

## Research Article

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## Occurrence, Sources and Prioritization of Per- and Polyfluoroalkyl Substances (PFASs) in Drinking Water from Yangtze River Delta, China: Focusing on Emerging PFASs

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### ABSTRACT

As regulations ban legacy PFASs, many emerging PFASs are being developed, leading to their release into the aquatic environment and drinking water. However, researches on these emerging PFASs in drinking water are limited, and current standards only cover a few legacy PFASs, leaving many emerging PFASs unregulated and their toxicity unknown. Therefore, a machine learning-based suspect screening method was employed to comprehensively identify and quantify both legacy and novel PFAS in drinking water from the Yangtze River Delta, and their potential sources of contamination were determined through pollutant profile analysis. Then, the identified PFASs were prioritized by integrating the PBT (persistence, bioaccumulation, and toxicity) properties of PFASs with environmental exposure data. A total of 30 PFASs were identified, including 16 legacy and 14 novel PFASs, categorized into 11 classes. The pollutant profile analysis suggested that PFASs in the Yangtze River Delta's drinking water are more likely to originate from pollution in the upper and middle reaches of the Yangtze River rather than from local industrial emissions. In the prioritization and risk assessment process, ten high-concern PFASs had Risk Index (RIs) higher than those of ref-PFOA and ref-PFOS, including eight legacy PFASs and two novel PFASs. The drinking water of Yangtze River Delta originates from the surface water of the lower Yangtze River, which accumulates pollutants from its upper and middle reaches, affecting the health of over 20 million people. Our findings indicated the presence of emerging PFASs in the region's drinking water and demonstrated conceptual models for integrating chemical information from suspect screening with toxicity prediction and risk assessment. Although the current levels of emerging PFASs are relatively low, legacy PFASs still dominate. Further research is needed to identify, monitor, and assess the health and environmental risks of emerging PFASs.

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**Received:** March 20, 2025; **Accepted:** March 24, 2025; **Published:** March 31, 2025

**Keyword:** Per- and Polyfluoroalkyl Substances, Suspect Screening, Drinking Water, Prioritization, Risk Assessment

### Introduction

Per- and Polyfluoroalkyl Substances (PFAS) are a class of anthropogenic synthetic compounds with high-energy covalent bonds, which are widely used in a variety of industrial and consumer applications due to their hydrophobicity, lipophobicity, high thermal and chemical stability [1]. Due to their widely application and exceptional physicochemical properties, PFAS are almost ubiquitous in the environment [2]. Numerous epidemiological and toxicological studies have demonstrated that exposure to PFAS can cause a series of adverse effects on human health, including developmental and reproductive toxicity, neurotoxicity, hepatotoxicity, genotoxicity, immunotoxicity, endocrine toxicity, and carcinogenicity [3-9]. The persistence and toxicity of PFAS have raised global concern about their potential environmental and health risks. In order to control the contamination and health hazards caused by PFAS, many countries and international organizations are implementing regulations to limit the manufacturing and utilization of some legacy PFASs, which has led to a growing

number of manufacturers turning to the production of new alternatives of PFASs [10]. However, adequately safety tests are lacking for these novel PFASs, and companies refuse to disclose their components citing trade secrets, making it difficult for regulatory agencies and the public to comprehensively assess their safety and long-term impacts [11]. Many of these novel PFASs are discharged into the environment and have been detected in both environmental and biological samples [10,12-15]. Previous studies have indicated that the toxicity of certain novel PFASs may be comparable to or even exceed that of legacy PFASs [16-19]. Many novel PFASs shown higher affinity in molecular docking experiments with PPAR $\alpha$  and ER $\alpha$  receptors than legacy PFASs [20].

Drinking water is one of the main pathways of human exposure to PFASs [21]. To safeguard public health, numerous countries and organizations have implemented standards for PFAS limits in drinking water. In 2024, the United States Environmental Protection Agency (EPA) established Maximum Contaminant Level (MCL) standards for six PFASs in drinking water. The specific standards are as follows: PFOA at 4 ng/L, PFOS at

4 ng/L, PFHxS at 10 ng/L, PFNA at 10 ng/L, and HFPO-DA (GenX) at 10 ng/L. Additionally, the Hazard Index MCLs for mixtures containing two or more of the substances PFHxS, PFNA, HFPO-DA, and PFBS should be less than 1 [22]. Chinese sanitary standards for drinking water stipulated that the concentrations of PFOA and PFOS should not exceed 80 ng/L and 40 ng/L, respectively. The current standards are primarily aimed at legacy PFASs and a small amount of novel PFAS. However, a significant portion of novel PFASs are not covered by existing standards of drinking water, and their toxicity remains largely unknown. Thus, comprehensively identifying and evaluating the risks of legacy and novel PFASs in drinking water is of utmost importance.

Many studies have demonstrated that target PFASs constitute only a minor portion of the total organic fluorine (TOF) present in living organisms and the environment. In fact, a significant portion of extractable organic fluorine (EOF) is attributed to unidentified PFAS [23]. EOF mass balance analysis revealed that target PFAS account for less than 36% of the EOF in drinking water from Shanghai [24]. This indicated the presence of a significant amount of unidentified organic fluorine compounds in the drinking water, underscoring the importance of identifying and studying these unknown pollutants. Currently, the majority of studies on PFAS in drinking water primarily focused on the routinely monitored targeted PFAS. However, the utilization of high-resolution mass spectrometry (HRMS) had enabled both suspect and non-targeted screening methods to identify unknown PFAS compounds within complex environmental samples [11]. Only a few researches had conducted suspect and non-targeted screening of PFASs in drinking water, but none of these studies had considered the toxicity and risk assessment of these emerging PFASs [24-27]. Linking the chemical information obtained from non-targeted screening to the toxicity of compounds is vital for risk assessment. This linkage is essential for identifying and evaluating potentially harmful substances that may not be regulated, thereby enhancing our ability to protect public health and the environment [28]. The Toxicological Prioritization Index (Toxpi) framework was designed to integrate multiple sources of information about exposure, compound properties, health and environmental risk, enabling a comprehensive prioritization of chemicals based on their hazards to facilitate informed decision-making [29-31]. It was frequently utilized for risk assessment of multiple emerging pollutants in food, biological samples and environmental media [12,32-35].

Yangtze River Delta is located in downstream of the Yangtze River, near its estuary. The local drinking water mainly relies on the surface water of the Yangtze River, which gather various pollutants from the upper and middle reaches of the Yangtze River and are vulnerable to further contamination [36]. With the rapid urbanization and industrial development along the region of Yangtze River, large amounts of industrial wastewater, domestic sewage, and agricultural runoff flow into the Yangtze River and its tributaries, resulting in a gradual deterioration of water quality [37]. Therefore, the safety of drinking water in Yangtze River Delta has become particularly crucial. As a major economic center in China, the drinking water safety in Yangtze River Delta directly affects the health of more than 20 million residents [24]. Ensuring the purity and safety of drinking water is not only an important measure to protect public health but also a key to maintaining regional economic stability and development.

In this study, a total of 49 drinking water samples were collected from Yangtze River Delta. The aims of the study were to (1) comprehensively investigate the occurrence and concentrations of legacy and novel PFASs in drinking water samples through the combination of target and suspect analyses; (2) identify the potential sources of PFAS in drinking water of Yangtze River Delta by analyzing the contamination profiles of PFAS in drinking water, surface water adjacent to potential industrial emission sources in the local area, the Taihu Basin, and the Yangtze River; (3) prioritize and risk assessment of identified PFASs according to their potential hazard effects and environmental exposure. The findings of our study would provide valuable insights into the prevalence of PFASs in drinking water from Yangtze River Delta and the associated exposure risks among the general public.

## Materials and Methods

### Chemicals and Standards

Authentic standards of 68 target PFASs along with their 13 corresponding internal standards were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada) for target analysis, and detailed information of these PFASs is provided in the Supporting Information (Table S1). The mixed standard solution and the mixed internal standard solution were prepared separately in methanol at 100 µg/L and stored at -20 °C. The ultrapure water was generated by a Milli Q system (18.2 Ω, TOC < 5 ppm, Merck, New South Wales, Australia) and HPLC grade methanol and acetonitrile were purchased from Sigma-Aldrich (New South Wales, Australia). Besides, all the other reagents (e.g. ammonium hydroxide, ammonium acetate) were purchased with HPLC grade from reliable suppliers.

### Samples Collections and Pretreatment

Samples were collected in August 2023. A total of 49 water samples were obtained from Yangtze River Delta, including 5 source water samples, 24 treated water samples, and 20 tap water samples. High-density polyethylene (HDPE) bottles (1L) and their caps were pre-rinsed using methanol (MeOH) and HPLC-grade water, then dried before use. Tap water samples were collected using the following procedure: operators wore laboratory nitrile gloves during sample collection, the tap water was allowed to flow for approximately 3 minutes, and the HDPE bottles were rinsed three times with tap water from the site before being filled. The collected water samples were stored at 4°C and extracted within 48 hours using the routine solid-phase extraction (SPE) method.

Before extraction, 100ml water samples were added 0.925g ammonium acetate (adjust PH to 6.8-7) and spiked with the internal standard solutions (20 µL of 100 µg/L each) before SPE. The extraction of water samples was performed by Oasis WAX Cartridges (150 mg, 6 mL, Waters). Briefly, the cartridges were preconditioned by 5 ml of 0.1% NH<sub>4</sub>OH in methanol, 5 mL of methanol and 10 mL of Milli-Q water in sequence, then the water samples were passed through the cartridges at a flow rate of approximately 5-10 mL/min. After sample loading, the cartridges were rinsed by 5mL 25mmol/L ammonium acetate solution and 10mL ultra-pure water and then dried under vacuum for about 15min. The WAX cartridges were hereafter eluted by 2mL of methanol and 4 mL of 0.1% NH<sub>4</sub>OH in methanol in succession. The eluents were nearly dried under a gentle stream of nitrogen and re-dissolved with 200ul 60% methanol aqueous solution. After vortex mixing, the sample were centrifuged at a rotating speed of 16000 RCF for 3min. Then, the supernatants were collected into the sample bottle for analysis.

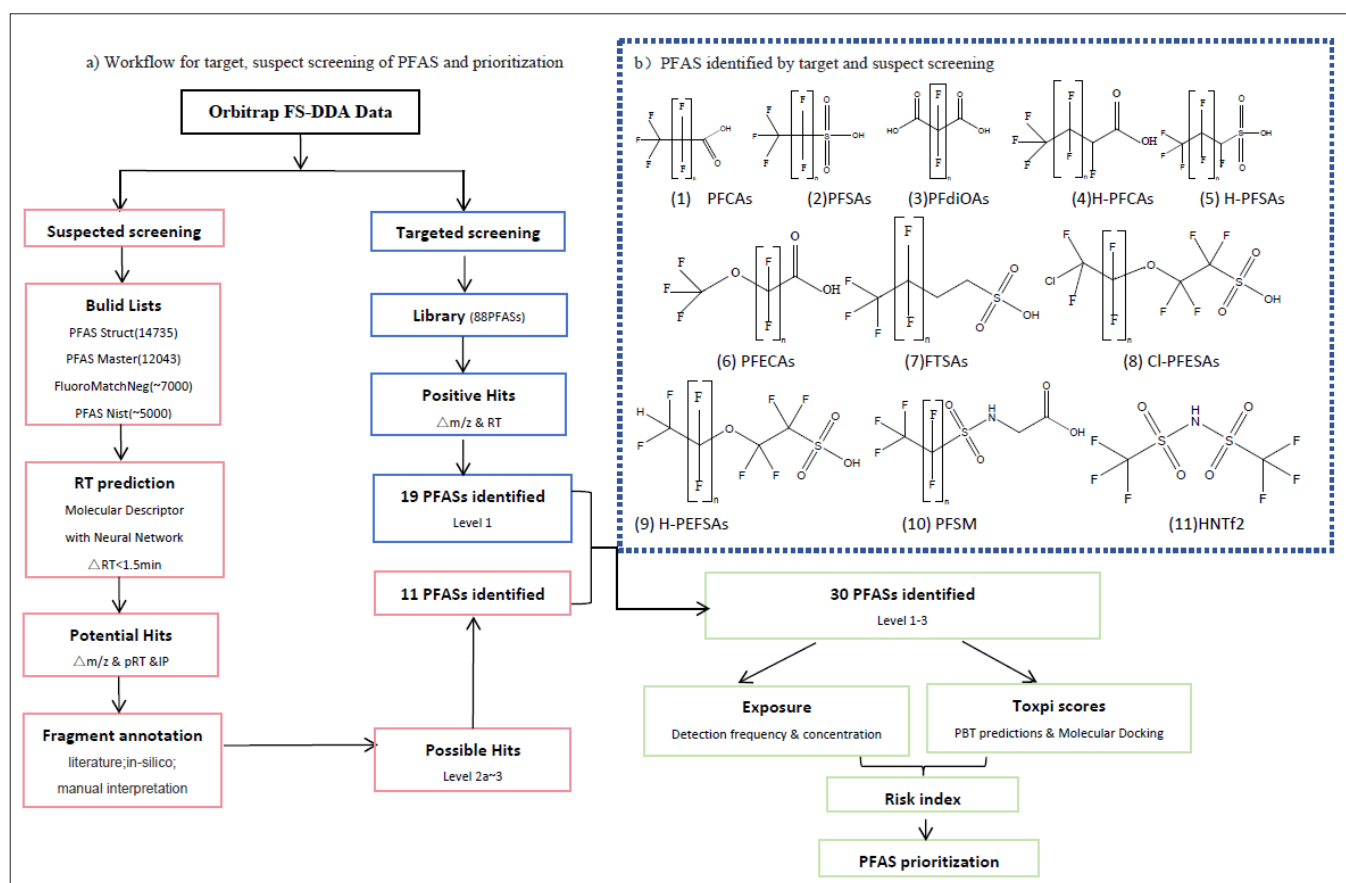
## Instrumental Analysis

Target and suspect screening of the water samples was performed using an Agilent 1290 Infinity II LC system coupled with an Orbitrap Exploris™ 240 Mass Spectrometer (Thermo Scientific, San Jose, CA, USA) with electrospray ionization (ESI) source. Samples (5  $\mu$ L) were injected onto an Agilent Infinity Lab Poroshell 120 EC-C18 analytical column (3.0  $\times$  150 mm  $\times$  2.7  $\mu$ m) with the column oven temperature set at 40  $^{\circ}$ C. Data acquisition was operated in negative ionization mode, utilizing both full scan (150–1500 Da) and data-dependent MS2 (ddMS2) scans to ensure comprehensive feature collection. The detailed parameters of chromatography and mass spectrometry parameters can be found in the Supporting Information (Text S1).

## PFAS Screening Workflow

The suspect screening process (Figure 1) was conducted in accordance with our previous research [13]. An in-house library was established using 68 reference standards. This library was utilized for target screening and the development of a machine learning-based retention time (RT) prediction model. We collected four lists for suspect screening, including PFASSTRUCT v5

(14,735 PFASs, 785 cationic PFASs, as of August 2022) and PFASMASTER (12,043 PFASs, 817 cationic PFASs, as of August 2021) from the US EPA CompTox Chemistry Dashboard, FluoroMatch v3.3 (7,206 PFASs, 493 cationic PFASs, accessed in September 2023), and “Suspect List of Possible PFAS” v1.7 (PFAS-Nist, 4,967 PFASs, 539 cationic PFASs, DOI: 10.18434/mds2-2387, January 2023). These lists were compiled to build a comprehensive database, which was then screened using Compound Discoverer 3.3 (Thermo Scientific, USA). Key criteria for feature filtering in raw data included precise  $m/z$  values ( $<5$  ppm), intensity  $>5$  times the intensity in the extraction blank, IPs (fit threshold  $>70\%$ , allowable intensity deviation  $<30\%$ , and mass deviation  $<5$  ppm), predicted retention time ( $<1.5$  min), and identification of at least one characteristic fragment ion ( $<10$  ppm). For structural confirmation, the formula-assigned features from suspect screening were further annotated by manual interpreting their fragment ions or comparing their MS2 spectra with literature. Positive identification required at least one characteristic fragment ion could be explained. The proposed structures were assigned three confidence levels (CL) based on criteria established in Charbonnet et al.’s study [38].



**Figure 1:** (a) Workflow for Target, Suspect Screening of PFAS and Prioritization and (b) Proposed Structures of PFAS Identified by the Target, Suspect Screening

## Semi-Quantification of Suspect PFASs

In our study, a neural network model was developed to semi-quantify 10 PFASs without authentic standards by predicting their response factors (RFs), which are the slopes of the linear regression lines in the calibration curves. The RF prediction model was constructed using 68 reference standards. A molecular descriptor set containing 3,874 descriptors was generated from the 2D structures (canonical SMILES from PubChem) of these standards using alvaDesc v2.0. The RFs and molecular descriptor set of all 68 standards were randomly divided into training and test sets in an 8:2 ratio for model training. The neural network algorithm, implemented in the Optuna mode (mljar-supervised v1.0, Python package), was employed to automatically tune the machine learning parameters. Over 5,000 models were generated with various parameters, and the model with the best RMSE was selected as the

optimized model. Using this optimized model, RFs for 10 PFASs without authentic standards were then predicted.

### Quality Assurance/Quality Control (QA/QC)

A 100mL volume of ultrapure water, spiked with the same internal standard solutions as the water samples, was used as a procedural blank to evaluate potential contamination during the extraction and analysis processes for each batch. The method LOD and LOQ values for 47 PFASs were detailed in the Supporting Information (Table S2). The calibration curves for each target compound exhibited high correlation coefficients ( $r^2 > 0.99$ ) (Table S2). Additionally, during the instrumental analysis, a standard mixture solution and methanol were sequentially injected following every six samples to act as calibration standards and blanks for data acquisition.

### Molecular Docking

Results from animal experiments, in vitro tests, and epidemiological studies suggested that some PFASs can disrupt the endocrine system, interfering with the secretion of sex hormones and thyroid hormones, and impacting normal reproductive ability, the nervous system, and immune function [39-43]. Receptor mediation is the primary mechanism by which compounds exert endocrine-disrupting activity [39]. Therefore, to assess the endocrine-disrupting effects of PFASs, six human hormone receptors (thyroid hormone receptors alpha (TR $\alpha$ , PDB ID: 3jzb) and beta (TR $\beta$ , PDB ID: 3gws), estrogen receptors alpha (ER $\alpha$ , PDB ID: 1ere) and beta (ER $\beta$ , PDB ID: 5toa), androgen receptor (AR, PDB ID: 3l3x) and peroxisome proliferators-activated receptors (PPAR $\alpha$ , PDB ID: 3vi8)) were selected for the receptor-ligand docking study. Molecular docking simulations were conducted using Biovia Discovery Studio 2021 software. The specific processes were referred to in our previous research [13]. The Libdock scores were utilized to assess the binding affinity between the compound and the active site of the receptor, with higher scores indicating stronger affinity.

### Prioritization and Risk Ranking of Identified PFASs

To prioritize the identified PFASs and pinpoint those of high concern, we utilized a prioritization approach that integrated data from multiple dimensions, including compounds properties, ecological and health hazards, and environmental exposure. This approach was based on the research by Hu et al., with some modifications [12]. There were three steps in our method: (1) Firstly, we used quantitative structure-activity relationship (QSAR) models and molecular docking to predict the PBT properties of PFASs (Table S5, S6). Based on these PBT properties, we calculated the ToxPi score for each PFAS using the ToxPi GUI software (Equation (1)); (2) Secondly, we evaluated the exposure to compounds from two aspects: detection frequency and concentration, as outlined in Equation (2) (3) (4); (3) Ultimately, we calculated the risk index (RI) for each PFAS by combining its ToxPi score with the normalized exposure value through multiplication (Eq. (5)) (Table S7) [30]. To further identify the compounds of high concern, we selected the Maximum Contaminant Level (MCL) standard set by the EPA for PFOS and PFOA, which is 4 ng/L, as a reference dose for risk assessment of the identified PFASs.

$$\text{Toxpi score}_i = W_p P + W_b B + W_t T \quad (1)$$

$$\text{Magnitude} = (C_i^p - C_{\min}^p) / (C_{\max}^p - C_{\min}^p) \quad (2)$$

$$\text{Exposure} = \text{DF} \times \text{Magnitude} \quad (3)$$

$$\text{Exposure}_{\text{normalized}} = (\text{Exposure}_i - \text{Exposure}_{\min}) / (\text{Exposure}_{\max} - \text{Exposure}_{\min}) \quad (4)$$

$$\text{RI} = \text{ToxPi Score} \times \text{Exposure}_{\text{normalized}} \quad (5)$$

### Data Analysis

Targeted and suspect analyses were performed using Trace Finder 5.0 software (Thermo Fisher Scientific, 2021, Waltham, MA, USA) and Compound Discoverer 3.3 software (Thermo Fisher Scientific, 2021, Waltham, MA, USA), respectively. The principal component analysis was conducted using SIMCA 17 software (Sartorius Stedim Data Analytics 2021, Umeå, Sweden).

### Results and Discussion

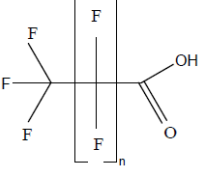
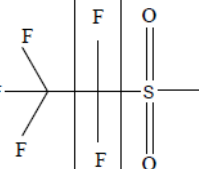
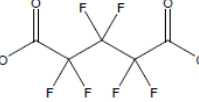
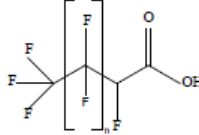
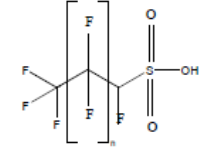
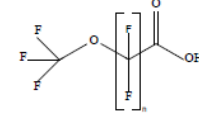
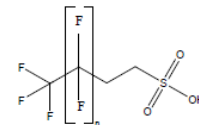
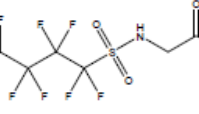
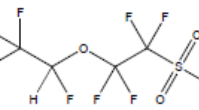
**Concentrations and Compositions of PFASs in Drinking Water**  
After preprocessing the raw data, we generated 414,397 peaks. Using suspect screening with blank subtraction and a retention time versus m/z filter, we identified 475 possible positive hits. Following the removal of duplicates and poorly shaped peaks, 88 peaks were selected for MS/MS spectra annotation. Through further structural elucidation based on diagnostic fragments, we ultimately identified 30 PFAS in the drinking water from Shanghai. Among these 30 PFAS, 12 legacy PFASs and 7 novel PFASs were confirmed using authentic standards.

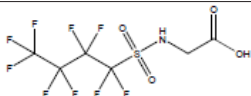
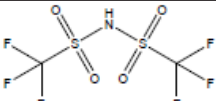
These 30 PFASs could be divided into 11 categories: (1) perfluoro carboxylic acid (PFCAs), (2) perfluoro sulfonic acid (PFASs), (3) perfluoroalkyl dioic acids (PFdOAs), (4) hydrogenated PFCAs (H-PFCAs), (5) hydrogenated PFASs (H-PFASs), (6) polyfluoroalkyl ether carboxylic acid (PFECAs), (7) fluorotelomer sulfonic acids (FTSAs), (8) chlorinated polyfluoroalkyl ether sulfonates (Cl-PFESAs), (9) hydrogenated polyfluoroalkyl ether sulfonates (H-PFESAs), (10) perfluoroalkyl sulfonamide (PFSMs), (11) HNTf $_2$ .

**PFCAs and PFASs:** By employing both targeted and suspect screening approaches, a total of 10 PFCA homologues (C3-C12) and 6 PFSA homologues (C3-C8) were identified. 14 of them were confirmed by authentic standards based on exact mass, retention time, and MS/MS spectra (level 1a). PFPrA and PFPrS were assigned to level 2b. In the MS/MS spectra of PFCAs, the neutral loss of CO $_2$  (m/z 43.98983) and the fragments [C $_n$ F $_{2n+1}$ ] $^-$  were commonly detected. The structures of PFASs were determined using characteristic fragments such as m/z 79.95736 [SO $_3$ ] $^-$ , m/z 82.96085 [SO $_2$ F] $^-$ , m/z 98.95577 [SO $_3$ F] $^-$ , and [C $_n$ F $_{2n+1}$ ] $^-$ . Among all the legacy PFASs identified, the maximum concentrations, in descending order, are as follows:

PFHxA (48.92 ng/L) > PFBA (44.83 ng/L) > PFOA (37.22 ng/L) > PFBS (26.77 ng/L) > PFPeA (8.75 ng/L) > PFHpA (8.42 ng/L) > PFNA (7.59 ng/L) > PFPrA (6.45 ng/L) > PFOS (6.10 ng/L) > PFPrS (5.32 ng/L) > PFDeA (4.40 ng/L) > PFHxS (3.73 ng/L) > PFUdA (2.25 ng/L) > PFPeS (0.16 ng/L) > PFHpS (0.14 ng/L) > PFDoA (0.09 ng/L) (Table 1). PFHxA and PFBS exhibited the highest detection frequencies and were detected in all water samples. The concentrations of PFOA and PFOS in drinking water of Shanghai remained lower than current Chinese Standards for drinking water (PFOA: 80 ng/L, PFOS: 40 ng/L), but above the MCL set by U.S. EPA (PFOA: 4 ng/L, PFOS: 4 ng/L) [22].

**Table 1: Structure of PFAS Identified at Level 3 or Above**

Category	Structure/Proposed Structure	Acronym	N	CL	DF	Max (ng/L)
PFCAs		PFPPrA	n 3	2b	63.27%	6.45
		PFBA	4	1	95.92%	44.83
		PFPeA	5	1	87.76%	8.75
		PFHxA	6	1	100.00%	48.92
		PFHpA	7	1	91.84%	8.42
		PFOA	8	1	97.96%	37.22
		PFNA	9	1	89.80%	7.59
		PFDeA	10	1	79.59%	4.40
		PFUdA	11	1	57.14%	2.25
		PFDoA	12	1	51.02%	0.09
		PFSAs		PFPPrS	n 3	2b
PFBS	4			1	100.00%	26.77
PFPeS	5			1	79.59%	0.16
PFHxS	6			1	91.84%	3.73
PFHpS	7			1	59.18%	0.14
PFOS	8			1	67.35%	6.10
PFdiOAs		PFGdiA	n 5	1	46.94%	1.51
H-PFCAs		HPFLCA_i n=4	n 4	3a	87.76%	0.03
		HPFLCA_i n=5	5	3a	77.55%	0.01
		HPFLCA_i n=6	6	3a	18.37%	0.00
H-PFSAs		HPFLSA_i n=4	n 4	2a	87.76%	7.42
PFECAs		PFMOAA	n 3	2a	10.20%	0.43
		PFMPA	4	2a	4.08%	0.81
		HFPO-DA	6	1	79.59%	3.20
FTSAs		4:2 FTSA	n 6	1	20.41%	0.75
		6:2 FTSA	8	1	83.67%	8.17
Cl-PFESAs		6:2 Cl-PFESA	n 8	1	10.20%	0.18
		HPFESA_i n=4	n 4	2a	12.24%	0.24

	PFSM-FSAA	n 6	2b	24.49%	5.11
	HNTf2	n 2	2b	87.76%	15.02

**CL:** Indicates the Confidence Level; **Max:** the Maximum Concentration; **DF:** Detection Frequency

**PFdiOAs:** One PFdiOA homologues (C5) were identified through suspect screening. In the MS/MS spectrum of this homologous series, a neutral loss of 108 Da, equivalent to the loss of  $(\text{CO}_2)_2\text{HF}$ , was observed. Additionally, fragments  $[\text{C}_3\text{F}_5]^-$  was detected in the spectrum (Fig S12). PFGdiA was detected in nearly half of the samples (DF=0.46), and its maximum concentration was semi-quantitatively determined to be 1.5 ng/L. PFdiOA homologues (C9- C15) were found in airborne particulate matter and serum samples in China [14,15]. At present, PFdiOA has not been reported in drinking water. Currently, specific studies on the toxicity of PFdiOA are relatively scarce, and more research is needed to determine its effects on health and the environment.

**H-PFCAs and H-PFSAs:** We tentatively identified 3 H-PFCAs (C4-C6) and 1H-PFSAs (C4) through suspect screening. The structures of H-PFCAs were identified using the predominant fragments  $[\text{M}-\text{CO}_2-\text{HF}]^-$ , which indicate neutral losses of  $\text{CO}_2$  and HF. The identification of H-PFSAs structures was based on the characteristic fragments of  $m/z$  79.95736  $[\text{SO}_3]^-$ ,  $m/z$  82.96085  $[\text{SO}_2\text{F}]^-$ ,  $m/z$  98.95577  $[\text{SO}_3\text{F}]^-$ , as well as fragments  $[\text{C}_n\text{F}_{2n-1}]^-$  (Fig S2,S3,S5,S6).

Among the four compounds, HPFLSA<sub>i</sub> n=4 had the highest detection frequency and concentration (DF=0.87, max=7.42ng/L), followed by HPFLCA<sub>in</sub>=4 (DF=0.87, max=0.03ng/L), HPFLCA<sub>i</sub> n=5 (DF=0.77, max=0.01ng/L), HPFLCA<sub>i</sub> n=6 (DF=0.20, max=0.01ng/L). At present, H-PFCAs and H-PFSAs have been detected in various environmental media, including land landfill leachate, rainwater, surface water and airborne particulate matter [10,13,14,44].

**PFECAs:** A total of 3 PFECAs (C3,4,6) were identified in our study. HFPO-DA were confirmed by authentic standards (level 1a), and other 2 PFECAs were identified as level 2a. For this homologue series, neutral loss of  $\text{CO}_2$  ( $m/z$  43.98983) and fragments  $[\text{C}_n\text{F}_{2n+1}\text{O}]^-$  were observed in the MS/MS spectrum (Fig S1). For these compounds, HFPO-DA had the highest detection frequency and concentration (DF=0.79, max=3.20ng/L), followed by PFMOAA (DF=0.10, max=0.43ng/L), PFMPA (DF=0.04, max=0.81ng/L). HFPO-DA has been extensively utilized as a replacement for PFOA in various applications, including as processing aid in fluoropolymer production and precursor for the synthesis of other fluorochemical compounds [45,46]. A study compared the developmental toxicity of HFPO-DA and PFOA found that they had different effects on zebrafish embryonic development, indicating distinct modes of action. Additionally, HFPO-DA exposure specifically affected lipid metabolism, the HPT axis, and neurodevelopment, suggested that HFPO-DA may not be a safe alternative to PFOA [45].

**FTSAs:** Two FTSAs, 6:2 FTSA and 4:2 FTSA, were identified through target screening. The characteristic fragments of this homologue series were  $m/z$  79.95736  $[\text{SO}_3]^-$ ,  $m/z$  82.96085  $[\text{SO}_2\text{F}]^-$  and  $[\text{M}-\text{HF}]^-$  were observed in the MS/MS spectrum (Fig S8). 6:2

FTSA was detected in approximately 80% of the water samples, with the highest concentration reaching 8.17ng/L. 4:2 FTSA had a detection frequency of 0.2 and a maximum concentration of 0.75 ng/L. 6:2 FTSA, as a replacement for PFOS, has been found to cause immunotoxicity in zebrafish embryos and hepatotoxicity in male mice according to studies [47,48]. These findings highlight the need for further investigation into the safety of 6:2 FTSA.

**Cl-PFESAs:** We identified one Cl-PFESAs (C8) by authentic standards in water samples. Structures of 6:2 Cl-PFESA was confirmed by fragments  $m/z$  79.95736  $[\text{SO}_3]^-$ ,  $m/z$  82.96085  $[\text{SO}_2\text{F}]^-$  and  $[\text{C}_6\text{ClF}_{12}\text{O}]^-$  (Fig S10). 6:2 Cl-PFESA was detected in 5 water samples (DF=0.1), with the highest concentration reaching 0.18 ng/L. 6:2 Cl-PFESA, as a substitute for PFOS, is widely used in multiple fields as a common fluorinated surfactant. Previous toxicological studies have indicated that 6:2 Cl-PFESA exhibits hepatotoxicity and endocrine toxicity, and its toxicity may be comparable to or even greater than that of PFOS [16,17,49].

**H-PFESAs:** One H-PFESA (C4) was confirmed by suspect screening and assigned to level 2a. The characteristic fragments of 2:2 PFESA were  $m/z$  79.95736  $[\text{SO}_3]^-$ ,  $m/z$  82.96085  $[\text{SO}_2\text{F}]^-$   $[\text{C}_2\text{F}_3\text{O}]^-$   $[\text{C}_2\text{HF}_4\text{O}]^-$  (Fig S13). HPFE<sub>A</sub><sub>i</sub> n=4 had a detection frequency of 0.12 and a maximum concentration of 0.75 ng/L.

**PFMSs:** One PFMS (C6) was identified by suspect screening. 4 characteristic fragments, including  $m/z$  79.95736  $[\text{SO}_3]^-$ ,  $m/z$  82.96085  $[\text{SO}_2\text{F}]^-$ ,  $[\text{C}_4\text{H}_9]^-$ ,  $[\text{C}_4\text{HF}_9\text{NO}_2\text{S}]^-$ , were observed in the MS/MS spectrum of FBSAA and assigned to level 2b (Fig S7). PFMSs have many derivatives. Its derivatives and homologues were previously detected in wastewater, landfill leachate, and surface water [12,13,50]. FBSAA was detected in approximately 24% of the water samples, with the highest concentration reaching 5.11ng/L.

**HNTf2:** The HNTf2 was identified by suspect screening and assigned to level 2b. Structures of HNTf2 were confirmed by 3 fragments  $m/z$  79.95736  $[\text{SO}_3]^-$ ,  $m/z$  82.96085  $[\text{SO}_2\text{F}]^-$  and  $[\text{CF}_3\text{SO}_2\text{N}]^-$  (Fig S4). It was detected in surface water in Beijing, drinking water and landfill leachate in Shanghai [12,13,24]. This compound was widely used as reagents, efficient catalysts or additives in numerous reactions and was categorized as a dangerous chemical of Category 3 due to relatively notable acute toxicity in administered rats [12,51].

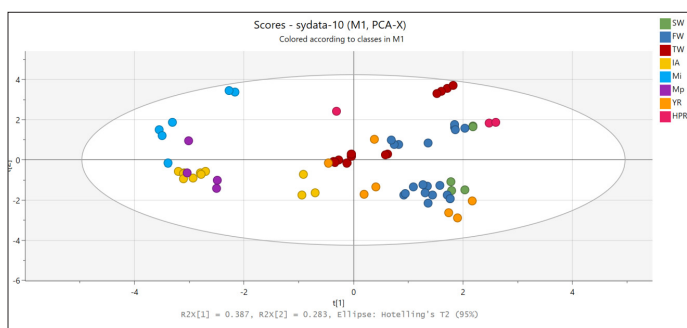
HNTf2 had the highest concentration among all emerging PFAS, with a detection frequency of 0.87 and a maximum concentration of 15.02 ng/L. However, there is a lack of comprehensive studies on their toxicity.

In our study, there are three limitations: (1) we only used Oasis WAX cartridge to extract PFAS in water samples, and some PFASs not retained by Oasis WAX cartridge could not be analysed. (2) For mass spectrometry analysis, we only selected the ESI(-)

ionization mode, which may result in our results only including anionic PFAS and zwitterionic PFAS. (3) during the pre-treatment process, we found that some PFAS with 14-16 carbon atoms had low recovery rates (<10%), likely due to significant adsorption on the tube walls caused by their low polarity, which could affect the detection of certain long-chain PFAS.

### Contamination Profiles Analysis

PFASs in drink water could originate from different pathways. To further identify the potential sources of PFAS contamination in drinking water from Yangtze River Delta, we collected data on the concentrations and types of PFASs in the Yangtze River, the Huangpu River, and surface waters adjacent to potential industrial emissions around Yangtze River Delta to conduct profile analysis[36, 37]. The PCA results indicated that the profiles of our drinking water samples were completely separated from that of the surface water near Shanghai airport, fluorochemical plants, and metal plating factories, suggesting significant differences in profiles. In contrast, the profiles of our drinking water samples were overlapping with that of the surface water from the Yangtze River and the Huangpu River (Figure 2). This suggested that the main source of PFASs contamination in drinking water from Yangtze River Delta is more likely to be from upstream discharges into the Yangtze River rather than local industrial emissions.



**Figure 2:** PCA Analysis of PFAS Profiles in Surface Water from Different Sources

SW: source water; FW: factory water; TW: tap water samples; IA: international airport; Mi: industrial fluorochemical plant, Mp: metal plating plant; YR Yangtze River; HPR: Huangpu River;

Among all the 30 identified PFASs, two novel PFAS, 6:2FTSA and HNTf2, had been reported in landfill leachate of Shanghai [13]. Notably, the concentration of 6:2FTSA reached as high as 69.2 ng/ml, suggesting that municipal waste management might be another significant source of novel PFAS in drinking water from Yangtze River Delta.

### Prioritization and Risk Assessment of Identified PFASs

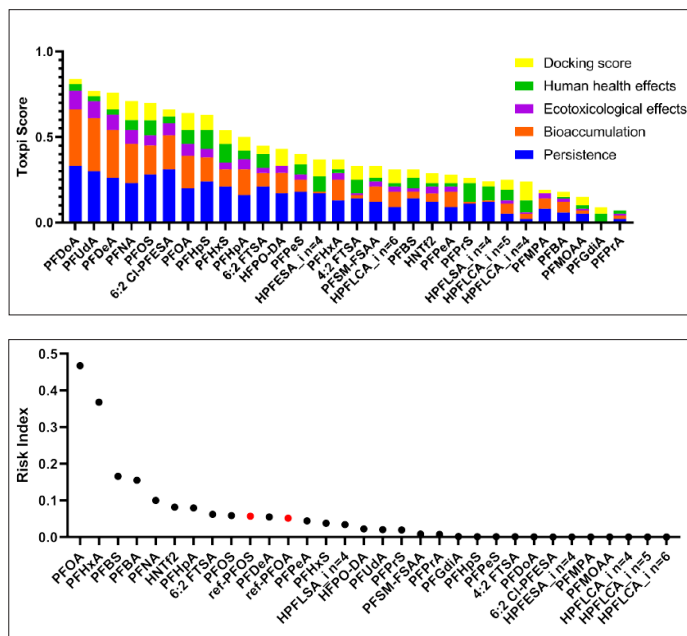
To prioritize the identified PFASs and pinpoint those of high concern, we utilized a risk-based prioritization approach based on the research by Hu et al, with some modifications. Multiple hazard attributes were incorporated in our model, including persistence, bioaccumulation, ecotoxicological effects, human health effects, molecular docking scores, detection frequencies and concentrations [12]. By integrating these multi-dimensional data, our model provided a comprehensive assessment of the potential environmental and health risks associated with the compounds, helping to prioritize those requiring immediate attention and regulatory action. A total of based on PBT properties and molecular docking scores. The ToxPi scores of the 30 identified PFASs ranged from 0.09 to 0.85(Figure 3a), with PFDoA having the

highest score, followed by PFUdA, PFDeA, and PFNA, all of which have higher scores than the traditional PFOA and PFOS. Generally, PFASs with longer carbon chains tend to have higher ToxPi scores. Among all the emerging PFASs, 6:2 Cl-PFESA has the highest ToxPi score (0.66), falling between the scores of PFOS (0.70) and PFOA (0.63). 6:2 Cl-PFESA, a proposed safe alternative for PFOS, may cause liver damage and induce lipid metabolism disorders in female mice through the action of PPAR- $\gamma$ . Previous studies documented that the hepatotoxicity of PFOS and 6:2 Cl-PFESA appears to be higher than that of PFOA. Previous studies documented that the hepatotoxicity of PFOS and 6:2 Cl-PFESA were higher than PFOA [52,53]. This indicated that we need to be mindful of the potential health and environmental hazards posed by these substitutes.

For persistence, the top 10 PFASs with the highest scores were PFDoA, 6:2 Cl-PFESA, PFUdA, PFOS, PFDeA, PFHpS, PFNA, 6:2 FTSA, PFHxS, PFOA (Table S7). Higher persistence scores indicated that these compounds were less biodegradable in the environment and might pose long-term risks to environmental and human health. There were a total of eight PFASs with persistence scores higher than PFOS and PFOA. Notably, the two substitutes for PFOS, 6:2 FTSA and 6:2 Cl-PFESA, had high persistence scores. Among them, 6:2 Cl-PFESA ranked second in persistence scores, even surpassing its predecessor, PFOS. For bioaccumulation, we selected logKow and BAF as the key attributes to evaluate the accumulation potential and associated risks of compounds within organisms. Among all the identified PFASs, the ones with the higher bioaccumulation potential were PFDoA, PFUdA, PFDeA, PFNA, and 6:2 Cl-PFESA. These compounds exhibited higher bioaccumulation than both PFOA and PFOS (Table S7). In terms of ecotoxicity, the PFASs with the higher toxicity scores were also PFDoA, PFUdA, PFDeA, PFNA, and 6:2 Cl-PFESA. We evaluated the human health effects of the compounds from five aspects: carcinogenicity, developmental toxicity, mutagenicity, skin sensitization, oral LD50 in rats and endocrine toxicity. The PFASs with the highest comprehensive score, in order, were PFHxS, PFPrS, PFHpS, HPFESA\_i n=4, PFOS, 4:2 FTSA, HPFLSA\_i n=4, PFOA(Table S7). We found 11 PFASs with possible carcinogenicity, 2 PFASs with potential developmental toxicity and 5 PFASs with potential skin sensitization (Table S6). Based on the molecular docking scores with six receptors, the compounds with the highest total scores were PFNA, HPFLCA\_i n=4, HFPO-DA, PFDeA, HPFESA\_i n=4, PFOS, and PFOA. The results indicated that some emerging PFASs exhibited higher affinity to receptors than PFOA and PFOS (Table S7). Additionally, we found that some long-chain PFASs, such as PFDoA, PFUdA, and PFDeA, had lower docking scores compared to PFNA, likely due to limitations imposed by their molecular size.

Based on the measured detection frequency and concentrations, we utilized toxpi scores to calculate the RIs, which represented a synthesized assessment for prioritizing chemicals based on potential health risks. The RIs ranged from 0-0.54 for 30 PFASs in water samples (Figure 3b). PFOA had the highest RI, followed by PFHxA, PFBS and PFBA. We found that some long-chain PFAS, such as PFDoA(Toxpi scores=0.85, DF=0.51,max=0.09 ng/L), PFUdA(Toxpi scores=0.77, DF=0.57,max=2.25 ng/L), exhibited higher ToxPi scores and posed significant environmental and health hazards, their RIs were not high when considering both detection frequencies and concentrations. Conversely, some short-chain PFAS, such as PFBA (Toxpi scores=0.17, DF=0.96, max=44.83ng/L) and PFBS (Toxpi scores=0.33, DF=1, max=26.77ng/L), despite had lower ToxPi scores, showed

higher health risks due to their higher detection frequencies and concentrations (Table S7). To further identify the compounds of high concern, we selected the Maximum Contaminant Level (MCL) standard set by the EPA for PFOS and PFOA, which is 4 ng/L, as a reference dose for risk assessment of the identified PFASs. A total of 10 high-risk PFASs were identified through our method, including 8 legacy PFASs and 2 emerging PFASs. Of these, PFOA had the highest RI (RI=0.46), indicating it poses the highest risk. PFOA was closely followed by PFHxA, PFBS and PFBA, suggesting that we need to be aware of the health risks posed by these short-chain PFAS. HNTf2 and 6:2 FTSA were the two emerging PFASs with the high risk. In the future, more researches on their toxicity are necessary (Figure 3b).



**Figure 3:** (a) ToxPi scores (b) risk index of 30 PFAS identified and quantified in drinking water from Yangtze River Delta

### Conclusion

In this study, target and suspect screening analysis using UPLC-Orbitrap HRMS was implemented to identify the occurrence and concentrations of legacy PFASs and novel PFASs in drinking water from Yangtze River Delta. A total of 30 PFASs with high confidence levels (>3) were identified through both target and suspect screening, including 16 legacy PFASs and 14 novel PFASs. By utilizing the ToxPi framework and RIs, we evaluated and prioritized the risks of identified PFAS in drinking water by integrating various data. A total of ten high concerns PFASs were identified through our method. Currently, legacy PFASs were the largest contributors to PFASs in drinking water of Yangtze River Delta, accounting for up to 83% of ΣPFASs, while the concentrations of emerging PFAS were relatively low. However, with some countries and organizations implementing regulations to ban the use of certain legacy PFASs, many companies are developing new alternatives of PFASs and producing novel PFASs. This could result in increased concentrations of emerging PFAS in drinking water in the future. Therefore, more researches are essential to comprehensively identify and monitor various emerging PFAS in drinking water and evaluate their potential health and environmental hazards.

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