Study of contamination and toxicity of PFAS in drinking water in the Fos-Berre area and analytical developments for atmospheric biomonitoring using lichens

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Per- and polyfluoroalkylated substances (PFAS)

More than 12 000 molecules

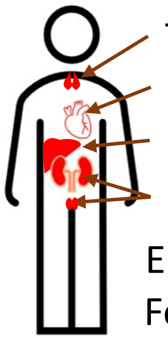
Anthropogenic origin

Synthesized since 1940'

Physico-chemical properties *[1]*

- Hydrophobicity
- Lipophobicity
- Surfactant
- Anti-adhesive
- Heat resistance and stability
- Chemical stability

Toxicity *[2, 3]*



Thyroid
Cholesterol
Liver
Cancers
Endocrine disruptors
Fertility & pregnancy

Many associated pathologies

November 2023 : 2 PFAS classified as **carcinogenic** and **possibly carcinogenic** to human *[4]*

Increasing regulations

STOCKHOLM CONVENTION
REACH COMPLIANCE

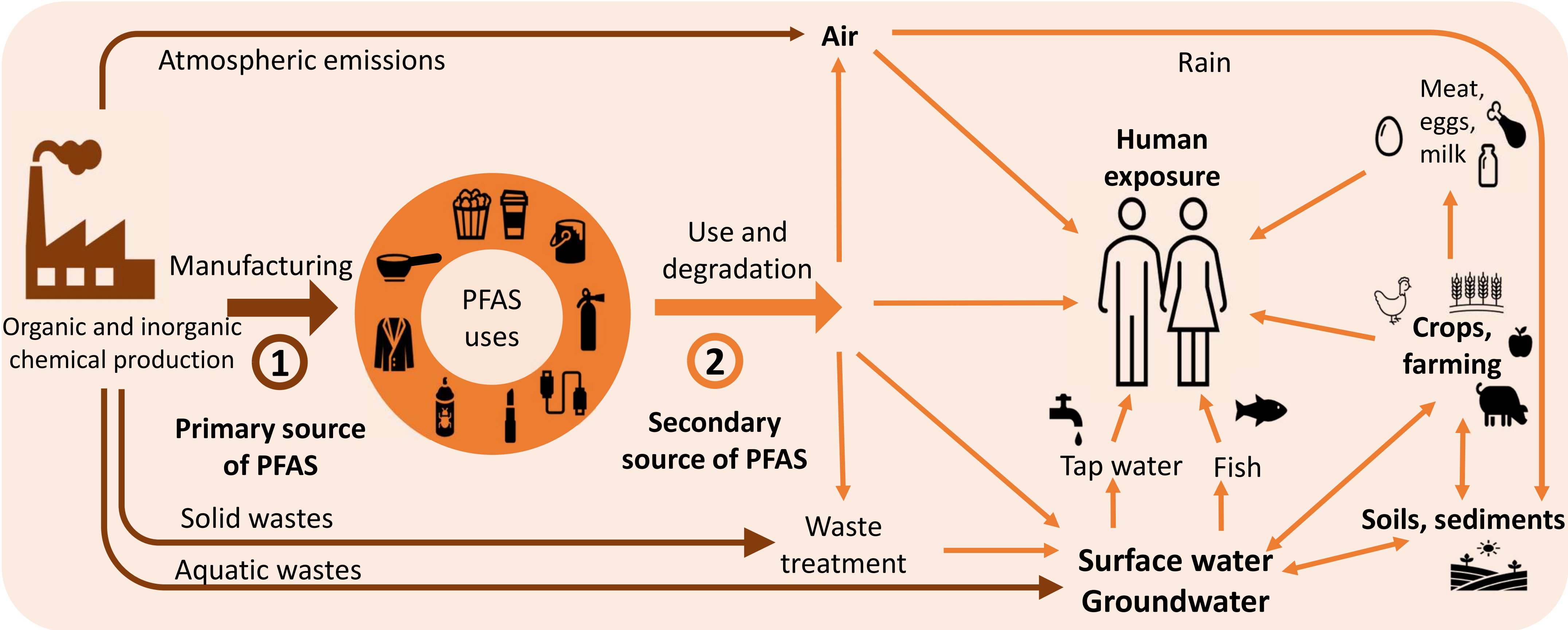
Drinking water (16/12/2020) *[5]*

Effluents (20/06/2023) *[6]*

Atmospheric emissions (31/10/2024) *[7]*

[1] 3M, The Science of Organic fluorochemistry, 1999 ; [2] Fenton et al., 2021 ; [3] EFSA 2020 ; [4] CIRC (12/2023) ; [5] Drinking water directive 2020/2184; [6] Plan d'action ministériel sur les PFAS, [7] Arrêté du 31 octobre 2024

PFAS exposure pathways



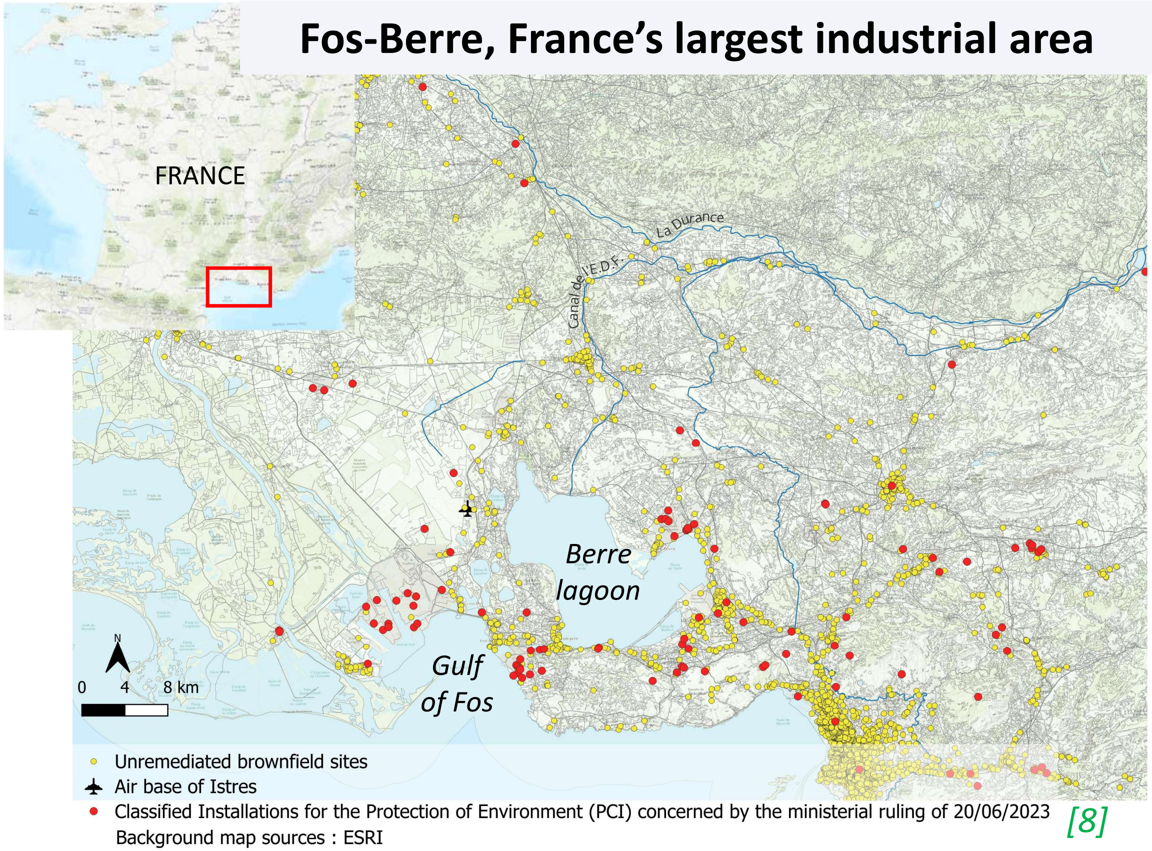
MATISSE Project : Multi-scale PFAS conTamInation Study in induStrial arEas

- 01/2024-12/2026
- Part of the project France 2030
- Financed by Amidex foundation and ERG foundation
- Budget: 1,43 million €

Occurence
- Study of targeted PFAS in drinking water, groundwater and lichens
- Suspected analysis

Multi-matrix transfers
- Soil, air, groundwater
- Comparison of a « manufacturing » site (Lyon) and a « user » site (Fos-Berre-Marseille)

Eco- and human toxicity
- Invertebrate and human cell exposure to mixtures of PFAS



[8] Géorisques

Lichen biomonitoring

Aim Evaluation of the atmospheric exposition (NF X43-904)

Advantages of the lichens

- Lack of root system → Representative of atmospheric contamination → Bioindicator
- Stationary → Spatial resolution
- Integration of the data over several months → Integrative bio-sampler
- Can live in urban, industrial, rural and extreme areas (deserts, Antartica) and on all types of substrate (trees, rocks, buildings) → Ubiquitous organisms
- Already studied for metals, pesticides, PCBs, PAHs, PCDD-F

Lichen biomonitoring

Techniques	PFAS in lichens	PFAS in other plant matrices [11]	Studied parameters
Extraction method	Solid/liquid extraction	Simple agitation, ultra-sonication, QuEChERS, assisted solvent extraction	Orbital agitation Ultra-sonication
Extraction solvent	Methanol	Methanol, acetonitrile, MTBE, ethyl acetate or a mixture of solvents, sometimes with formic acid, ammonium acetate or ammonium hydroxide	Methanol, with and without formic acid or ammonium hydroxide
Purification	Sampli Q carbon [9] Activated carbon [10]	Carbon (solid phase extraction or dispersive (d)SPE) → graphitic carbon (ENVI-Carb™), carbon, C_{18}, Ionic exchange (WAX)	Captiva EMR PFAS Food I (*Agilent*) ENVI-Carb™ (*Supelco®*)

[9] Muller et al., 2011, [10] Alava et al., 2015, [11] Nassazzi et al., 2022

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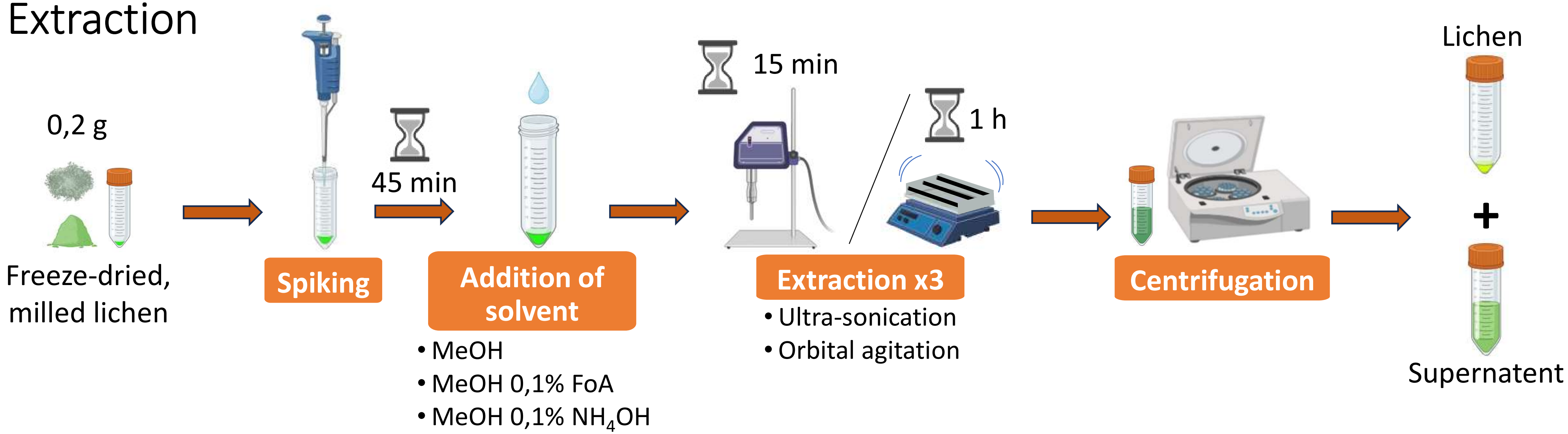
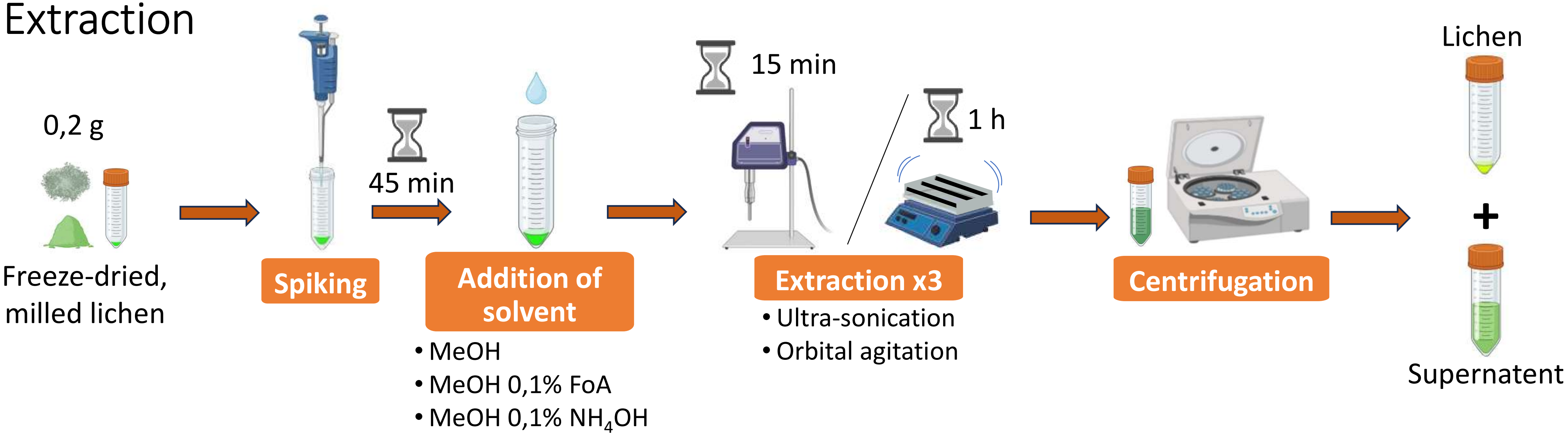
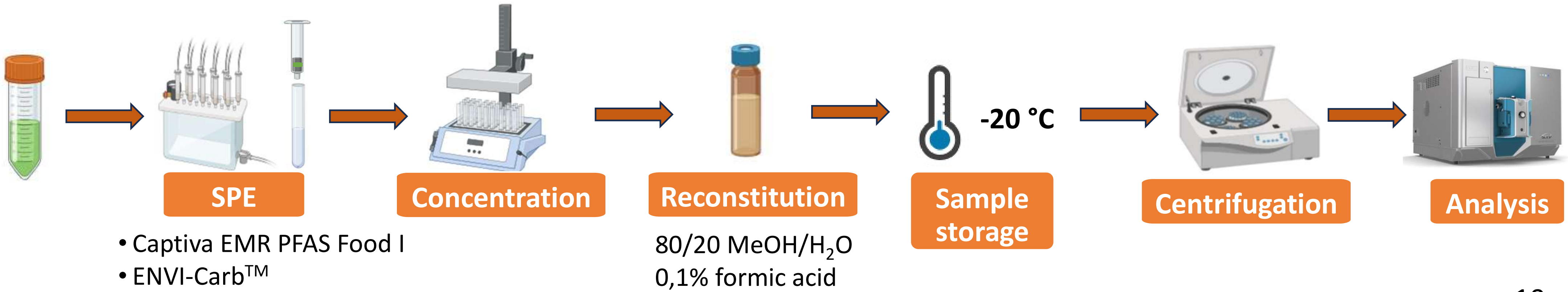
Extraction


0,2 g
Freeze-dried, milled lichen
Spiking
45 min
Addition of solvent
• MeOH
• MeOH 0,1% FoA
• MeOH 0,1% NH_4OH
15 min
1 h
Extraction x3
• Ultra-sonication
• Orbital agitation
Centrifugation
Lichen
+
Supernatent

Extraction



Purification



Analysis

LC-QqTrap 7500 (Sciex)

- Method developed for 49 PFAS (PFCAs, PFSAs, Gen-X, ADONA, FTS, diPAPs, FASAs)
- LC-MS/MS method not sensitive enough for volatile PFAS (FTOHs)

Conclusion ➔ Comparison of extraction recoveries and matrix effects

Purification:

- EMR Captiva Food I → Ionic PFAS retained on the cartridge and could not be eluted
- ENVI-Carb™ → Good purification recoveries (70-120%)
  - Extracts were further diluted to reduce matrix effect

Extraction solvent:

- MeOH 0,1% NH_4OH → Best extraction recovery
- MeOH 0,1% FoA
- MeOH

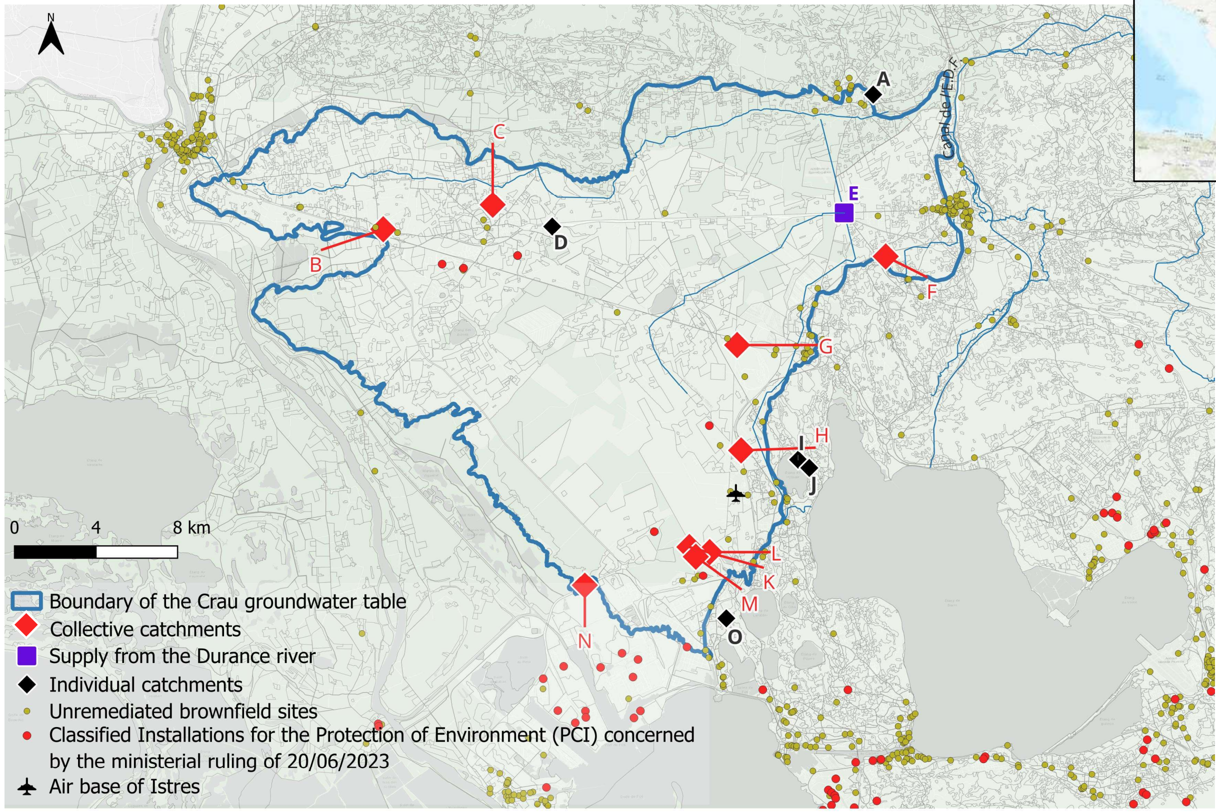
Extraction technique:

- Orbital agitation
- Ultra-sonication → Good extraction recoveries (70-120%), better repeatability and lower matrix effects

Limit of quantification of the method : < 1 $ng.g^{-1}$ (dw) for most PFAS

Method to be validated and applied to a sampling campaign

Drinking water campaign



Background map sources : ESRI

Sampling carried out with the help of the participatory science network of the IECP

- **28 tap waters**

from the Crau groundwater table or the Durance river

- **2 groundwaters**

private wells from the Crau groundwater table

- **1 field blank** <u>per sampling site</u> (MilliQ water)

Sampling and analytical method

Analytical challenges: contamination, loss, degradation *[12]*

➔ **Adaptation of the protocols**

	👍	👎
Sampling	• HDPE bottles	• Water-repellent clothes • Food packagings • Fluoropolymers • PFAS containing chemicals
Conservation	• Addition of a preservative agent • Frozen (-20 °C)	
Preparation	• Dilution in 60% MeOH *[13]*	
LC-MS/MS Analysis	• Within 2 weeks • Adaptation of analytical chain	

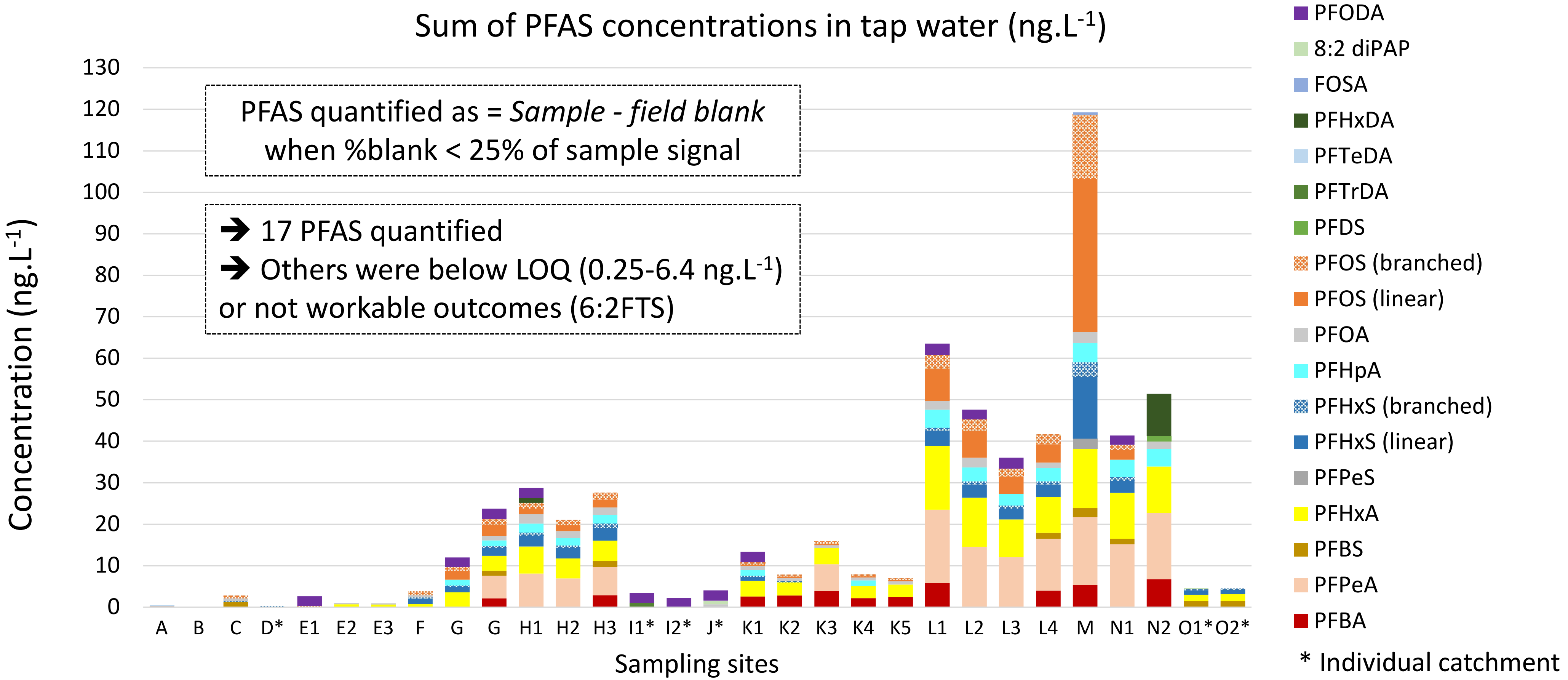
35 PFAS
Sciex Qtrap 7500

Evaluation of the method accuracy:

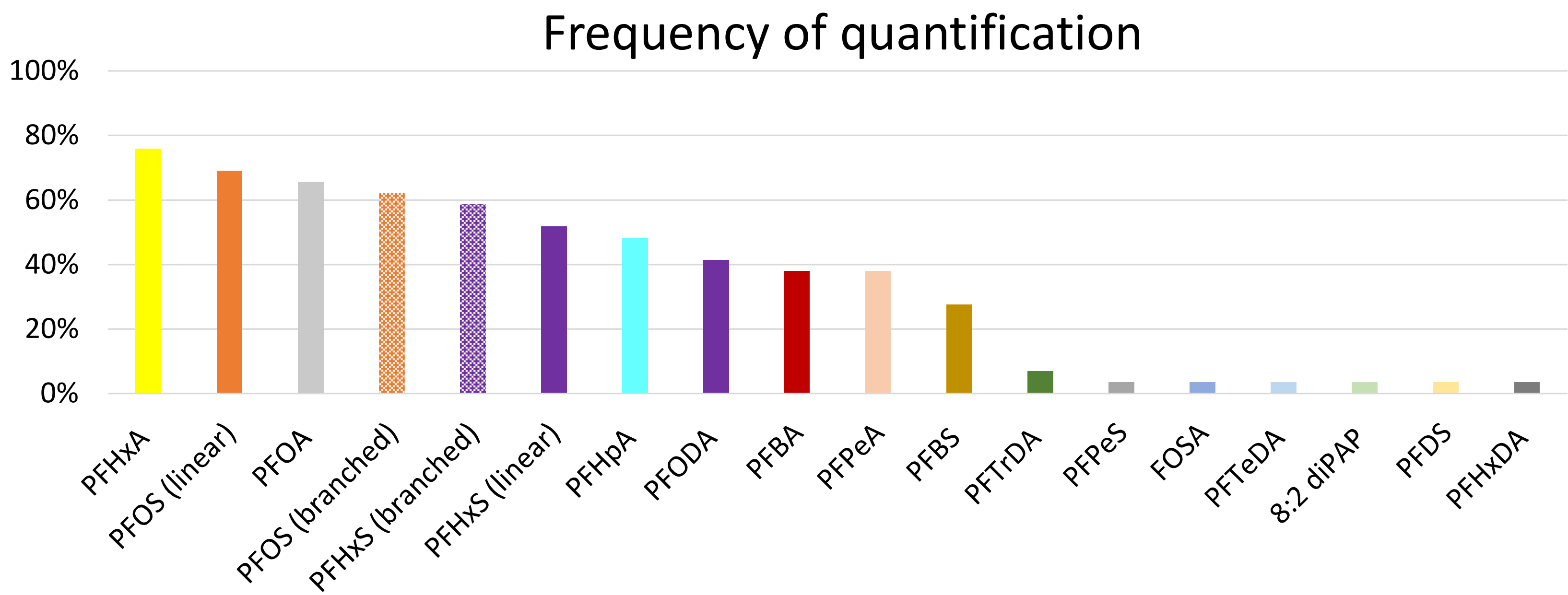
- Water Certified Reference Material (CRM IRMM-428)
  - ➢ All results within 30% of the certified values
- 5 duplicated samples analysed by a certified laboratory (Eurofins)
  - ➢ 90% of the results within 30% of the certified values

[12] Zhang et al., 2022, [13] Mancini et al., 2023

PFAS investigation in drinking water



PFAS investigation in drinking water

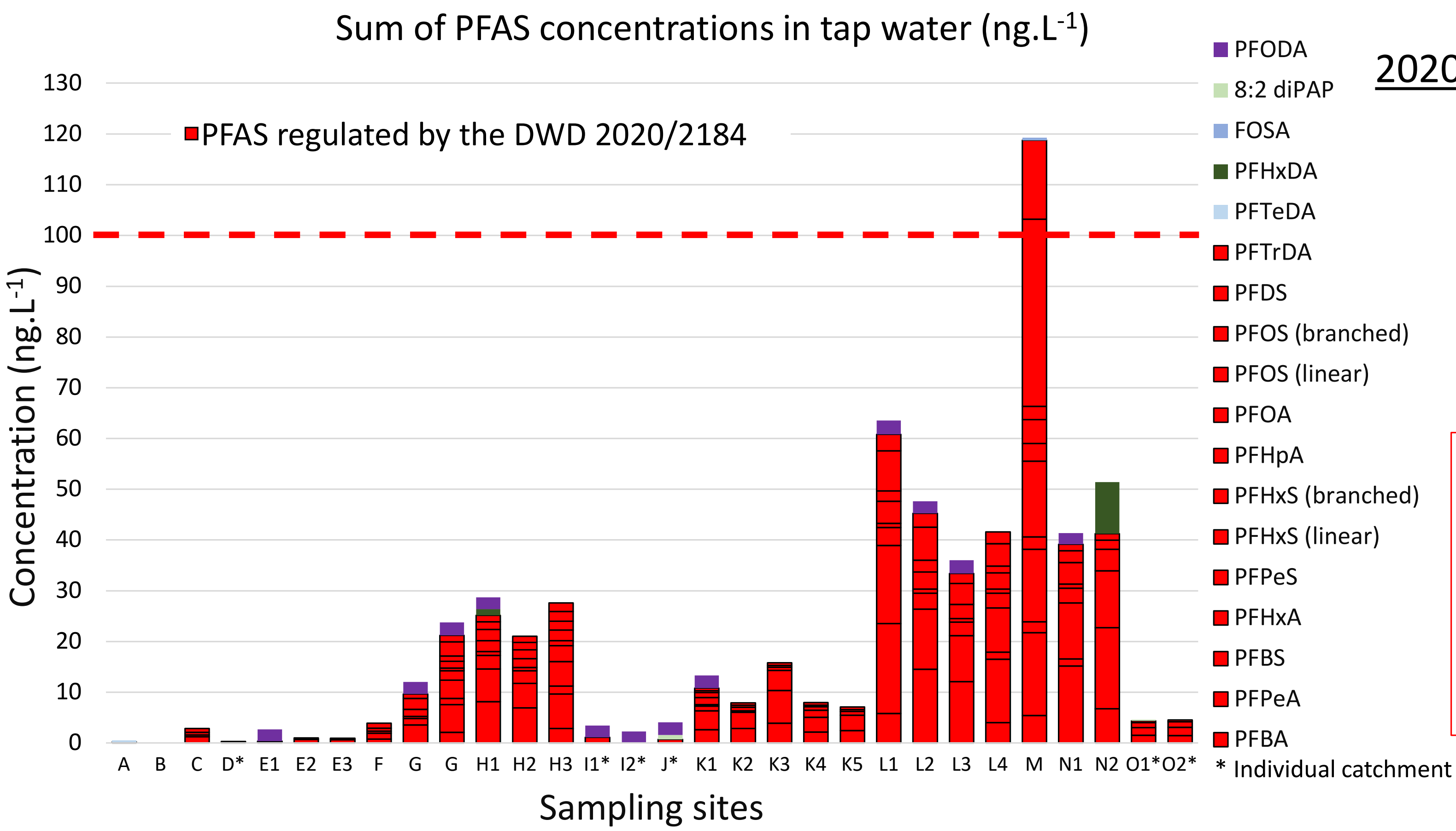


➔ Higher frequency of quantification for short-chain PFAS (3-8 carbons)

- Higher solubility in water
- Used in replacement of long-chain PFAS
- Degradation products of long-chain PFAS precursors

Main uses of the PFAS found in the samples

Comparison to the European regulation



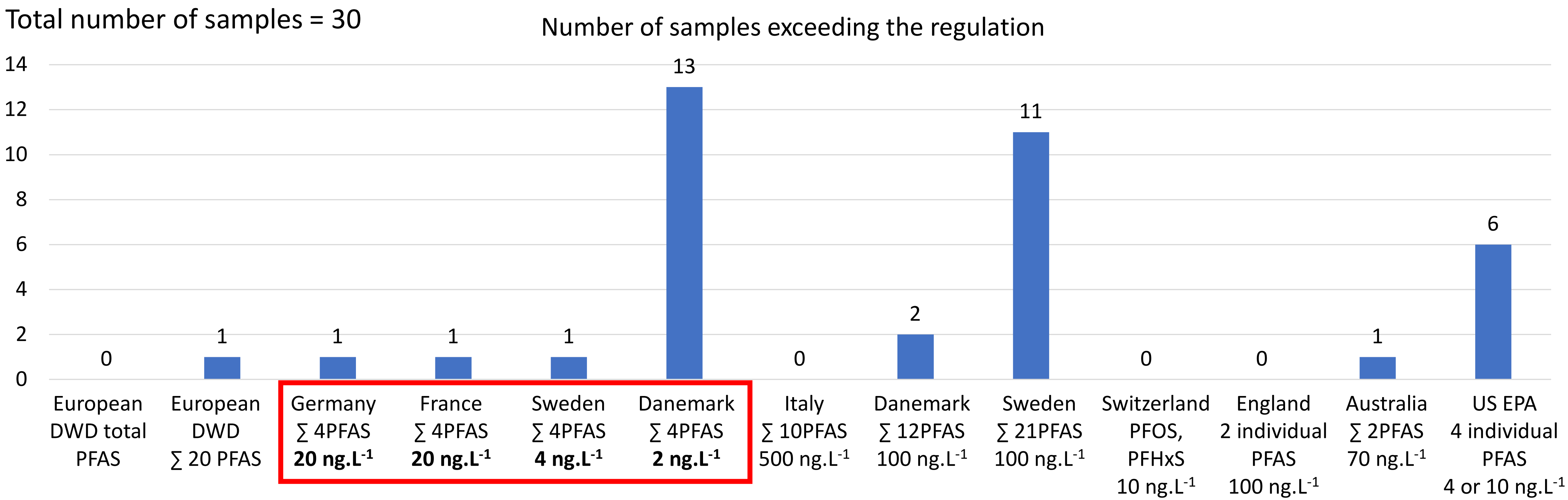
2020/2184 European Drinking Water Directive (16/12/2020)

∑ 20 PFAS: 100 $ng.L^{-1}$

Total PFAS: 500 $ng.L^{-1}$

- ➔ 10 of the quantified PFAS are part of the 20 regulated PFAS in drinking water
- ➔ 1 site above the European regulation

Alarming results ? A regulation-dependant interpretation



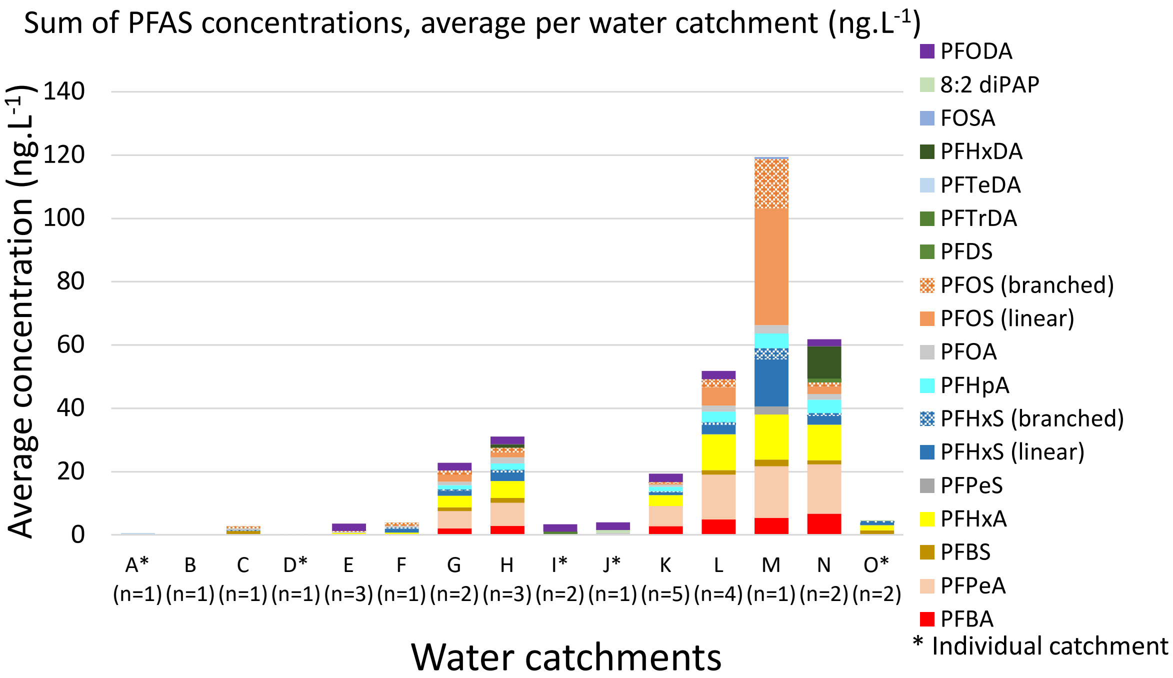
New French recommandation : ∑4 PFAS (PFOA + PFOS + PFNA + PFHxS) < 20 $ng.L^{-1}$ *[14]*

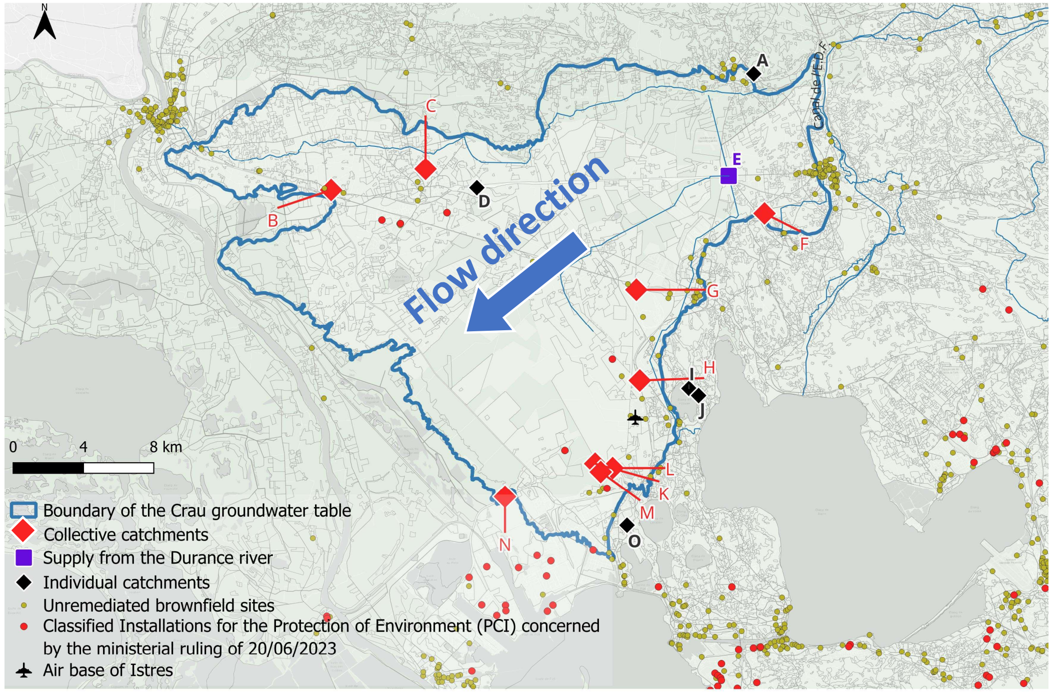
➔ The same sample exceeds the recommandation

[14] HCSP Haut conseil de la santé publique, institution française (19/02/2025)

Conclusions for drinking water analysis

➔ Concentrations impacted by the location of the water catchment, explaining the **variability** in PFAS contamination **within** and **between** towns





Background map sources : ESRI

➔ Higher contamination for water catchments located downstream of the groundwater table, except for individual catchments

➔ Contamination is not higher in individual catchments than in collective catchments (despite additional treatments)

➔ Difference in contamination between neighbouring catchments is not yet explained

Selecting PFAS for the study of toxicological and ecotoxicological properties

⇨ Based on

- **Occurrence**
- **Abundance**
- Presence or absence of **existing literature**
- **Ability to recreate the mixtures**

Final choices

❖ **3 PFAS for toxicological assays** :

PFOS, PFHxA and PFPeA

❖ **9 PFAS for ecotoxicological assays** :

PFODA, PFOS, PFOA, PFHpA, PFHxS, PFHxA, PFBS, PFPeA, PFBA

Toxicological assay

Aim — Evaluate the potential effects of an exposure to PFAS for 24 h via the digestive tract

Model — Caco-2 cell line (enterocyte phenotype)

Technique — RT-qPCR

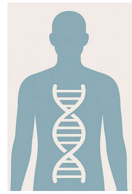
Exposure concentrations

- ✓ PFOS : 7.7 ng/L
- ✓ PFHxA : 0.35 ng/L
- ✓ PFPeA : 90 ng/L
- ✓ **Mix of the 3**

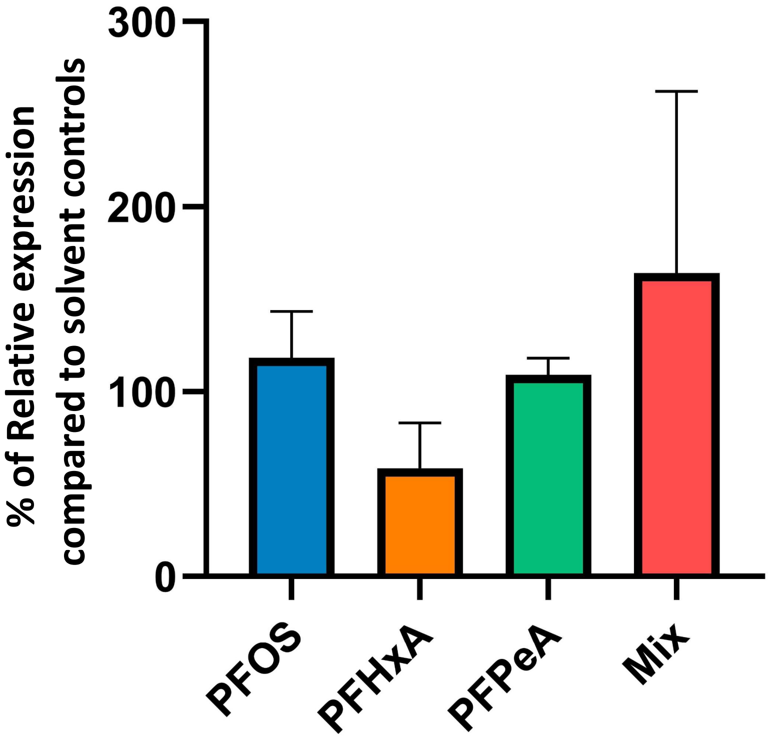
Genes of interest

- **Inflammation** (IL1b, IL6, TNFa)
- **Detoxification** (CYP1A, AhR)
- **Oxidative stress** (CAT, SOD2, GPx)
- **Epithelium integrity** (CLDN1 & CLDN 3)

Toxicological assay : Preliminary results



Relative expression of SOD2 (superoxide dismutase) in Caco2



→ No significant differences compared to solvent controls ($P > 0.05$)

→ Increase the number of replicates to strengthen statistical power

Ecotoxicological assays

Advantages of using *Hydra vulgaris* as a model

- **Invertebrate** (3R → replacing vertebrate models)
- **Diploblastic organism**→ full exposure to the aquatic medium
- **Ability to regenerate** → similar to a hydra embryo, indication of the potential teratogenicity of a substance *[15, 16]*
- **Easy to rear** in a laboratory
- **Sequenced genome**
- **Multi-scale biological responses** : molecular / organism / population

[15] Mathieu-Denoncourt et al., 2015 ; [16] Cera et al., 2020

Ecotoxicological assays

Morphology and teratogenicity studies

Gene expression levels

Metabolomic approach

Next steps in the project

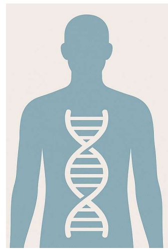
1) Drinking water

- Study of the contamination profiles to identify contamination sources
- Study of PFAS contamination in groundwaters (passive sampling)
- Establishing a local fingerprint of PFAS contamination in waters

2) Lichens

- Validation of the method
- Analysis of samples from a sampling campaign in Fos-Berre and Lyon

3) Toxicity



- Study of the effect of higher concentrations on target genes
- Ecotoxicological assays on aquatic organisms

4) Multi-matrix tranfers

- Study of PFAS contamination in soils, plants and groundwater collected in Lyon
- Implementation of a similar campaign in Fos to compare production and usage sites

Thank you for listening

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- toxicology : melissa.denton@imbe.fr

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