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Executive Summary

This deliverable presents the outcomes of the PROMISCES task dedicated to the implementation of advanced monitoring strategies for PFAS and iPM(T) compounds. Despite significant challenges inherent in detecting and quantifying these substances in complex matrices such as sludge, wastewater, and environmental waters, notable progress was achieved. Sensitive and reliable analytical methods were developed, including high-precision quantitative analyses, non-target screening approaches to uncover unknown contaminants, and passive sampling techniques tailored for various environmental contexts such as industrial effluents, surface waters, and groundwater systems.

In parallel, a comprehensive battery of *in vitro* bioassays was established to assess key toxicological endpoints, covering cytotoxicity, genotoxicity, endocrine disruption (e.g., thyroid hormone disruption), oxidative stress, and metabolic disorders. These methods allowed a broader and more integrated evaluation of chemical hazards. Furthermore, advanced *in silico* models were applied to prioritize compounds based on their predicted binding potential to nuclear hormone receptors (PPARs, TRs), offering a valuable tool to complement empirical screening and guide monitoring efforts towards the most problematic substances.

The tools developed within PROMISCES were successfully deployed across a wide range of complex case studies, addressing major environmental challenges such as wastewater treatment efficiency and water reuse potential, identification and characterization of PFAS and iPM(T) sources in the urban water cycle, contaminant accumulation in sludge, fate and transport modelling in soil-sediment-water systems, and the evaluation of treatment technologies for water and soil remediation. Their application in diverse real-world contexts not only confirmed the scientific robustness and relevance of the approaches but also revealed important operational insights regarding sampling design, method selection, data interpretation, and the scalability of analytical techniques.

The experience gathered through these deployments allowed the identification of several key recommendations to further enhance future monitoring strategies. These include: implementing a tiered monitoring approach, beginning with broad non-targeted screening to establish an initial comprehensive overview, followed by focused targeted analyses at key locations; continuously improving analytical methods to achieve lower detection limits, greater selectivity, and higher robustness to meet evolving regulatory needs; systematically involving analytical and toxicological experts from the early stages of monitoring strategy design to ensure the selection of appropriate tools and accurate interpretation of complex results; developing and adopting harmonized methods and certified reference materials to enable consistent and comparable data generation across laboratories and studies; integrating predictive toxicology and *in silico* modelling to better prioritize substances of concern in large chemical datasets; and applying rigorous quality assurance and quality control (QA/QC) protocols at every stage of the monitoring process to safeguard data reliability and reproducibility.

Overall, the lessons learned from the field experience within PROMISCES highlight the urgent need for adaptable, scientifically robust, and resource-efficient monitoring frameworks to address the complex challenges posed by PFAS and iPM(T) contamination. They also emphasize the importance of bridging advanced scientific developments with practical operational deployment to support effective environmental risk management and regulatory decision-making.

List of abbreviations

AFFF : Aqueous film-forming foams
AOF : Adsorbable Organic Fluorine
AP: Ammonium phosphate
CALUX : Chemically Activated LUciferase gene eXpression
CIC : Combustion Ion Chromatography
DI Direct Injection
DW: drinking waters
EFF: WWTP effluent
EOF: Extractable Organic Fluorine
EQS : environmental quality standards
FT : fluoro-telomerization
GW: Groundwaters
INF: WWTP influent
iPM(T) : industrial Persistent, Mobile (and Toxic) compounds
NAM : Non Animal Method
POCIS : polar organic chemical integrative samplers
PR : Phosphorus recovery
QA/QC : Quality control/ Quality assurance
RC : Collection Ratio
RS : Sampling Rate
RPF : Relative Potency Factor
RSD : relative standard deviation
SD: Speedisk
SLE: Solid Liquid Extraction
SPE: Solid Phase Extraction
SS: Sewage sludge
SW : surface waters
TOF : Total Organic Fluorine
TPS: Tube Passive Sampler
TTR : Transthyretin Thyroid Receptor
USC-PFAS : Ultra-short chain PFAS
WWTP : Waste Water Treatment Plant

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1 Introduction

This deliverable reports on the activities conducted in Task 1.4, focusing on the implementation of advanced monitoring strategies, with innovative methodologies and practical feedback.

Recognizing the critical need to assess the accumulation of chemicals and the associated risks within the framework of the Circular Economy, the Horizon 2020 project PROMISCES specifically addresses iPM(T)S and the so-called "forever chemicals", such as per- and polyfluoroalkyl substances (PFAS).

The project investigates five key Circular Economy routes: (i) semi-closed water cycles for drinking water (DW), (ii) wastewater reuse in agriculture, (iii) nutrient recovery from sewage sludge, (iv) material recovery from dredged sediments, and (v) groundwater and land remediation for safe reuse. These routes highlight the complexity of monitoring contaminants in various environmental matrices and underscore the need for robust analytical approaches.

Despite the inherent challenges of detecting and quantifying PFAS and certain other iPM(T)s in complex matrices such as sludge and wastewater, significant progress has been made in developing sensitive and reliable analytical methods. These include quantitative analysis of PFAS and iPM(T) compounds, non-target screening techniques, and comprehensive assessments of organic fluorinated compounds. In addition, passive samplers have been designed and deployed across diverse environmental contexts to detect PFAS in industrial effluents, characterize contamination in lakes, and support large-scale monitoring experiments.

A comparative summary of all methods developed is shown in [Table 1](#). Details are presented in deliverable D1.1. In summary the methods cover 62 different PFAS and 12 different types of matrices. The method ACEA_1 has been extended to biochar and solid sorbents following additional assays and specific developments related to stack emissions (biooil, syngas) have been achieved, as described in deliverable D4.6.

Table 1: Comparative summary of all PFAS methods; (*matrices or methods added in a second time)

	ACEA 1	ACEA 2	BRGM 1	BRGM 2	BWB 1	BWB 2	CSIC 1	CSIC 2	TU Wien 1	TU Wien 2
Matrix	Sediment, sludge, biochar*, biosolids*	Leachate, concentrate, liquid waste	Drinking water, Ground water, WWTP effluent, process water	Sediment, soil, sludge	Drinking water, ground water	Surface water, wastewater	Drinking water, ground water, WWTP in- & effluent, process water	Sediment, soil, sludge, lettuce	Aqueous matrices	Sludge, sediment
Separation	UPLC	UPLC	UPLC	UPLC	UPLC	UPLC	UPLC	UPLC	HPLC	HPLC
Analyser	Triple quadrupole	Triple quadrupole	Triple quadrupole	Triple quadrupole	Triple quadrupole	Orbitrap (HRMS)	Orbitrap (HRMS)	Orbitrap (HRMS)	Triple quadrupole	Triple quadrupole
Sample preparation	Ultrasonic SLE	Dilution or DI	DI	DI	dilution or SPE	Online-SPE	SLE	SPE	Online-SPE or DI)	Ultrasonic SLE
# PFAS analytes	30	30	56	56	30	27	29	29	34	34
# extracted internal standards	1					19	20	20	24	24
# non-extracted internal calibration standards	19	19	22	22	19		2	2	7	7
LoQ in ng/L		15 - 75 (washing water, leachate) 1000 - 10000 (liquid waste)	15 - 100 (process water, WWTP effluent) 2 - 15 (groundwater, surface water)		1 - 5 (0.01 - 0.05 w/ automated SPE)	25 - 100	0.13 - 5.44 (surface water) 0.15 - 12.40 (effluent water) 0.69 - 49.60 (influent water)		1 - 10	
LoQ in µg/kg	10 - 50 (sludge) 0.050 - 0.250 (sediment)			0.040 - 0.300 (soil) 160 - 1200 (sludge)			0.13 - 4.96 (sediment) 0.04 - 9.92 (lettuce)			0.1 - 0.5 (soil) 1 - 20 (sludge)

SLE: Solid Liquid Extraction; DI Direct Injection; SPE: Solid Phase Extraction; detail in Deliverable [D1.1](#)

To better consider the PFAS group, global methods have been developed (as described in deliverable [D1.3](#)) and implemented for the PROMISCES project:

- TOP assay on groundwaters, surface waters, wastewaters, landfill leachates and sludge;
- Adsorbable organic fluorine (AOF) on surface waters, wastewaters;
- Total Organic Fluorine content (TOF).

To gain a more comprehensive understanding of the broad universe of iPM(T), suspect screening and database-assisted analysis workflows have been developed and are described in deliverable [D1.4](#). Five workflows have been designed, utilizing different analytical setups to analyse various environmental and biological matrices, including groundwater, surface water, wastewater, landfill leachates, lettuce, and sediment. Two workflows rely on liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) and are specifically tailored for the screening of PFAS compounds in landfill leachates, as well as their transformation products in groundwater, surface

water, wastewater treatment plant effluents, lettuce, and sediment. Two additional LC-HRMS workflows focus on the identification of iPM(T)s in surface water and wastewater. In complement, a gas chromatography-mass spectrometry (GC-MS)-based method, has enabled the screening of more non-polar compounds that are not amenable to most LC-MS methods.

Based on the deployment of these methods, main compounds of interest were selected, and targeted analytical methods (Table 2) were then developed, for a total of 84 compounds, covering industrial chemicals, but also pharmaceuticals and pesticides of concern.

Table 2: Comparative summary of the four methods developed for iPM(T) in waters

	BAFG	BWB	CSIC 1	CSIC 2
Matrix	Surface water	Drinking, ground, surface water, wastewater	WWTP effluent	Groundwater
Separation	UPLC	UPLC	UPLC	GC
Analyser	Triple quadrupole	Orbitrap (HRMS)	Q-TOF (HRMS)	Single quadrupole MS
Sample preparation	Direct injection	Online-Solid phase extraction	Online-Solid phase extraction	Purge and Trap
# PM(T)s analytes	26	21	42	59
# Internal standards	16	8	22	3
LOQ (ng/L)	1-200	12 – 369	0.02 - 321	8.4-6400

In order to close the PFAS assessment gap, in vitro and in silico methodologies for toxicological assessment of environmental media/drinking water were improved ([Deliverable D1.5](#)), parallel to the development of chemical analysis methods.

Regarding the parallel in vitro bioassay toxicity analysis, a standardized in vitro test battery—including assessments for cytotoxicity, genotoxicity, neurotoxicity, oxidative stress, obesity, thyroid hormone interfering activities (inhibition and transport competition) and early warning indicators—was applied to meet specific PFAS/iPM(T) criteria, such as endpoint relevance, sensitivity, and specificity.

Building upon existing and improved CALUX bioassays, various toxicological endpoints were assessed to characterize the PM(T) properties of chemicals. These included metabolic syndrome-related pathways (e.g., obesity-related PPAR activation), endocrine disruption (EAT testing), genotoxicity (p53-related response), and general toxicity pathways (e.g., early warning PXR activation and oxidative stress Nrf2).

The in vitro toxicity profiling followed a stepwise approach, by first testing the potential reference compounds PFOA and PFOS as well as industrial mixtures (e.g., GenX and ADONA) with a wide range of in vitro methods. Based on these results, the most suitable in vitro toxicity endpoints have been chosen for the rest of the selected PFAS compounds as well as for selected complex mixture testing (e.g., water, soil).

The same approach has been applied for iPM(T)s, firstly on several key PM(T)s (e.g., bisphenol A, triclosan, methyl-paraben, 5-chlorobenzotriazole, benzothiazole) then on a larger set of the PM(T) compounds as well as for testing complex mixtures (e.g., water, soil).

Potency factors were also determined for 40 PFAS tested in the in vitro human cell-based bioassays. In addition, up to 22 PM(T) chemicals were tested using a combination of bioassays (six endpoints) and general toxicity in vitro bioassays (four endpoints).

On the other hand, faced with the broad universe of compounds, in-silico modelling was conducted on several thousand chemicals to find criteria of prioritization or identification of new PFAS of concern. The in-silico study specifically assessed the binding potential of PFAS to nuclear hormone receptors (NHRs), including peroxisome proliferator-activated receptors (PPARs) α , β , and γ , as well as thyroid hormone receptors (TRs) α and β . In the first step, the developed QSAR models were applied to screen a large dataset of 4,464 compounds from the NORMAN Database for interactions with these five NHRs. Next a comparison of in vitro and 3 in silico models was performed on 45 PFAS.

All of these developments have been implemented across various case studies (i.e. CS# 1 to CS#7) to address specific challenges such as:

- **Wastewater treatment efficiency and water reuse:** The removal efficiency of PFAS in wastewater treatment plants (WWTPs) remains a concern, particularly when these facilities receive substantial contributions from industrial wastewater streams. As a result, until new treatment solutions are deployed, PFAS hotspots may not be able to implement wastewater reuse (e.g., for irrigation).
- **Better understanding of urban water cycle** by investigating PFAS and iPM(T) sources for groundwater and surface water (industrial wastewater, urban runoff and stormwater) , in order to improve the management of the urban water cycle.
- **PFAS accumulation in sludge:** During wastewater and landfill leachate treatment, long-chain PFAS may accumulate in sludge. While only low levels of targeted PFAS compounds have been quantified, the presence of precursors in sludge is suspected and could hinder its agronomic valorization.
- **Modelling PFAS fluxes, fate and transfer** in the Soil-Sediment-Water system, to provide more accurate estimate of the expected concentrations in soil and groundwaters and the human exposure
- **Identifying sources and emission pathways of PFAS and iPM(T) compounds** within a large water catchment area, with the objective of developing a model train for catchment-scale emission modelling to support environmental risk assessment and the evaluation of control and mitigation measures.
- **Water and soil treatments:** Given the ubiquity of PFAS and the complexity of their treatment, with processes covering environmental waters, drinking water, soils, etc., it is necessary to develop innovative treatment technologies and to verify their efficiency.

The overview of the case studies related with their main objectives is illustrated in the Figure 1.



Figure 1 : Schematic description of the PROMISCES case studies.

To effectively address these challenges, it is critical to implement robust and reliable analytical methods, alongside clearly defined monitoring strategies. These strategies should encompass the design of appropriate sampling plans and the selection of the most suitable and validated analytical techniques. As part of the PROMISCES project, a range of analytical and monitoring tools have been developed and subsequently applied across multiple case studies to address various scientific and regulatory questions. This deliverable presents selected examples, illustrating how these tools have, in certain instances, improved the quality, precision, and interpretability of the information generated. Moreover, the feedback gathered through these applications highlights several critical considerations for future research and operational deployments. These include lessons learned from the dissemination of results and challenges encountered during the experimental implementation, particularly when working with highly complex substances such as PFAS. The insights derived from this experience are intended to inform the refinement of future methodologies and to contribute to the advancement of best practices in environmental monitoring and assessment.

2 What is the water quality in the investigated area and what are the sources of PM(T)s and PFAS?

2.1 Application of a Semi-Passive Sampler for Monitoring Fluctuating Trace Compounds in Wastewater Effluent, urban run-off and stormwater

Sampling of industrial contaminants such as (i)PM(T)s or PFAS requires long-term and integrative monitoring approaches that capture temporal fluctuations and episodic discharges that may be missed by traditional grab sampling. The latter only provides snapshot knowledge but no information on concentration variations prior or after the specific grab sample time. Passive samplers, such as polar organic chemical integrative samplers (POCIS), commercially available SPE-devices like

Speedisks, and a newly developed semi-passive integrative tube passive sampler (TPS, Hensel et al. 2024) offer promising capabilities for continuous monitoring over extended periods. These tools enable the detection of low-concentration contaminants via enrichment and can provide time-weighted average concentrations, improving the reliability and interpretability of monitoring data. In comparison to continuous composite sampling, they offer much simpler handling, reduce sample numbers dramatically, and therefore are substantially less cumbersome.

In the first testing study, a novel tube passive sampler (TPS) approach was evaluated for monitoring organic micropollutants (OMPs), i.e. pharmaceuticals and industrial chemicals in the effluent of a Berlin wastewater treatment plant (WWTP) (Hensel et al. 2024). The system features a miniaturized flow-through design, housing standard 47 mm SPE discs and enables precise volumetric flow control (Figure 2). This configuration allows for both accurate calibration and continuous sampling in pressurized pipe systems—an important advancement compared to classical passive samplers typically deployed in open water bodies with uncontrolled flow conditions.

Following an introductory load-dependent calibration using consecutive and partially time-varied exposure experiments, the TPS was able to quantitatively determine average effluent concentrations for a broad range of OMPs. These results closely matched data obtained from conventional composite sampling, demonstrating the method’s potential for representative and reliable monitoring under real-world conditions. In Figure 2, two calibration strategies—time-dependent and load-dependent—were evaluated.

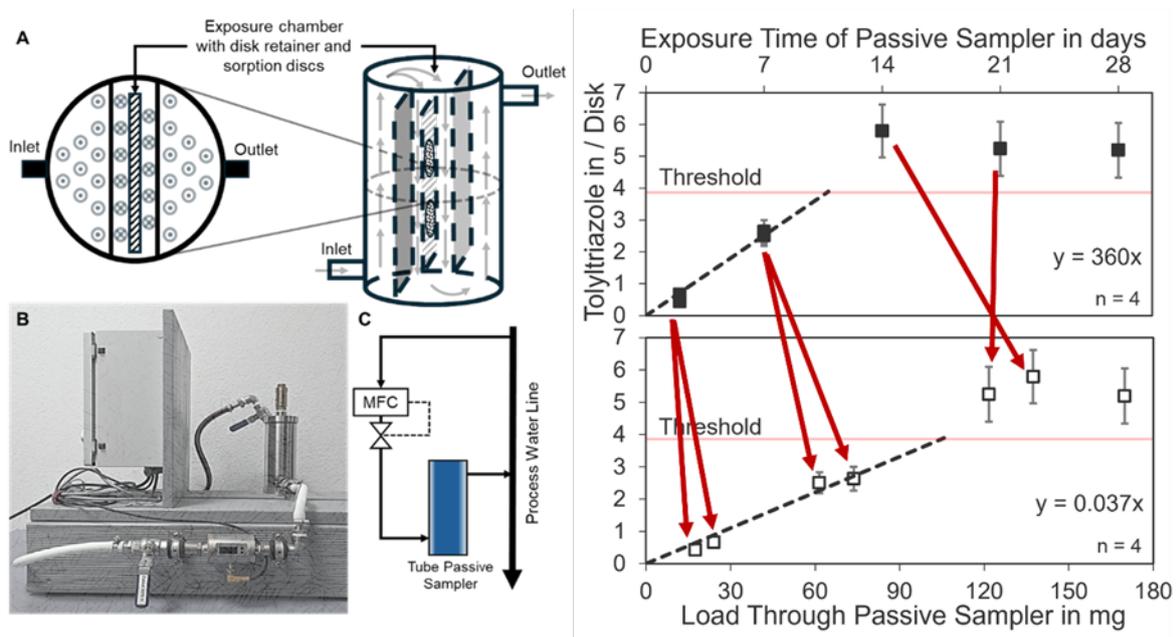


Figure 2: Left: TPS design; Right: Time- vs. load-dependent calibration curves for selected OMPs (e.g., metoprolol, tolyltriazole). From Hensel et al. 2024, ES&T Water

While both correlated well with constant contaminant concentrations, the load-dependent calibration showed particular strength in handling OMPs with strongly fluctuating concentrations like industrial chemical tolyltriazole, by directly linking passive sampler uptake to the actual chemical load in the feedwater, as determined through the available data from consecutive composite sampling. A

comparison of the load-dependent and time-dependent calibration approaches and the corresponding data from composite samples is provided in Table 3.

The ability to decouple exposure duration from concentration dynamics gives the TPS a distinct advantage, especially in environments with temporal variability in contaminant presence. This includes diurnal, weekly, or event-based fluctuations in WWTP effluents. Furthermore, the consistent flow conditions within the TPS allowed for reproducible experiments and accurate regression-based quantification, using both the newly introduced collection ratio (RC) and classical sampling rate (RS) metrics.

Table 3: Overview of TPS units installed at the Berlin WWTP and monitored OMPs from Hensel et al. 2024.

Compound	C_w in $\mu\text{g/L}$								
	time-dependent ^a			load-dependent ^a			auto-sampler ^b		
Phenazone	0.23	±	0.01	0.21	±	0.01	0.33	±	0.01
AAA	2.8	±	0.2	2.8	±	0.2	3.0	±	0.2
AMPH	0.19	±	0.01	0.19	±	0.01	0.35	±	0.01
FAA	9.5	±	0.7	9.4	±	0.7	11.6	±	0.9
Diclofenac	3.8	±	0.3	3.8	±	0.3	4.0	±	0.3
Metoprolol	2.0	±	0.1	1.9	±	0.1	1.7	±	0.1
DEET	0.12	±	0.01	0.11	±	0.01	0.13	±	0.01
Benzotriazole	42.8	±	1	35.5	±	0.9	16.6	±	0.4
Tolytriazole	11.5	±	0.1	10.1	±	0.1	7.9	±	0.1

^a Calculated value ± absolute error, based on the individual error,

^b Mean value ± absolute error, based on the individual error.

Due to its straightforward installation, good quantitative performance, and independence from external autosamplers, the TPS presents a valuable tool for long-term, integrative monitoring of wastewater discharges. Its versatility suggests further applications in regulatory monitoring and process control, especially where continuous quantification is required or where grab samples and/or composite samples are too laborious and/or costly.

Complementary Monitoring of PFAS in Industrial Wastewater Using Composite and Passive Sampling

While the semi-passive TPS provided quantitative results for WWTP effluent matrices, WWTPs taken as sampling locations obviously can only yield data over all wastewater influents to a given WWTP, that are mixed and without actual source allocation. Therefore, additional experiments further covered raw industrial wastewater dischargers as sampling locations to gain insight into the potential point sources of PFAS to wastewater. Three passive sampling techniques were tested and complemented alongside automated composite aqueous sampling. Sampling was conducted at the discharge points of three indirect dischargers from distinct industrial sectors—each contributing over 10,000 m³ annually—within an urban sewer network. To our knowledge, passive sampling for PFAS in industrial process water from indirect dischargers has not yet been examined in the scientific literature. The tested devices included a polar organic chemical integrative sampler (POCIS), Speedisk (SD), and the semi-passive tube passive sampler (TPS), each installed at the industrial outlets. Concomitantly, 8-hour flow-proportional composite samples were collected over two workweeks using automated samplers.

The aqueous composite samples provided quantitative data for 3 to 18 individual PFAS (depending on the examined industry), revealing substantial variability in concentrations and daily loads—from 0.12 mg/day to 8.8 mg/day—across different facilities, including a semiconductor manufacturer and an industrial laundry. Noteworthy, passive samplers detected up to 12 additional PFAS not identified in the aqueous samples, underscoring their utility for qualitative screening and source identification in complex industrial matrices (Figure 3). This discrepancy highlights the PS’s ability for in-situ enrichment, offering sensitive detection across variable timeframes.

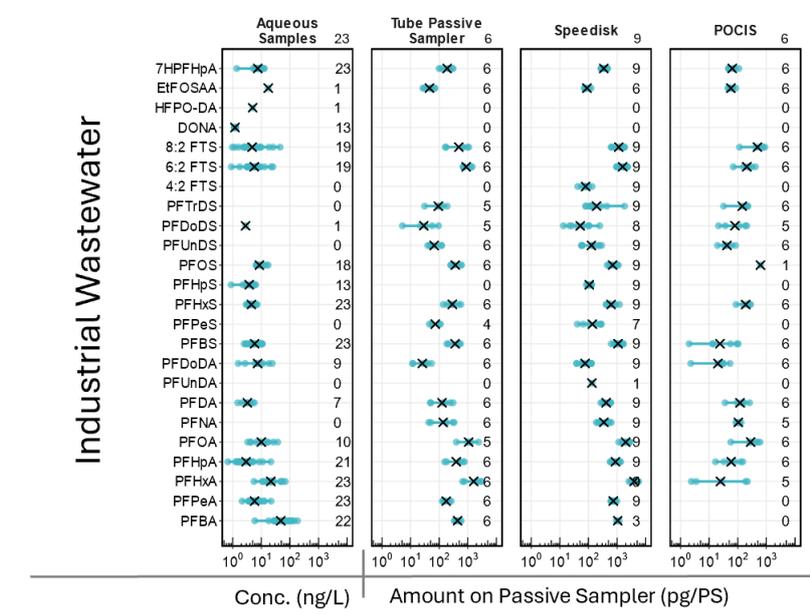


Figure 3: Comparison of PFAS concentrations in aqueous composite samples vs. amount of PFAS on passive samplers (POCIS, SD, TPS). From Hensel et al., submitted).

The findings underscore the complementary strengths of both approaches: while composite sampling enables robust quantitative analysis and time-integrative concentration data, passive samplers serve as effective tools for expanding the detectable PFAS spectrum and identifying

potential point sources. However, the complex nature of industrial wastewater—with its varying flow, pH, and matrix effects—currently limits the use of passive samplers for precise quantification. Thus, while PS provide valuable qualitative insight and screening potentials, composite aqueous sampling remains essential for regulatory compliance and quantitative assessments. However, it must be noted that most current industrial discharge surveillance bases on (qualified) grab sampling (e.g. according to the German Wastewater Ordinance). Thus, the currently applied sampling approach also does not provide quantitative data, except for a single moment in time. In this respect, PS would logically outperform grab samples in case of fluctuating (i)PM(T)s despite their current imperfections concerning quantification.

Given the complexity and diversity of trace contaminant sources and behaviors in the aquatic environment, a multi-faceted monitoring framework—combining advanced analytical techniques with passive and direct sampling strategies—currently appears the most feasible, especially for extremely challenging matrices like raw industrial wastewaters. In the context of indirect discharge monitoring, we currently propose a combined sampling strategy. Together, passive and automated composite samplers can deliver a more comprehensive picture of PFAS emissions, inform source tracking, and support future regulatory developments. Future studies should further explore the potentials of quantitative passive sampling – which proved successful in WWTP effluents but still suffers from the conditions in more harsh matrices. Nonetheless, the findings demonstrate that industrial dischargers are particularly important sources of (i)PM(T)s and are, nowadays, still mostly overlooked and should obtain much more attention. With current legislation on industrial wastewater surveillance only covering a handful of general parameters (COD, nitrogen, pH, etc.), no clear picture as to the actual point sources of (i)PM(T)s can be drawn. The sampling/monitoring results within CS#1 clearly highlight important research gaps that need to be thoroughly addressed.

2.2 Monitoring strategies on an identified hot-spot

In the Berlin semi-closed water cycle case study (CS#1), additional methods were also used to acquire more information about a contaminated hotspot. Knowing that the lake Flughafensee was the receiving body for urban runoff, and that it is located close to a PFAS hotspot from the former Tegel Airport, it was selected for further source tracking investigations to characterize and differentiate different sources contributing to PFAS contamination in the environment to provide a PFAS fingerprint.

To examine its role as a potential source of PFAS into the adjacent aquifer and drinking water source catchment, input and output pathways of the lake (i.e. urban stormwater runoff and groundwater, as well as lake sediments) were investigated to identify sources based on their PFAS patterns.

Stormwater runoff from two industrial catchments with potential PFAS usages (e.g. pharmaceutical industry, plastic production, metal industry, dry cleaning) discharging into the lake were analyzed. 24 storm events were sampled from separate storm sewers using automated samplers connected to flow measurement devices. For each storm event, a flow proportional sample was analysed, and these were tested for presence of 26 PFAS. Results showed concentrations above LOQ for at least one PFAS compound (10 detected in total) in 36 of 47 samples: PFOA (33/47), PFHxA (30/45) and PFHpA (26/47) and PFOS (17/47) were most often detected. Median PFAS-20 concentrations for all samples from both sites were 7 ng/L (max 35 ng/L).

The lake water was sampled three times at four locations and two depths (1 m and 6-10m). Samples were analysed for 26 PFAS. Nine PFAS compounds were found in concentrations above LOQ in all

samples, and mean PFAS-20 concentrations for all samples were 72 ng/L, i.e., one order of magnitude higher compared to stormwater runoff inputs.

Sediment cores from the four locations at the bottom of the lake were analysed for 39 PFAS using liquid chromatography combined with tandem quadrupole mass spectrometry (LC-MS/MS) and for extractable organic fluorine (EOF) using high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-CS-GFMAS). EOF extraction from the dried sediment samples was conducted with organic solvents. From the undisturbed sediment cores, consisting of sapropel occurring in anoxic environments, 16 samples from depths up to a maximum of 38 cm were taken. Results revealed quantifiable PFAS concentrations at all sampling locations but not all sampling depths. In 50% of sampling locations, increasing PFAS occurrences and concentrations were measured with increasing depth, with highest PFAS concentrations close to the runoff inlet into the lake. PFOS and its precursors N-MeFOSAA and N-EtFOSAA were found at highest concentrations, suggesting that the historical contamination originated either from industrial discharges, urban runoff, or firefighting foam.

The Lake Flughafensee is assumed to hydraulically gain on its eastern shore and hydraulically lose on its western shore. Groundwater was sampled at three locations on three occasions in one monitoring well up-gradient and two monitoring wells down-gradient of the lake. In the up-gradient monitoring well, concentrations of PFAS substances were lower compared to both down-gradient locations. At the up-gradient location, longer chain PFAS made up about 45% of concentrations, whereas shorter chain PFAS contributed about 36%. In contrast, the down-gradient groundwater composition showed much more PFOS (76%) and PFHxS (12%) presence, and even longer chain PFAS compounds were also detected.

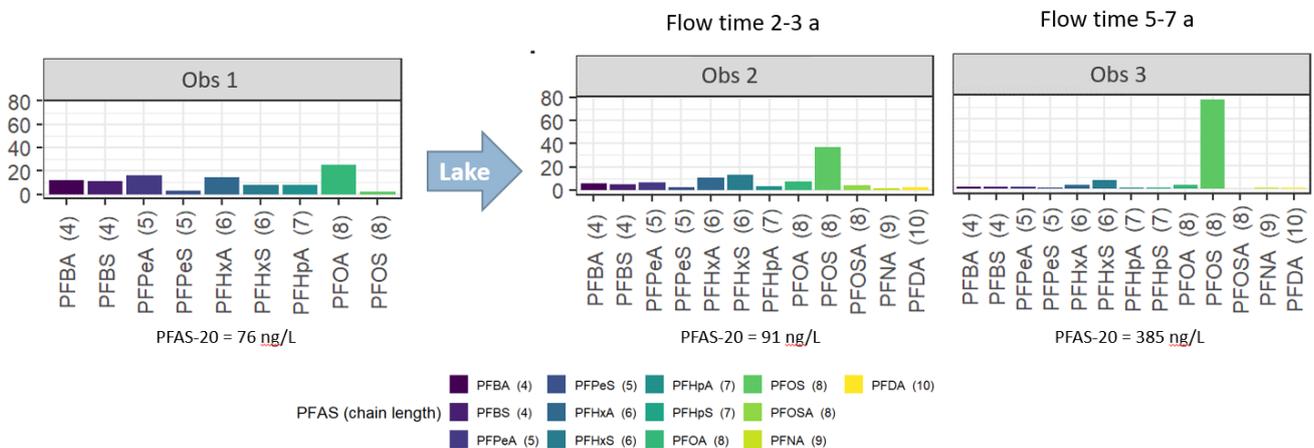


Figure 4: Examples of PFAS fingerprints in groundwater monitoring wells upgradient (Obs 1) and downgradient (Obs 2 and 3) from lake Flughafensee.

By combining monitoring of multiple environmental media (urban runoff, surface water, groundwater, and sediments), it could be concluded that the PFAS contamination in the lake is more likely linked to up-gradient groundwater contamination and potential remobilization from sediments rather than stormwater runoff. This was accomplished through the combination of methods to provide and compare PFAS ‘fingerprints’ (Figure 4) for the different environmental media within the location. This approach enabled an improved understanding of the complex situation regarding PFAS

at this location. Understanding the full scope of contamination sources requires further investigation of groundwater pathways, sediment characteristics, and potential long-term contamination from past sources. This should be supplemented by complementary analytical approaches (sum-parameters, target analysis) to obtain a full understanding of the situation and close PFAS mass balances.

2.3 Identification of pollutants and characterisation of sources on a catchment scale : from non-target screening to quantitative monitoring

Within the large catchment scale monitoring altogether about 300 samples have been collected from about 50 locations all over the Upper Danube Basin (CS#2). Sampling locations include surface water from the Danube itself and its tributaries, groundwater from bank filtration, inflow and outflow of municipal and industrial wastewater treatment plants, landfill leachate, surface runoff and atmospheric deposition. Targeted analysis of 32 PFAS revealed varying occurrences of PFAS contamination in the upper Danube catchment, originating from certain industries and other local hotspots. Furthermore, results indicate a limited retention at wastewater treatment plants and bank filtration. Further details can be found in (Liu et al. 2023, Obeid et al. 2023).

Monitoring of further iPM(T)s was performed by means of a database-assisted suspected target screening with 127 samples using the BAFG workflow (Deliverable [D1.4](#)). Data acquisition was done with LC-HRMS using a Q-ToF-MS/MS and chromatographic separation by means of a C18 column. Being part of a non-target approach, in principle all acquired signals might be of interest for further evaluation. Therefore, peak picking of all signals is performed by an algorithm written in R, which is described in detail in (Dietrich 2022). After completion of peak picking in all samples, an alignment of all features is performed: features detected in different samples having the same mass and retention time within a given tolerance are regarded as the same feature and are grouped accordingly. Identification of suspects is achieved by matching high resolution mass, MS² spectra and retention time of the aligned features with an in-house database, which was fed with the respective information from analyses of authentic reference standards on the same machine.

In total, 280 different compounds have been tentatively identified in samples of the Danube catchment, with a large share of typical wastewater-borne micropollutants such as pharmaceuticals and cosmetics, about 40 different pesticides and about 75 industrial compounds. Those numbers cannot be given as exact numbers as a distinct classification is not always possible: In many cases compounds might be used in industrial processes and for non-industrial purposes. Table 4 provides the TOP10 of the most frequently identified industrial compounds within the Danube monitoring. A complete list of all substances tentatively identified in samples from CS#2 can be found in Zenodo (<https://zenodo.org/records/14011987>).

Table 4: TOP 10 industrial substances most frequently identified via suspect screening of CS#2 samples

Compound	CAS	Classification	Identified use
1,3-Diphenylguanidine	102-06-7	Industrial	vulcanization additive
Benzothiazole-2-sulfonic acid	941-57-1	Industrial	industrial byproduct or metabolite
4- Isopropyl-benzenesulfonic acid	16066-35-6	Industrial	industrial byproduct
Tris(1-chloro-2-propyl) phosphate	13674-84-5	Industrial	widespread use in plastics production, flame retardant
2,4-Dinitrophenol	51-28-5	Industrial	Organic synthesis
2-Naphthalenesulfonic acid	120-18-3	Industrial	Organic synthesis
Triisopropanolamine	122-20-3	Industrial	widespread use in plastics production, corrosion inhibitor
Didecyl-dimethylammonium	20256-56-8	Industrial	Disinfection agent
Triphenylphosphine oxide	791-28-6	Industrial	industrial byproduct
Benzotriazole	95-14-7	Industrial	corrosion inhibitor

First results of the suspected target screening at CS#2 have been further used for the development of a respective targeted method (Deliverable D1.2). The list of target compounds of this method and the frequency of detection using database-assisted suspect screening within the Danube monitoring campaign is provided in Table 5.

The comparison of the targeted and the suspected target method demonstrates the advantages and limitations of both approaches. Targeted methods can never provide a complete overview of all potentially relevant substances. Non-targeted methods can be an additional tool here to at least partially fill this gap. On the other hand, most non-targeted methods suffer from decreased sensitivity compared to targeted methods, which might be one reason why some of the compounds listed in Table 5 have not been detected via suspect screening. Furthermore, typical non-target approaches work without calibration and thus cannot provide robust information on concentrations.

Untargeted methods can never replace a robust target analysis, but the combination of both approaches can significantly increase the analytical window and permit an efficient prioritization of most relevant compounds on which dedicated target analysis methods can be implemented.

Table 5 : Target compounds included in the BAFG LC-MS/MS method (Deliverable D1.2) and respective frequency of detection (FD%) via suspect screening.

Compound	CAS	Identified use	FD %
Benzotriazole	95-14-7	Corrosion inhibitor	91
1-Hydroxy-Benzotriazole	123333-53-9	Organic synthesis	57
Diglyme	111-96-6	Organic synthesis, solvent	0
Triglyme	203-977-3	Organic synthesis, solvent	0
Tetraglyme	143-24-8	Organic synthesis, solvent	86
Ethyltriphenyl phosphonium	1530-32-1	Organic synthesis	0
Methyltriphenyl phosphonium	1779-49-3	Organic synthesis	0
Tetrabutyl phosphonium	2304-30-5	Organic synthesis	0
(Methoxymethyl)triphenyl phosphonium	4009-98-7	Organic synthesis	0
Tetrabutyl ammonium	1643-19-2	Organic synthesis, solvent, plasticizer	41
Tetrapropyl ammonium	1941-30-6	Organic synthesis, solvent, plasticizer	2
Caffeine	58-08-2	Food industry	53
Acesulfame	55589-62-3	Food industry	0
Diphenyl urea	102-07-8	Intermediate	9
Saccharine	81-07-2	Food industry	0
Sucralose	56038-13-2	Food industry	0
Denatonium	3734-33-6	Additive for toxic liquids and solvents (bitter taste)	86
Tolyl biguanide	93-69-6	Antioxidant also used in paints and coatings	6
Propiconazole	60207-90-1	Fungicide also used for wooden construction material	5
Tebuconazole	107534-96-3	Fungicide also used for wooden construction material	19
Terbutryne	886-50-0	Algicide for paints	5
Cybutryne	28159-98-0	Antifouling agent	0
Triphenyl phosphineoxide	791-28-6	Byproduct from organic synthesis	93
Cyclamate	139-05-9	Food industry	80
Acridone	578-95-0	Fluorescence dye	3
Acridine9-carboxylic acid	332927-03-4	Fluorescence dye	0

3 How characterize the fate and transport of PFAS and iPM(T) compounds?

Experimentation and modelling for plume investigation

BRGM's Pluri-Metric Pilot (PMP) was used to reproduce, in controlled conditions, AFFF contamination within the soil-groundwater continuum (CS#6, Deliverable D2.4).

The monitoring set-up consists of probes and sampling devices that allow the monitoring of water table depth, soil moisture and soil water potential in the vadose zone, as well as water sampling in the vadose and saturated zones. To collect water samples, 80 suction cups were positioned along the main flow lines in different locations in the PMP and at different depths from -0.5m to -2.5m. Water samples were also collected in 9 piezometers at 3.5m depth. Weekly sampling campaigns were performed for 8 months, leading to the collection of nearly 2500 water samples.

Both total organic fluorine concentrations (CIC based; Combustion Ion Chromatography) and targeted analysis (LC/MS/MS based) were performed. Targeted analysis identified 7 PFAS in the AFFF solution, which correspond to approximately 85% of the total organic fluorine concentration measured in the AFFF solution. No inorganic fluorine was detected. Therefore, total organic fluorine analysis was used to perform a comprehensive initial screening of the contamination plume during the experiment. Next, targeted analyses were conducted on some of the water samples to gain a more refined understanding of the fate and transport of the AFFF-induced contamination within the soil-groundwater continuum. This monitoring scheme was also selected because no sample prep is required for total organic fluorine analysis. 50 to 200 μ L of the collected samples are directly placed on a ceramic support and injected in the CIC. Rapid measurement (25 min) and efficient data treatment enabled the analysis of more than 30 samples a day. This is particularly useful in the case of large-scale experiments which generate a large number of water samples. Furthermore, the rapid sample analysis allows piloting the experiment in near real-time. On a subset of the samples for which total organic fluorine was measured, targeted PFAS analysis was performed to quantify concentrations of the main 7 PFAS present in the AFFF. This list was defined further to prior analysis on a pure AFFF solution. The main PFAS found in the AFFF are 6:2 FTSA, 6:2 FTAB and 6:2 FTSAam (1525.0 mg/L, 3409.0 mg/L, 780 mg/L, respectively) and, to a lesser extent, PFHxA, 8:2 FTSA, 4:2 FTSA and 10:2 FTSA (8.9 mg/L, 3.8 mg/L, 3.4 mg/L, 1.7 mg/L). During the entire experiment, the total organic fluorine concentrations were measured on 700 samples. Among them, a targeted analysis was done on 250 samples.

A cross-comparison has been conducted to validate that the total fluorine organic concentrations are in line with the sum of the PFAS concentrations measured by targeted analysis and to validate the monitoring scheme used during the experiment (Figure 5). A 1:1 correlation was found for total organic fluorine concentrations ranging from 1.5 mg/L to 25 mg/L. An absence of correlation is found for total organic fluorine concentration below 1.5 mg/L corresponding to diffuse contamination. These results demonstrate the efficiency of the monitoring strategy and the complementary of the two types of analyses used in the experiment to characterize the contamination plume in soil-groundwater continuum induced by infiltration of AFFF. Indeed, weekly monitoring was performed using CIC based measurements allowing to assess that plume contamination is proceeding as planned based on theoretical calculations performed using the model train developed in PROMISCES (Deliverables D2.3 and D2.4). This near real-time monitoring allows also to detect any problems during the experiment and helps to detect the most appropriate mitigation solutions. The targeted

analysis conducted on a smaller subset of samples allows us to gain a more refined understanding of PFAS transfer in the soil-groundwater continuum, notably a distinct residence time between the three most abundant PFAS in the AFFF: 6:2 FTSA, 6:2 FTAB and 6:2 FTSAam. The retardation factor of the former is low, implying a residence time close to the one defined for a non-reactive tracer while high retardation factors are found for the two latter, implying that they are strongly sorbed below the area where AFFF enters into the soil-groundwater continuum and slowly migrates towards the groundwater (Deliverable D2.3).

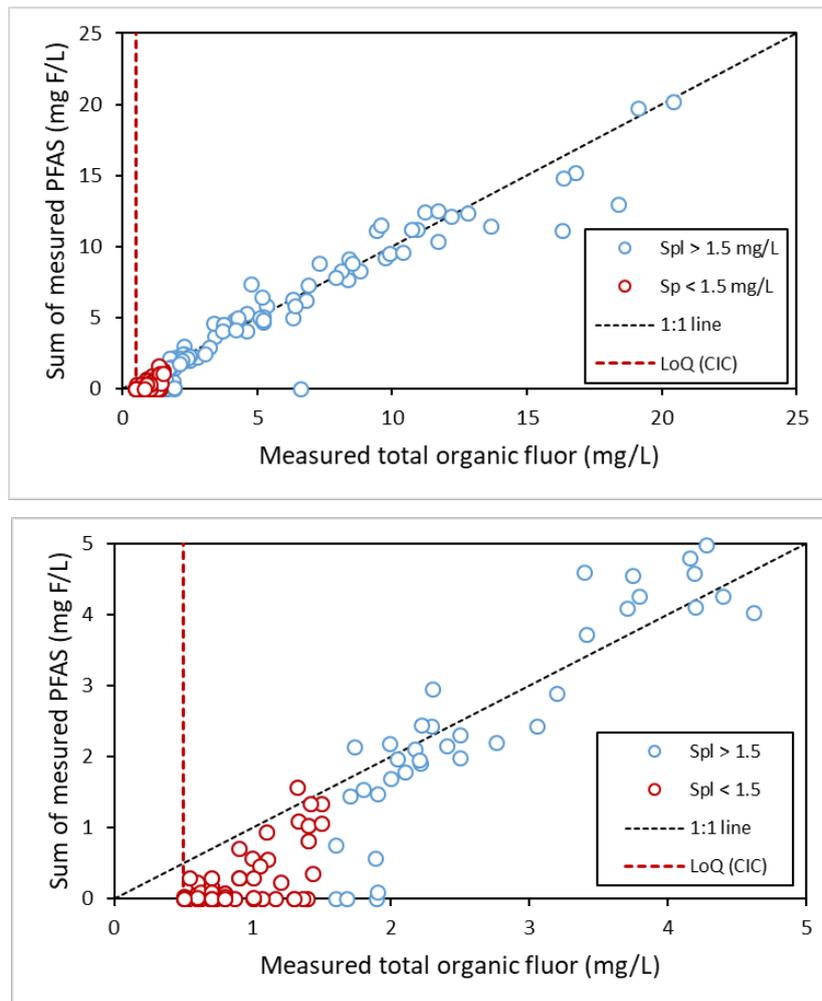


Figure 5: Cross-correlation between total organic fluorine concentration and the sum of concentrations of 7 PFAS (6:2 FTAB, 6:2 FTSA, 6:2 FTSAam, PFHxA, 8:2 FTSA, 4:2 FTSA and 10:2 FTSA). The blue and red dots correspond to samples with a total organic fluorine concentration higher or lower than 1.5 mg/L, respectively. The black line is the 1:1 line and the red dotted line is the theoretical LoQ for CIC.

Limitations

A specific point of attention associated with the monitoring strategy developed for the PMP experiment is the risk of cross-contamination between samples during CIC-based measurements: high amounts of fluorine in a sample can affect subsequent samples. To avoid such cross-contamination, systematic blank samples should be added between each sample suspected to have a high level of contamination. This procedure limits the number of samples analysed per day but ensures robustness of produced results.

As the TF analysis method cannot discriminate between PFAS, it can only be used to monitor the evolution of the plume and identify the most relevant samples for quantitative analysis of the PFAS of interest. The second stage, carried out by targeted analysis, was used to calibrate the PFAS reactive transport model in the pilot ([Deliverable D2.3](#)). In addition, the initial concentration in inorganic fluorine must be measured prior in the circulating water to better discriminate the results provided by CIC-based measurements.

4 How to assess treatment efficiency?

4.1 Monitoring Wastewater Treatment for Reclaimed Water Production and Crop Uptake Assessment

The objective of CS#3 was to evaluate new wastewater treatment methods based on electrochemical advanced oxidation processes (EAOPs) and constructed wetlands (CWs) for the production of reclaimed water for agricultural irrigation. To assess the efficiency of these methods in removing iPM(T)s and PFAS from wastewater and their uptake by crops (lettuce) irrigated with treated wastewater, several analytical strategies were employed.

In the case of iPM(T)s, since information on their occurrence in WWTP effluents was largely unknown, a suspect screening methodology was initially applied to identify the most concerning iPM(T)s present. To this end, three different sample preparation methods (lyophilization, direct injection, and online solid-phase extraction) were tested on effluent wastewater samples collected on three different weekdays, prior to analysis by liquid chromatography–high-resolution tandem mass spectrometry using a hybrid quadrupole time-of-flight analyzer (LC-QToF-MS/MS). This approach enabled the identification of 119 PM(T)s, 22 of which were confirmed with reference standards. Industrial compounds were the second most detected category of contaminants (after pharmaceuticals), with a total of 33 compounds/transformation products. The identified compounds were then prioritized based on their persistence, bioaccumulation, mobility, and toxicity. For the 25 most relevant compounds, along with 16 additional ones selected in a similar study conducted at another WWTP, a targeted methodology based on isotope dilution, online solid-phase extraction (online SPE)-LC-QToF-MS/MS, and positive ionization was developed. This targeted approach provided reliable results with satisfactory sensitivity (LODs ranging from 0.04 to 166 ng/L), reproducibility (RSD; relative standard deviation <15% for most compounds), and recoveries (absolute values generally between 60% and 140%). Detailed information on specific conditions is available in Deliverable [D1.4](#). This overall strategy proved to be highly valuable for identifying and prioritizing iPM(T)s for further water treatment studies.

For the analysis of the same 41 iPM(T)s in lettuce, another targeted methodology was developed. This method was based on a QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction method, followed by LC-QToF-MS/MS analysis using the same experimental conditions as those applied to water.

In the case of PFAS, analytical methods for the determination of 43 compounds in both water and solid matrices (sediments and plants) were already available at CSIC. These targeted methods were complemented with suspect screening approaches, enabling the identification of a broader range of PFAS and transformation products (TPs)—up to 1,280 compounds included in a proprietary library—during the water remediation studies (details in Deliverable [D1.4](#)).

The application of these analytical methodologies in various experiments conducted in CS#3 made possible to optimize the operational conditions of the EAOP and to evaluate the removal efficiency of both the EAOP pilot plant and the constructed wetland (CW) in the field. These studies concluded that the removal efficiency of iPM(T)s—generally satisfactory—primarily depends on the type of compound (its chemical structure and susceptibility to oxidation processes) and its concentration in the source water, as described in Deliverable [D4.3](#). For PFAS, removal rates were generally low, and no additional PFAS compounds beyond those covered by the targeted methods were detected, underscoring the relevance and comprehensiveness of the selected analytes.

Finally, an assessment of the occurrence and associated health risks of iPM(T)s and PFAS in lettuce irrigated with WWTP effluent and reclaimed water (post-EAOP + CW treatment) revealed no health risk from iPM(T)s, but a potential risk from PFAS—only when irrigating with effluent from secondary treatment.

Based on these findings, the water treatment technology tested in CS#3 addresses the water scarcity challenges commonly faced in Mediterranean regions, positioning reclaimed water as a sustainable and viable solution for agricultural irrigation.

The analytical approaches employed have enabled the tracking of the removal and uptake of iPM(T)s and PFASs throughout the various experiments in a comprehensive and cost-effective manner, covering a wide range of pollutants and focusing for quantitative purposes on the most relevant ones. Nonetheless, in the case of PFAS, the application of more general analytical techniques—such as AOF, EOF, and TOP—could provide a more complete picture.

On the other hand, the contamination profile observed in WWTP effluent through suspect screening suggests that pharmaceuticals and pesticides are also relevant contaminants that must be studied alongside iPM(T)s. Additionally, for more robust conclusions regarding removal efficiencies, careful consideration must be given to hydraulic retention time when planning water sampling, as well as to potential contamination sources (e.g., geotextile material used in CWs) or operational conditions that may affect analytical results. In uptake studies, soil analysis is also necessary.

Finally, to assess compliance with Directive 2024/3019—which sets standards for the advanced treatment of effluents from urban WWTPs and requires a minimum 80% average removal of specific micropollutants—analysis of all or some of the listed micropollutants should be conducted:

- Category 1 (easily treatable substances): amisulpride, carbamazepine, citalopram, clarithromycin, diclofenac, hydrochlorothiazide, metoprolol, and venlafaxine;
- Category 2 (easily removable substances): benzotriazole, candesartan, irbesartan, and a mixture of 4-methylbenzotriazole and 5-methylbenzotriazole.

During pilot plant operation, four of these substances—carbamazepine, venlafaxine, benzotriazole, and the 4-/5-methylbenzotriazole mixture—were monitored. With the exception of venlafaxine, all other substances exhibited removal rates below 80%. However, among the 41 PM(T)s analyzed in CS#3, 11 achieved removal rates above 80% using the EAOP + CW system.

4.2 Sludge recovery and WWTP effluent characterization

PROMISCES aims also to evaluate PFAS transfer during nutrient recovery processes, especially the conversion of digested sludge into ammonium- and phosphate-based fertilizers.

For that several methods developed have been applied, including targeted LC-MS/MS analysis for 58 PFAS, TOP assay and Combustion Ion Chromatography (CIC) for measuring extractable organic fluorine (EOF) and total fluorine (TF) contents.

The samples were supplied by various partners engaged in the PROMISCES project : Sewage sludges, related fertiliser (struvite) produced from the digested sludge have been delivered only by WWTP A., some compost or intermediate products have been characterized all along the project (Idjaton et al., 2024), for answering the objective of the task (detailed results are presented in Deliverable D3.5.), but also to validate the robustness, complementarity and relevance of the different analytical approaches.

In all the cases where the different approaches were compared on WWTP samples (solid or liquid), the relative proportion of fluorinated compounds explained by the different methods remained of the same order, as illustrated in the Figure 6.

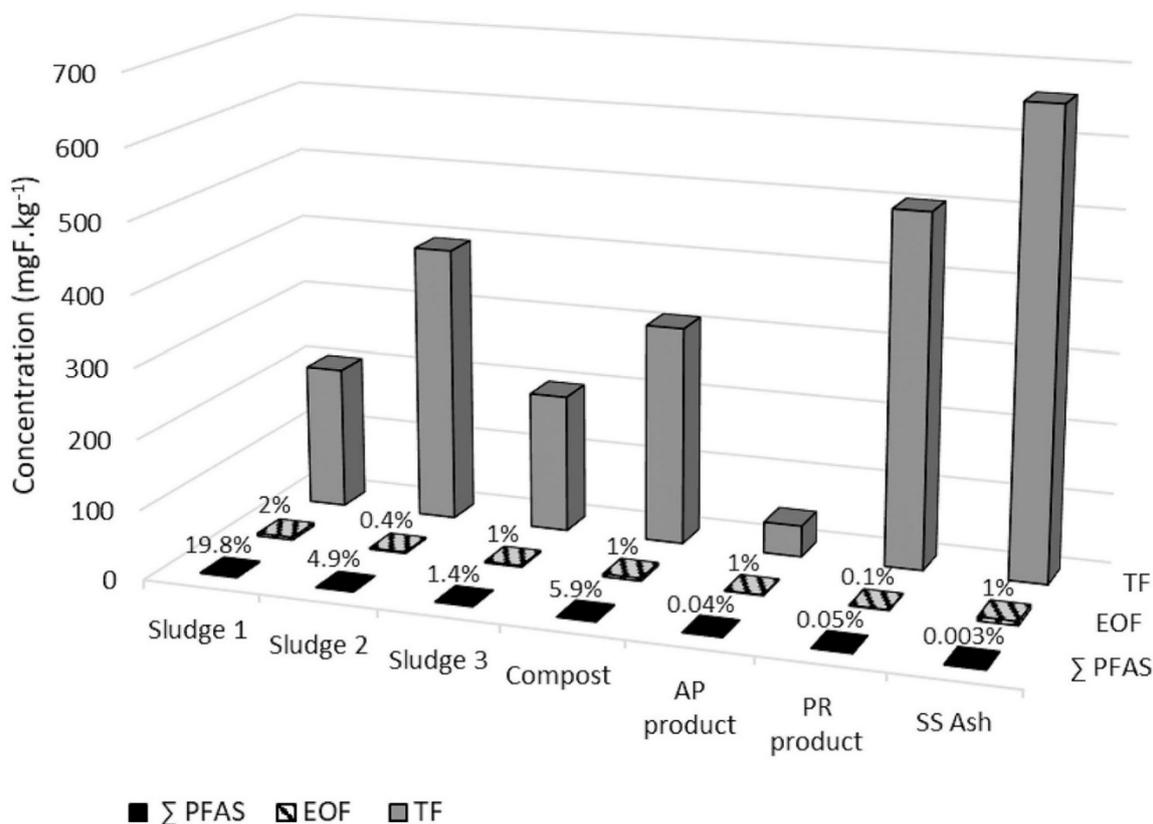


Figure 6: Comparison between TOF, EOF and Σ PFAS analysed by LC-MS/MS expressed as mg F.kg⁻¹ on solid samples. (AP: ammonium phosphate; PR: phosphorus recovery; SS: sewage sludge). Percentages associated correspond for EOF, to the percentage of TF explained by EOF and for Σ PFAS, to the percentage of EOF explained by Σ PFAS (Idjaton et al., 2024).

The gap between EOF and TF results suggests that some fluorinated compounds in solid matrices are not captured by the EOF method. The main hypothesis is a partial extraction of the fluorinated compounds that remain trapped in the solid matrix. Specifically, the presence of fluorinated polymers is one of the serious avenues to be explored, but this would require new analytical techniques not deployed in the PROMISCES project (e.g. 19F NMR). As the TF includes inorganic fluorine at this stage, a step to remove it before analysis must be added to really determine the proportion of organic fluorine. Work is in progress, in particular as part of the expertise group on the standardisation of an EOF method.

Nevertheless, global analysis provides consistent information concerning the efficiency of nutrient recovery processes like struvite precipitation for reducing PFAS transfer to agricultural soils, as demonstrated in the study of distribution and transfer of per- and polyfluoroalkyls (PFAS) in wastewater treatment plants (WWTPs) and ammonium/phosphate recovery (struvite) unit in Deliverable D3.5.

The TF and EOF concentrations range from 44 mg F/kg dry weight(dw) to 58 mg-F/kg dw and 0.13 mg F/kg dw to 0.15 mgF/kg dw, respectively for struvite, when the TF and EOF concentrations range from 161 mg F/kg dw to 189 mgF/kg dw and 0.6 mg F/kg dw to 0.7 mg F/kg dw, respectively in related digested sludges from the same WWTP.

For the characterization of WWTP effluents, the added value of TOP assay is clearly demonstrated. Direct analysis of the samples led to the quantification of only three PFAS compounds in WWTP A (6:2 FTAB, PFECBS, and PFHxS), while no PFAS were detected in WWTPs B and C. Conversely, as illustrated in Figure 7, the results of the TOP assay revealed a different scenario, highlighting the presence of PFAS precursors that became detectable after the oxidation step.

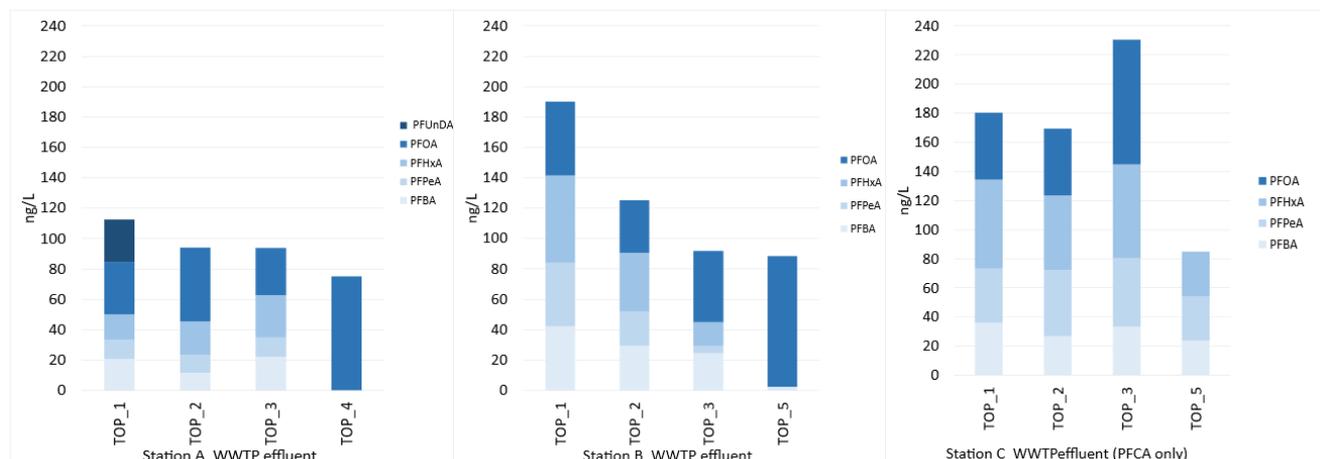


Figure 7: TOP assay results on PFCA concentrations on three WWTP effluents weekly sampled. PFCA are classified from the shortest chain (light blue) to the longest (dark blue).

Although these results are encouraging, certain limitations of the method have also been identified, particularly concerning its robustness when applied to highly complex matrices such as wastewater treatment plant effluents. Additional efforts are required to further refine and frame the procedure, and the formal standardization of the method is necessary to harmonize practices and ensure its operational deployment, enabling consistent and comparable results across a broad range of laboratories.

4.3 Assessment of remediation technologies using complementary approaches

Given the strong persistence of PFAS, experiments were conducted using plasma reactors for PFAS remediation. These experiments combined various approaches, from target analysis of PFAS, TOP assay tests, AOF measurements for global organic fluorine content, as well as toxicological assessment using CALUX bioassays. Different processes have been compared, with argon plasma treatment, with argon-oxygen and with argon-hydrogen. The results are not all consistent, requiring further investigation to better differentiate whether the discrepancies are due to the experimental and analysis protocols or to the actual treatment methods.

As an example, in all 3 conditions, quantitative analysis of PFAS demonstrate a removal of targeted PFAS. At the same time, adsorbable organic fluorine (AOF) levels increased in all samples. However, when PFAS undergo mineralization, a decrease in AOF levels is expected, as fluorine is released as fluorine. This unexpected increase could indicate a transformation of the PFAS into other organic fluorine compounds that adsorb more readily, or into inorganic complexes (such as CaF_2) that are not easily removed during the AOF washing step. Therefore, the toxicological potential increased after plasma treatment while the concentrations of PFAS analysed decreased. This suggests the formation of more toxic transformation products. However, the exact cause of this increase remains unclear.

and needs further investigation. In this example, the complexity of the matrix is a challenge for methods whose robustness has yet to be tested but highlight the need to use complementary approaches to increase confidence in highly exploratory treatment methods for compounds for which much remains unknown.

5 Risk assessment of PFAS and iPM(T) using bioassays

The extremely broad diversity and complexity of PFAS (per- and polyfluoroalkyl substances) means that relying solely on chemical monitoring is insufficient to guide regulatory or remediation actions. To improve the prioritisation of substances and better understand their associated risks, multiple complementary approaches have been explored. Among these, *in vitro* and *in silico* toxicity assessments have emerged as particularly powerful tools, enabling the evaluation of large numbers of compounds rapidly and systematically.

5.1 Toxicological assessment to better characterise PFAS and iPM(T) compounds

As an example, based on the 40 PFAS on which *in vitro* based RPFs have been obtained, three most active PFAS compounds identified in the *in vitro* TTR-TRb CALUX assay (PFOA, P37-DMOA, and 6:2 FTAB) were used in *in silico* modelling to identify structurally analogous PFAS. This analysis identified 12 structural analogues for PFOA, three (3) for P3,7DMOA, and seven (7) for 6:2 FTAB. The uses, tonnage and potential for release into the environment of these substances could be particularly well documented, to identify if there is a specific risk associated with them. Given the current desire for a strict ban on PFAS, and their restriction to essential uses, this criterion could be used for a systematic screening of substances at stake, or to enable choices to be made between several substances. In parallel, a specific effort has been done in PROMISCES to propose a tiered *in vitro*/*in silico* approach to screen the toxicity of PFAS (related to disruption of thyroid hormone transport). Applied to 12,654 PFAS, this workflow offers a way of tackling the scale of the task ahead (Sosnowska et al., 2025).

Several PFAS and iPM(T)s (such as benzotriazoles, parabens, ...) have been characterised in Deliverable D1.5 by *in vitro* toxicity profiling of CALUX bioassays and have been described in CWA 18201 (2025). Bioassays have been deployed to characterize samples used in different case studies for remediation technique assessments.

The panel of *in vitro* CALUX bioassays evaluated here was also used to analyze the AFFF-FT mixtures employed in the project for PFAS treatment and in case study CS#6. Concentrated fire-fighting foam (AFFF-FT based) was dissolved in DMSO to obtain a final concentration of 5%. The stock concentration is given in table 1.

Table 6: Sample coding and stock concentration prepared

Sample code (-)	BDS code (-)	stock prepared (mg/ml)	stock prepared (%)
Concentrated Fire Fighting Foam (AFFF-FT Based)	46131	50.69	5.07

Following preparation of serial dilution series of the final sample extracts, the bioactivity in a tailored set of CALUX bioassays was determined. In figure 8, the sample dose-response curves of the PFAS CALUX bioanalysis results of the concentrated Fire Fighting Foam (AFFF-FT Based) are presented, in comparison with the reference compound response, showing a lower activity (or no activity) for this mixture compared to the reference compound of each assay.

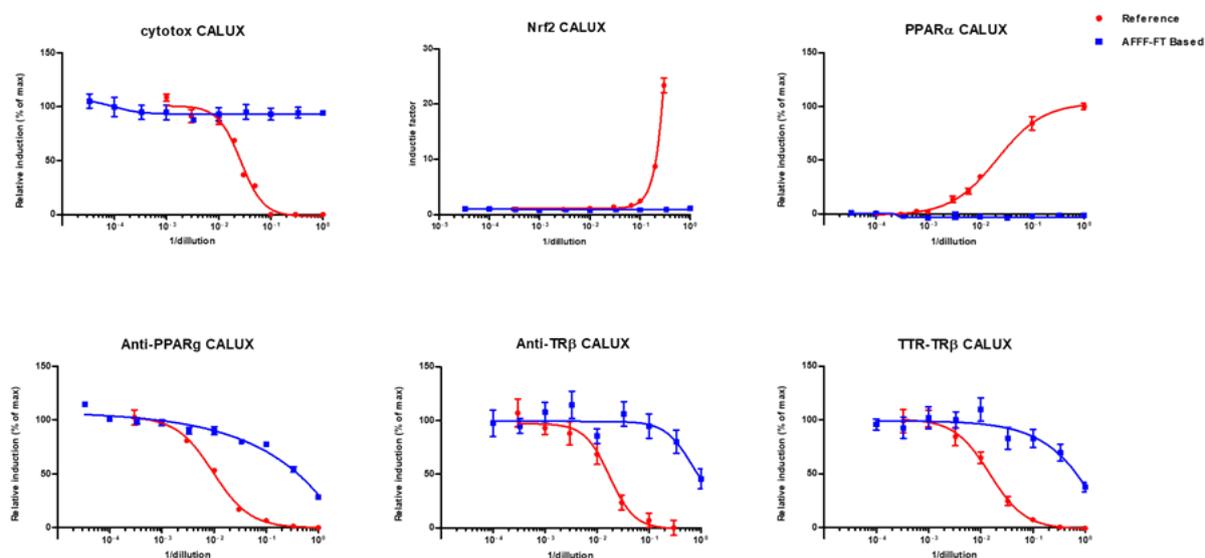


Figure 8: Graphical representation of CALUX analysis results of Fire Fighting Foam (AFFF-FT Based) on a tailored set of CALUX bioassays

In Table 7, the quantified CALUX bioassay analysis results of the analysed Fire Fighting Foam (AFFF-FT Based) is summarised.

Table 7 : CALUX bioanalysis results of Fire Fighting Foam (AFFF-FT Based)

Bioassay	activity	LOQ	Unit	LOEC	Unit
cytotox CALUX	<LOQ	92	ug TBT/g sample	0.51	g sample/l medium
Nrf2 CALUX	<LOQ	4496	ug Curcumine/g sample	> 0.51	g sample/l medium
PPARα CALUX	<LOQ	1.3	ug GW7647/g sample	> 0.05	g sample/l medium
anti-PPARγ CALUX	47	1.9	ug GW 9662/g sample	0.0021	g sample/l medium
anti-TRβ CALUX	1781	597	ug Diclazuril/g sample	0.017	g sample/l medium
TTR-TRβ CALUX	947	454	ug PFOA/g sample	0.24	g sample/l medium

Bioassays can therefore be applied to comprehensively assess the toxicity of mixtures or individual compounds, enabling their prioritization based on a broad range of biological endpoints associated with organic pollutants.

How to act before having sufficient toxicological data available is a key point. The principle of the Health-Related Indicator Value (HRIV) concept, used in Germany, allows to close the time gap before the appearance of a robust limit value. Based on data obtained on in vitro assays and in silico model, an enhanced HRIV between 0.1 µg/l and 0.01 µg/l has been proposed for individual PFAS based on effects on the inhibition of thyroid hormone transport ([Deliverable D1.6](#)) as illustrated in Figure 9.

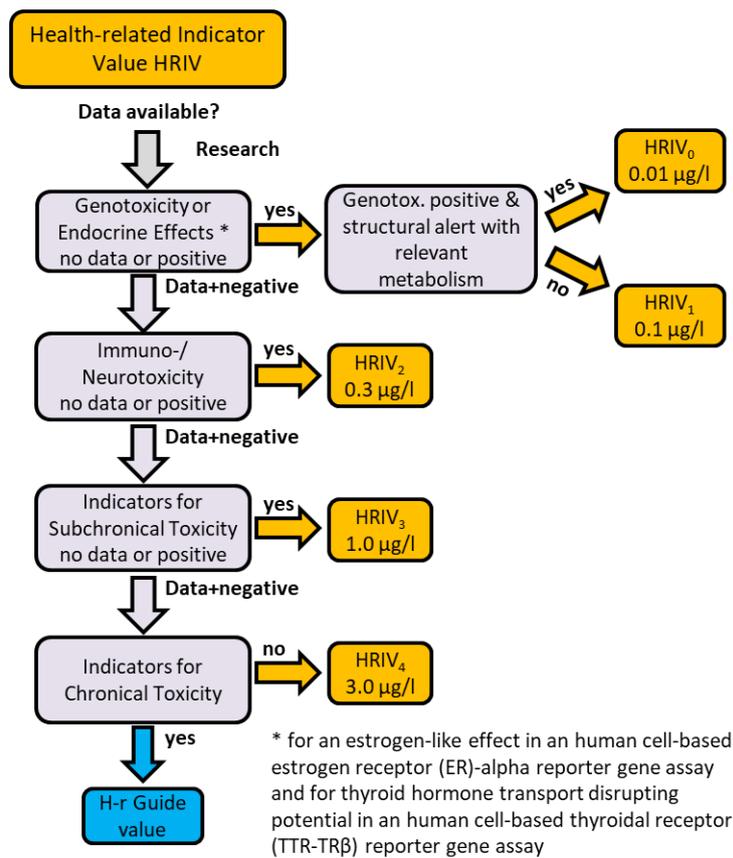


Figure 9: Proposal for an enhanced HRIV concept in Germany for individual PFAS (Kuckelkorn et al., D1.6).

5.2 Highlighting relevant situation based on toxicological criteria.

The added value of bioassays has been also illustrated on a large set of environmental samples. Considering Relative Potency Factor obtained for 45 PFAS, the calculation of a PFAS CALUX concentration has been proposed (expressed in $\mu\text{g PFOA eq./L}$). When results from 100 water samples are compiled (Figure 10), the most problematic concentration ranges and sample types can be identified, and a warning level can be set for each sample type, above which more detailed investigations should be carried out.

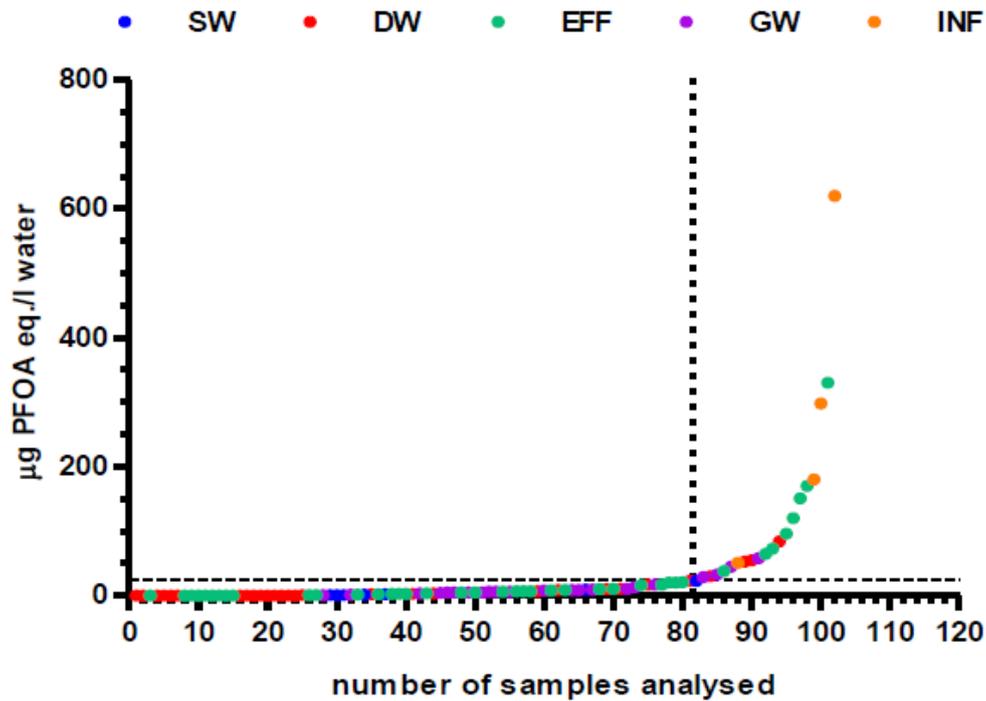


Figure 10: PFAS CALUX activity distribution by origin from ca. 100 water samples (GW: Groundwaters, SW : surface waters, DW: drinking waters, INF: WWTP influent, EFF: WWTP effluent). Dotted line corresponds to 80% of the samples.

As CALUX activity is not specific to PFAS compounds, the highlighted situation can concern other compounds than PFAS, such as pharmaceuticals compounds, industrial compounds or others. The results should be regarded as an early warning, necessitating the initiation of more in-depth investigations to identify the responsible compound(s) and their origin, thereby facilitating the development and implementation of effective management strategies.

6 Conclusions and recommendations

Although persistent challenges remain and several analytical and methodological approaches still require thorough validation before regulatory deployment, the contribution of advanced tools developed in PROMISCES is already considerable. These tools have significantly enhanced our understanding of the pathways, fate, transport, and transformation of major pollutants, notably per- and polyfluoroalkyl substances (PFAS) and iPM(T) compounds.

The PROMISCES project represents a major scientific milestone. Its achievements, when integrated with forthcoming European and international standardisation efforts, lay the foundation for a more comprehensive and robust management of complex micropollutant challenges. In particular, PROMISCES has developed and validated analytical methods enabling the quantification of 62 PFAS across more than twelve environmental and biological matrices—substantially enriching the regulatory and research toolbox.

Nevertheless, it must be recognised that certain methodologies (Non target screening, TOP assays...) require a high level of technical expertise, specialised instrumentation, and substantial financial and infrastructural resources. Therefore, their strategic application lies primarily in early-stage investigations (such as prioritising substances or contaminated sites) and in informing the design of targeted, scalable monitoring strategies that could subsequently be adopted by routine laboratories.

A clear and urgent need persists for further metrological development, particularly regarding method harmonisation, the implementation of inter-laboratory comparison studies, and the production of certified reference materials representative of the diversity of PFAS and their transformation products. Ensuring the reproducibility, traceability, and comparability of data is essential for supporting both research and regulatory activities. Recent developments, including work on standardising an EOF method to distinguish inorganic fluorine, are important steps toward this objective.

Importantly, several tools and integrated approaches (bioassays, target analysis, AOF) have now reached sufficient maturity to justify immediate application in addressing pressing environmental and public health issues. Initial examples of operational deployment already exist, demonstrating that the integration of advanced analytical, toxicological, and modelling approaches into monitoring and regulatory frameworks is both feasible and necessary, if associated with scientific expertise for the interpretation.

The Table 8 is a summary of the main benefits and remaining actions after the PROMISCES project to facilitate the implementation of the developed methods or approaches in environmental monitoring.

Table 8 : Summary of the added values and remaining actions following PROMISCES actions on chemical and toxicological developments

Methods	Added values from PROMISCES project	Remaining actions
Targeted analysis	Expanded list of PFAS with validated methods	Need to continuously expand the PFAS list costly
	Broader array of tested matrices	
AOF	Better insight into method efficiency /limitation	Limit of quantification below 1 µg/L remains challenging
	Improvement of method robustness	Some PFAS are not taken into account (USC-PFAS)
	Demonstration of the added value of the method for assessing PFAS pollution	
TOP assay	Better understanding of the efficiency /limitation of the method	Method harmonization across laboratories still needed
	Demonstration of the added value of the method for PFAS pollution characterization	
Non target screening	Demonstration of the added value of the method for identifying new compounds of interest	Development of data sharing (common databases...)
Bioassays	Increase in the number of PFAS available for RPF CALUX	Further discussion needed on how to interpret and use CALUX toxicological data
	Demonstration of the added value for assessing PFAS impact	
	Highlighted benefits of combining chemical and toxicological methods.	

Given the environmental ubiquity, chemical diversity, and poorly characterised nature of PFAS and iPM(T)s, a paradigm shift is needed. This shift should move away from conventional targeted monitoring toward broader, integrative strategies that combine non-targeted analysis, effect-based screening, and predictive toxicology. These new approaches allow for a more holistic understanding of the hazards and risks associated with PFAS.

Recent developments include the establishment of Relative Potency Factor (RPF)-based bioassays for 45 PFAS and 22 industrial (iPM(T)) compounds. Complementarily, predictive modelling approaches have been proposed, identifying approximately 100 PFAS compounds potentially exhibiting higher toxicity than regulated PFAS among a database of over 14,000 screened substances. Such integrative efforts—coupling empirical data with computational modelling—underscore the necessity of prioritising compounds of concern effectively.

Finally, advancing knowledge on the environmental fate of transformation products, improving validation of remediation mass balances, and systematically integrating toxicity screening into monitoring strategies remain essential to optimise risk management, enhance public health protection, and achieve sustainable management of PFAS contamination.

When considering the use of these methods across various regulatory frameworks, a strong heterogeneity can be observed. This heterogeneity arises either from the long implementation timelines of the regulations — as the methods did not yet exist at the time of their revision — or from the fact that the methodological requirements still appear to be incompatible with regulatory expectations.

Table 9 : Overview of European legislation mentioning PFAS parameters and available methods

EU Directive / Regulation	Environmental compartment	PFAS mention / scope		Method fit for purpose
Drinking Water Directive (EU) 2020/2184	Drinking water	Introduces two PFAS parameters: "Sum of 20 PFAS" and "PFAS Total".	Sum of 20 PFAS	Quantitative measurement via LC/MS/MS
			PFAS Total	No validated method; Imperfect proxy : AOF, TOP assay
Water Framework Directive (2000/60/EC) and Priority Substances Directive (2013/39/EU)	Surface and groundwater	PFOS listed as priority substance; environmental quality standards (EQS) established.	PFOS	Quantitative measurement via LC/MS/MS
Revision of Sewage Sludge Directive (86/278/EEC)	Sewage sludge (agricultural use)	No explicit mention of PFAS; focuses on heavy metals.	-	-
Urban Wastewater Treatment Directive (recast 2024)	Wastewater treatment plants (WWTP)	Monitoring required for PFAS and other micropollutants in treated effluents.	No defined list	Quantitative measurement via LC/MS/MS discussion on EOF or TF
Soil Monitoring Directive (2025 agreement)	Soils	PFAS included among the chemical descriptors as emerging contaminants.	List under discussion	Quantitative measurement via LC/MS/MS
Revision of Water Framework Directive	Surface and groundwater	Expansion of the PFAS list	Threshold weighting via RPFs	Quantitative measurement via LC/MS/MS + toxicological criteria (RPFs)

However, some recently tested methods could be introduced to address the shortcomings, such as the already discussed PFAS Total parameter, for which conventional quantitative methods are unable to adequately address.

The need to better integrate effects into regulatory parameters is still poorly reflected in the texts, with the exception of the ongoing revision of the Water Framework Directive (WFD). This revision highlights the need to develop effect-based monitoring, for which the toxicological methods developed in PROMISCES are highly relevant.

Similarly, ongoing discussions within the framework of PFAS restrictions, for example regarding PFHxA and its derivatives, raise the question of incorporating methods such as the TOP assay, which could account for all molecules that can degrade into PFHxA.

One of the challenges is the interpretation and comparison to threshold values of these methods, which do not target individual molecules, but groups of molecules. This challenge can be partly addressed thanks to feedback from experiences carried out in PROMISCES.

Recommendations

Building on the achievements of PROMISCES and considering the remaining challenges, several key recommendations emerge:

- **Strengthen broad monitoring before focused analysis**

The proposed tools facilitate an extensive screening of the situation, encompassing spatial and temporal dimensions, while employing a diverse range of investigative methods. After this initial step, which provides a global overview, it becomes possible to focus the monitoring on points of interest, whether based on toxicity criteria or occurrence. This gradual approach can limit costs while enabling the most relevant data to be acquired to characterise or monitor the necessary management actions.

- **Enhance Analytical Methods**

Continuous improvement of analytical techniques is necessary to detect pollutants at trace levels across multiple matrices and to adapt to evolving regulatory thresholds (e.g., watch lists). Analytical methods must offer low quantification limits, be robust, cost-effective, and suitable for large-scale deployment.

- **Involve analytical and toxicological experts in the definition of the monitoring strategies to implement.**

There are numerous analytical methods available, often complementary, each with their own advantages and limitations. Involving analytical and toxicological experts in the design of monitoring strategies would allow for the selection of the most appropriate methods to address specific challenges. Their participation is also essential for the accurate interpretation of results. Given the complexity of studying PFAS, experts must support the various stakeholders to ensure a better understanding and appropriation of the results, especially in the current period where knowledge is continuously evolving.

- **Develop Standardised Metrics**

It is an urgent priority to establish reproducible and harmonised analytical methods for measuring PFAS concentrations and total PFAS-related toxicity, ensuring consistent data generation across laboratories and regulations. Even if the golden standard for “Total-PFAS” is not reachable, several methods can partially answer the question but need to be well defined and harmonized to allow obtaining comparable data.

- **Contribute to Standardisation Initiatives**

Continued involvement in standardisation efforts (such as development of an EOF method to separate inorganic fluorine) is essential to support regulatory implementation and harmonisation across countries.

- **Promote Non-Animal Methods (NAMs)**

Developing and validating in vitro toxicity assays (such as PFAS TTR-TR CALUX bioassays) is critical for screening the large number of unknown or poorly characterised PFAS. These methods should also be applied to assess the cumulative effects of complex environmental samples, as proposed in CWA CEN 18201 (2025).

- **Integrate Predictive Modelling Approaches**

Relative Potency Factor (RPF)-based bioassays and in silico predictive models can be used to identify and prioritise the most toxic PFAS among the thousands of existing compounds. Such integrative tools are critical for efficient risk-based prioritisation.

- **Implement Rigorous Quality Assurance Protocols**

Given the ubiquitous presence, high persistence, and contamination potential of PFAS, strict QA/QC measures must be implemented at every stage—from experimental design through sampling, analysis, and data interpretation—to avoid artefacts and ensure the reliability of results. In this context, well-defined experimental designs incorporating a higher degree of control than typically applied are crucial to guarantee the interpretability and robustness of the findings.

- **Validate Remediation Strategies with Mass Balance and Toxicity Assessment, and Advance Knowledge on Transformation Products**

Future studies must include full mass balance evaluations of PFAS remediation processes to ensure complete removal, destruction, or thorough characterization of the produced transformation by-products. A major research focus should also be the identification, characterization, and monitoring of these transformation products, particularly during remediation. This is essential to avoid implementing processes that generate by-products that are equally or more toxic, and to accurately assess the true effectiveness of treatment technologies. Therefore, toxicological assessment remains a crucial step to prevent secondary environmental contamination.

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