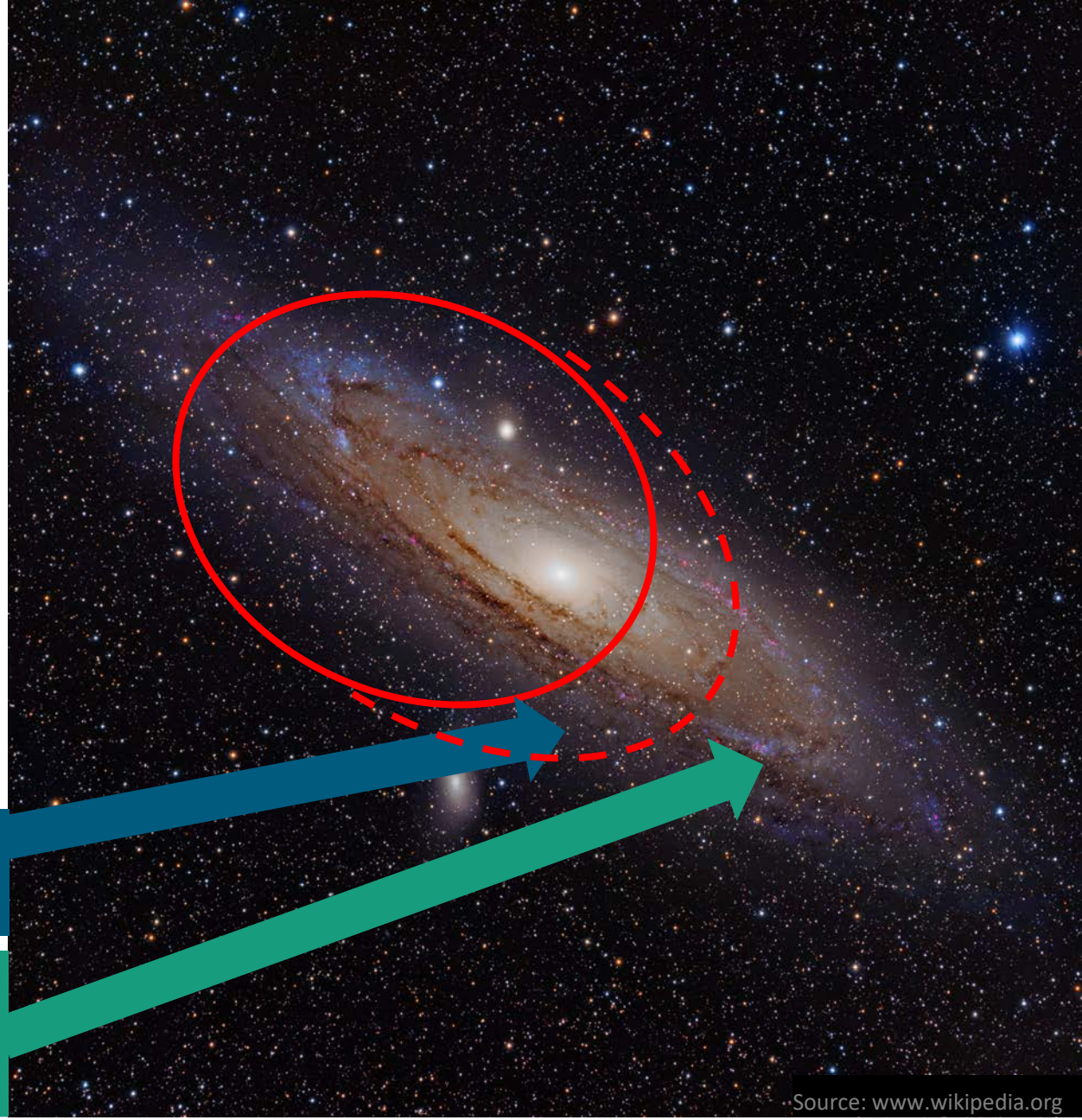
dTOP Assay
in the PFAS galaxy

Pros

+ Quantitative assessment of unknown PFAS (i.e. precursors)
+ Highly selective for PFAS
+ Semi-destructive: Hints towards mobility/accumulation potential
+ Includes non-extractable PFAS
+ Allows application for complex samples

Cons

– Does not include all PFAS
– Reduced sensitivity



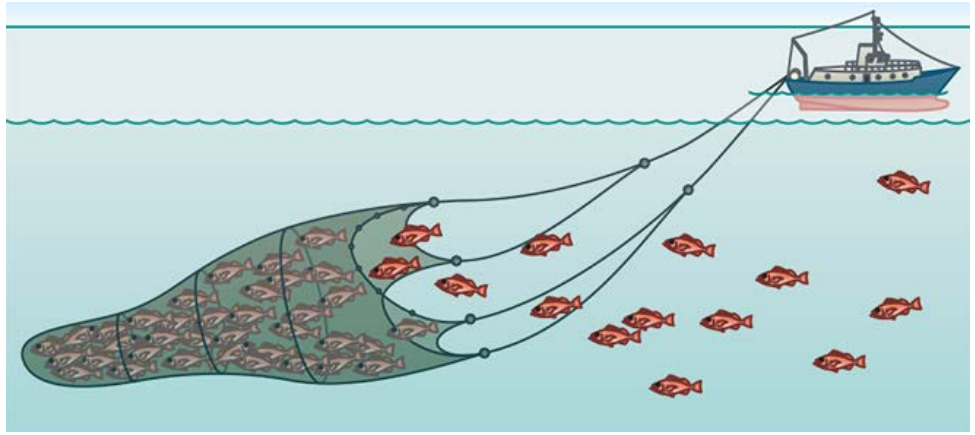
Source: www.wikipedia.org

Suspect & Non-Target Screening

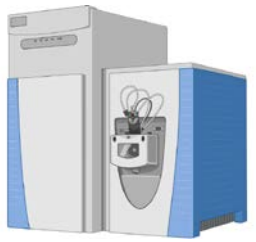
Method overview

"Catch them all"

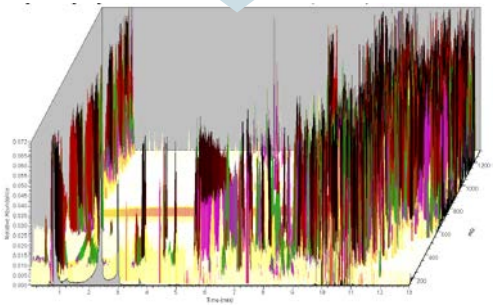
- High-resolution mass spectrometry (HRMS)
- Liquid (LC) and gas chromatography (GC)
- Broad-band scan of masses and fragments



Source: www.worldoceanreview.com

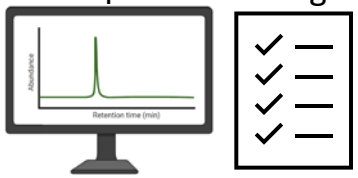


HRMS analysis



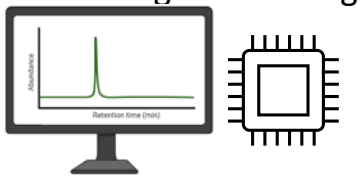
complex data set

Suspect Screening



suspect list check

Non-Target Screening



peak picking algorithms, prioritization & identification

List of detected substances ((semi-)qualitative)

List of prioritized features ((semi-)qualitative)

Partially created in https://BioRender.com

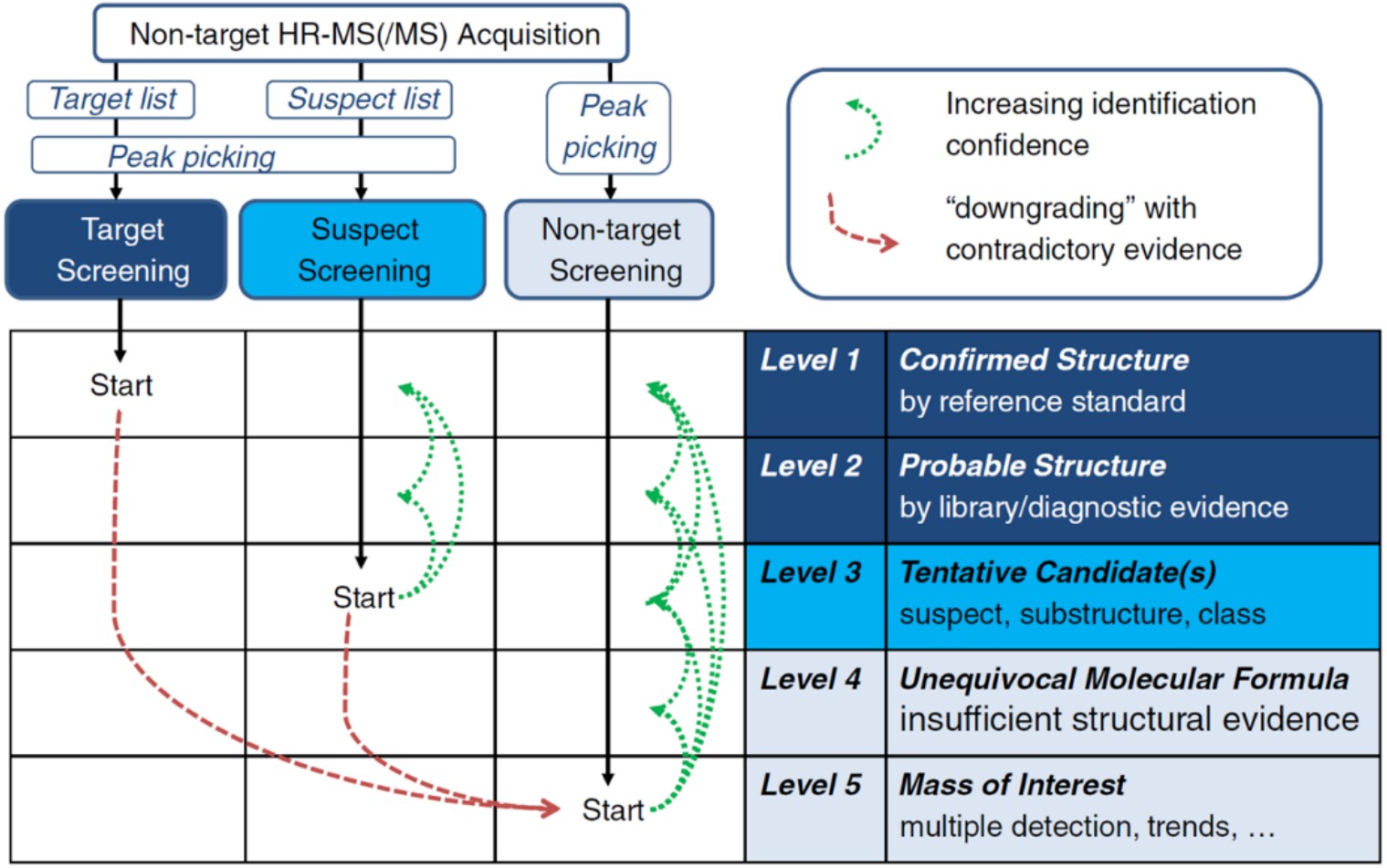
Suspect & Non-Target Screening

Method overview

"Catch them all"

- High-resolution mass spectrometry (HRMS)
- Liquid (LC) and gas chromatography (GC)
- Broad-band scan of masses and fragments

- Confidence assessment of findings
  - Identification level classification scheme by **Schymanski et al 2015**



Source: Haglund & Veenaas 2019: DOI: 10.13140/RG.2.2.35033.47209

Suspect & Non-Target Screening

Method overview

“Catch them all”

- High-resolution mass spectrometry (HRMS)
- Liquid (LC) and gas chromatography (GC)
- Broad-band scan of masses and fragments

- Confidence assessment of findings
  - Identification level classification scheme by **Schymanski et al 2015**
  - Alternative, more detailed and PFAS-specific scheme by **Charbonnet et al 2022**

Level	Identification Confidence	Accurate Mass	Mass Defect	Isotopic Pattern Match	Consistent RT	Homologue (number; level)	MS^2 Fragments (number; type)	Library MS^2	Reference Standard
Level 1a	Confirmed by reference standard	✓	✓	✓	✓			✓	✓
Level 1b	Indistinguishable from reference standard	✓	✓	✓	✓			✓	✓
Level 2a	Probable by library spec. match	✓	✓	✓	✓			✓	
Level 2b	Probable by diagnostic fragmentation evidence	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 3; diagnostic**		
Level 2c	Probable by diagnostic homologue evidence	✓	✓	✓	✓	**≥ 2; ≥ level 2a**	**≥ 2; diagnostic**		
Level 3a	Positional isomer candidates	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 1; subclass-aligned**		
Level 3b	Fragmentation-based candidate	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 1; subclass-aligned**		
Level 3c	Circumstantial candidate with fragmentation evidence	✓	✓	✓	✓	*≥ 1; ≥ level 3*	**≥ 1; subclass-aligned (in silico)**		
Level 3d	Circumstantial candidate with homologue evidence	✓	✓	✓	✓	**≥ 2; ≥ level 2a**			
Level 4	Unequivocal molecular formula	✓	✓	✓					
Level 5a	PFAS suspect screening exact mass match	**✓ (suspect list match)**							
Level 5b	Nontarget PFAS exact mass of interest	✓	✓			*≥ 3*	*≥ 2; subclass-aligned (in silico)*		

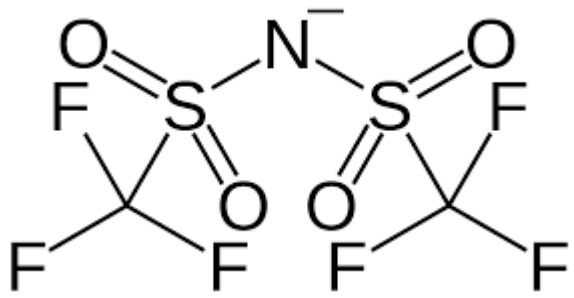
Source: Charbonnet et al 2022: https://doi.org/10.1021/acs.estlett.2c00206

Suspect & Non-Target Screening

Examples

Suspect Screening in Water Samples

- Surface, waste, and tap water from Lyon region with known industrial PFAS production
- Suspect screening identified various compound classes that are typically not included in target analyses
- Often detected: **Bistriflimide**
  - Used as ionic liquids for lithium-ion batteries



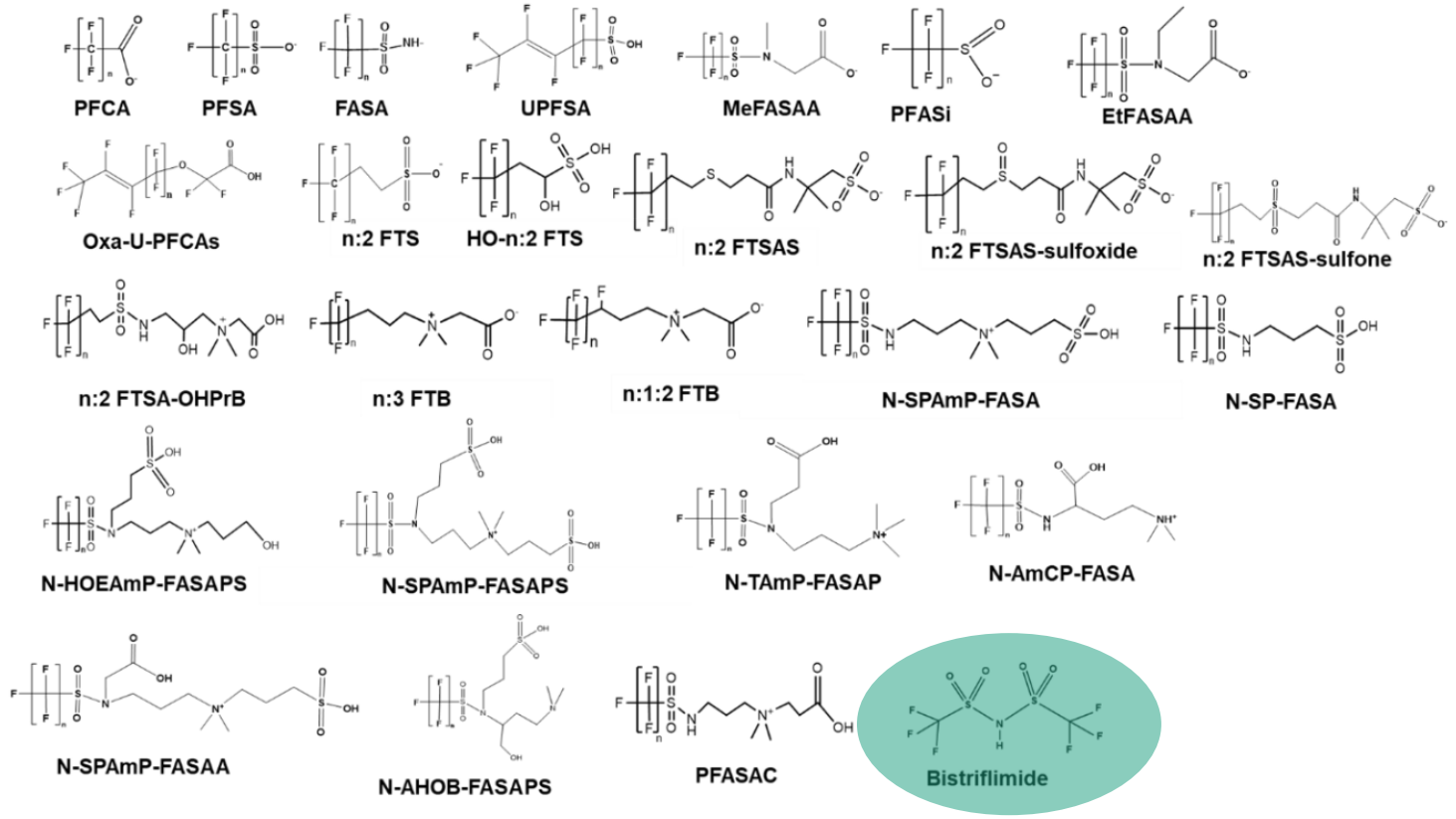


Figure S8. Proposed structures of bistriflimide and other key PFAS classes analyzed in this study.

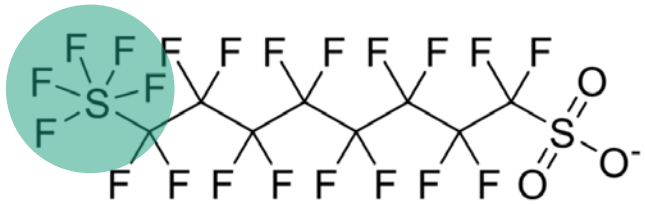
Source: Teymoorian et al 2025: https://doi.org/10.1021/acs.estlett.4c01126

Suspect & Non-Target Screening

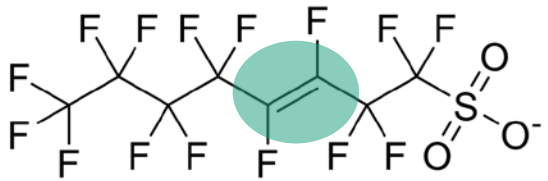
Examples

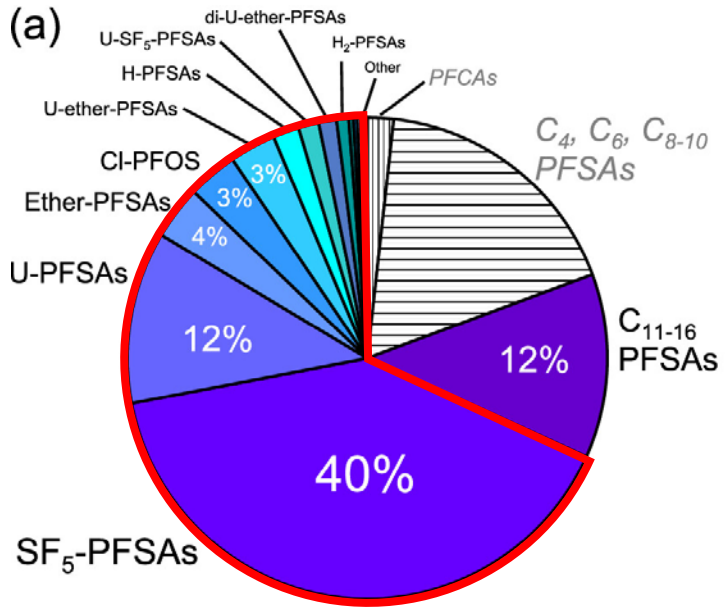
Soil from contamination site

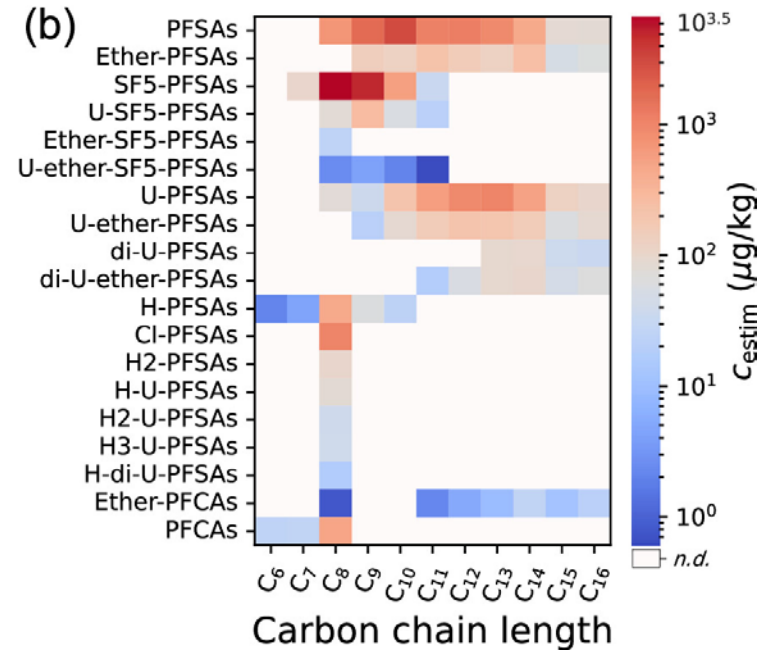
- Non-Target Screening
- Identification of several PFAS classes with various chain-lengths
- Semi-quantitative estimation of concentrations
- Most abundant:
  - SF_5-PFSA



  - Unsaturated PFSA







Source: Zweigle et al 2023: https://doi.org/10.1021/acs.est.2c07969

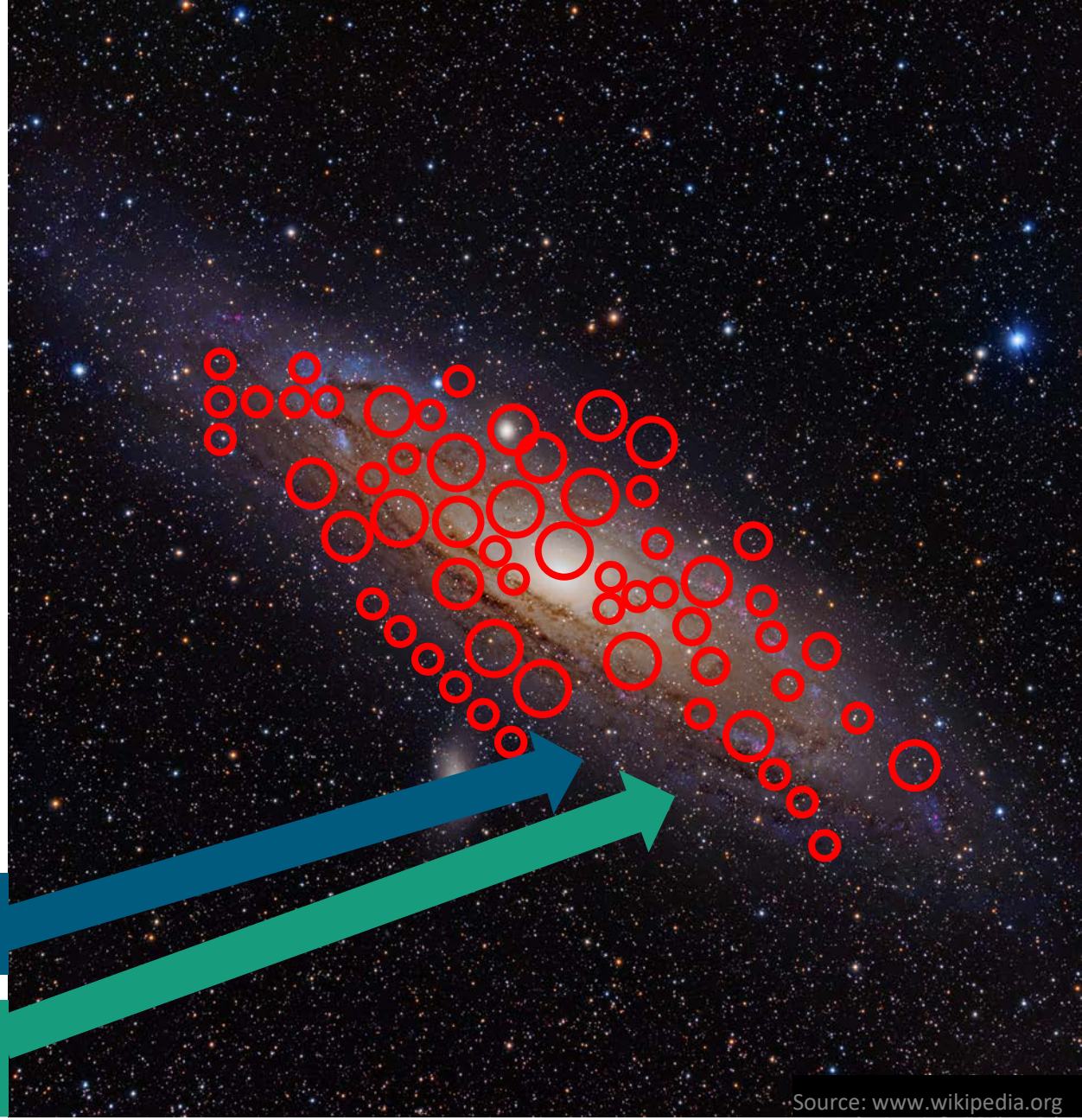
Suspect & Non-Target Screening
in the PFAS galaxy

Pros

+ Lots of potential to identify unknown PFAS
+ (semi-)qualitative
+ Digital freezing of samples for future questions

Cons (as of today!)

– Not quantitative (but lots of progress)
– Not easy to implement, no to little standardization, needs lots of experience
– Still no full picture of the PFAS galaxy



Source: www.wikipedia.org

Conclusions

There is no perfect method

- No method covers all necessary aspects
  - Comprehensive but selective
  - Sensitive
  - Quantitative
- There is no perfect “total PFAS” analysis method!

	Target	TOP	dTOP	TF	EOF/AOF (CIC)	EOF/AOF (HR-GFMAS)	SS	NTS	19F-NMR
Quantitative?	very good	very good	very good	very good	very good	very good	very bad	very bad	very good
Sensitivity?	very good	good	fair	fair	fair	fair	good	good	very bad
No. of PFAS covered?	very bad	fair	fair	good	good	good	good	good	very good
Includes all PFAS?	very bad	very bad	very bad	very good	very good	very good	very bad	very bad	very good
Includes non-PFAS?	very good	very good	very good	very bad	very bad	very bad	very good	very good	very good
Includes non-extractables?	very bad	very bad	very good	very good	very bad	very bad	very bad	very bad	very good
Structure information?	very good	fair	fair	very bad	very bad	very bad	good	good	good
Costs?	very good	good	good	fair	fair	good	very bad	very bad	very bad
Degree of implementation?	very good	good	fair	fair	good	bad	bad	bad	very bad
Standardized?	very good	fair	very bad	very bad	fair	very bad	very bad	very bad	very bad
Time per sample?	very good	good	good	good	good	very good	bad	bad	very bad

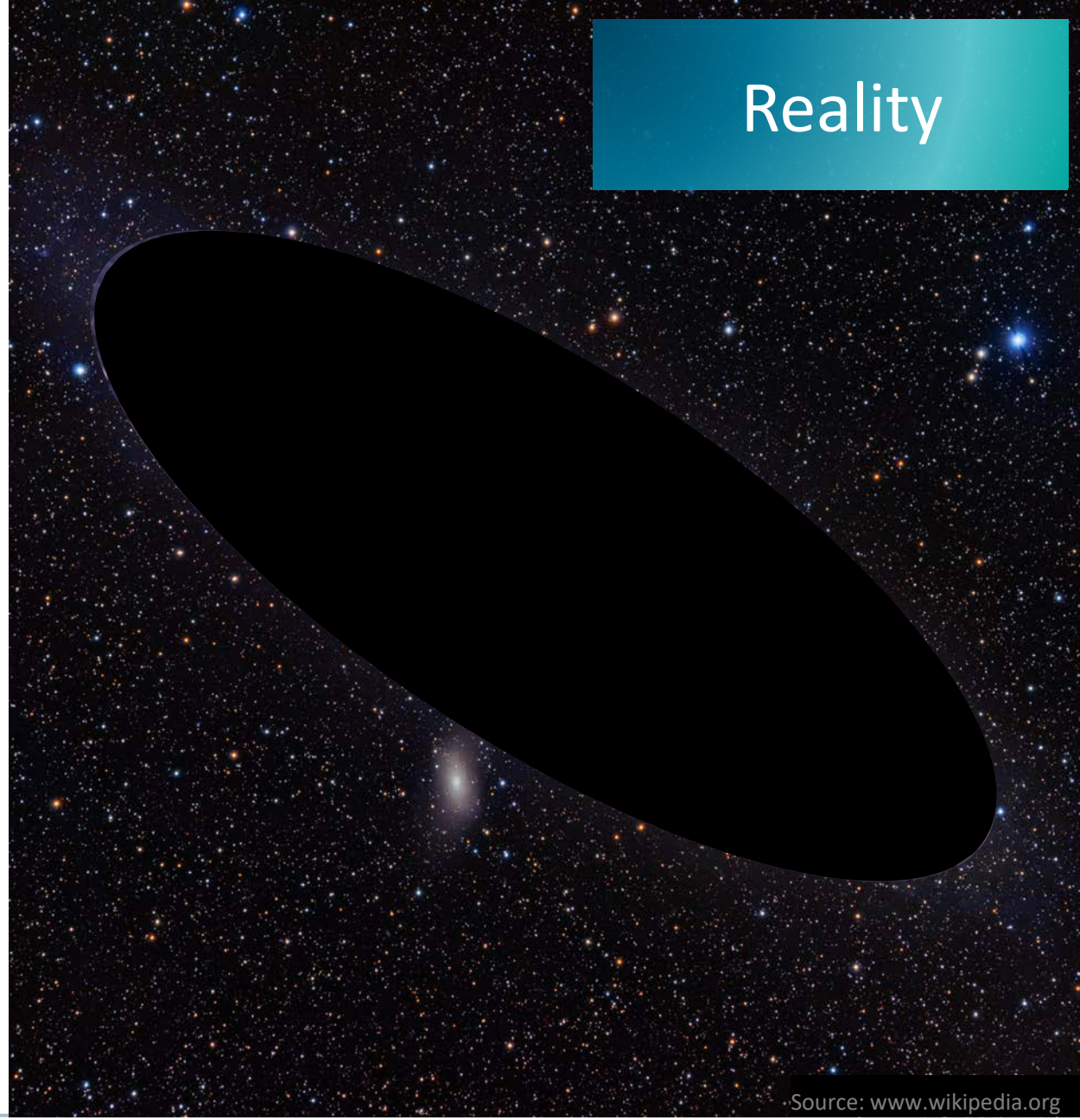
very good
good
fair
bad
very bad

Conclusions

The perfect method?

- No method covers all necessary aspects
  - Comprehensive but selective
  - Sensitive
  - Quantitative

- There is no perfect “total PFAS” analysis method!



Source: www.wikipedia.org

Many thanks for your attention!

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