



Review

Per- and poly-fluoroalkyl substances: A review of sources, properties, chromatographic detection, and toxicological implications

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Abstract: Emerging per and poly-fluoroalkyl substances (PFASs) are very resistant to degradation and have negative impacts on human and environmental health at very low concentrations. The initial stage in removing PFASs from contaminated locations is their detection and quantification. Particularly utilized in this context are Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), and High-Performance Liquid Chromatography (HPLC). In this review, we seek to contribute to our understanding of the state-of-the-art in emerging PFAS by offering a complete analysis of PFAS in environments, taking into consideration their sources, classes, and properties. Polyfluoroalkyl ether substances (PFAES), short-chain PFA, replacement PFA, and fluorotelomer-based substances can bioaccumulate in living species, making their detection even more necessary. We intend to provide researchers with an overview of the current state of research on PFASs in environments, encompassing the toxicological effects and their

detection and quantification methods, serving as a guide for current and future studies.

Keywords: emerging PFAS; chromatography; spectrometry; toxicological impacts; physicochemical properties

1. Introduction

Per- and poly-fluoroalkyl substances (PFASs) are a global concern because of their persistence in the environment and possible health risks associated with exposure [1]. The two most extensively researched perfluoroalkyl acids (PFAAs) are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) [2]. Perfluoroalkyl sulfonates, which are also being researched, include substances like perfluorobutanesulfonate (PFBS) and perfluorohexanesulfonate (PFHxS). Moreover, the environment contains fluorotelomer alcohols (FTOHs), which function as precursors for breaking down into PFAAs [3]. Furthermore, compounds like Teflon, which may produce PFAS during manufacturing or breakdown, are examples of polyfluorinated polymers found in the environment [4]. PFASs persist in the environment owing to the strong carbon-fluorine bonds, which provide this stability. Moreover, because PFAS compounds can lower the surface tension of liquids, they are useful surfactants and crucial components of aqueous film-forming foams [5,6]. The majority of PFASs exhibit both hydrophobic and hydrophilic properties, which enable them to interact with a diverse array of chemicals [7]. Long perfluorocarbon chain PFASs like PFOA and PFOS are particularly hydrophobic, which helps them repel water and shield surfaces from water damage [8].

Owing to distinct physicochemical characteristics, PFAS are used in a wide range of applications and persist in the environment. Their enduring nature is partly attributed to their exceptional resistance to deterioration by chemicals, heat, and microbes [9]. The strong carbon-fluorine bonds, which are among the strongest single bonds in organic chemistry, are what provide this stability. Because PFAS compounds can lower surface tension in liquids, they are useful surfactants and crucial parts of aqueous film-forming foams (AFFFs), which are employed in combating fires [10]. PFAS can behave as repellents and surfactants [9,10]. The majority of PFAS exhibit both hydrophobic and hydrophilic areas, which enable them to interact with a diverse array of chemicals [11]. Because of this characteristic, PFAS can behave as repellents and surfactants. Long perfluorocarbon chains PFAS like PFOA and PFOS are particularly hydrophobic, which helps them repel water and shield surfaces from water damage [12].

Certain PFASs with shorter chains can exhibit lipophilicity [13], which enables them to interact with biological tissues that are rich in lipids. These interactions lead to PFAS bioaccumulation and toxicity in living things. For these characteristics, numerous health concerns, including liver and thyroid disorders, reproductive damage, immune system suppression, and an elevated risk of some malignancies, have been linked in studies to PFAS exposure [14]. Global regulatory bodies have begun enforcing more stringent norms and procedures to lower the use of and contamination from PFAS. Even while PFAS contamination is becoming more well-recognized, there are many things we do not know about these chemicals, especially the newer ones. First, there is little to no toxicological information available on many newly discovered PFAS [9]. Also, the environmental fate and movement of emerging PFASs are frequently not fully captured by studies [10]. Thus, it is unclear how

they degrade, what transformation products they produce, and how they interact with environmental media [11]. The absence of standardized analytical techniques that can identify the diverse array of PFASs found in the environment exacerbates this constraint even further [12,13]. Furthermore, rather than addressing the cumulative effects of PFAS combinations, a large portion of PFAS research concentrates on individual chemicals [14]. Complex PFAS combinations that individuals and ecosystems encounter in real-world situations may have additive or synergistic effects that are not sufficiently captured in single-compound research. Moreover, the rapid creation of novel PFASs has outpaced regulatory systems [15]. There is little to no regulation for most developing PFASs; however, strict limitations have been put in place for some legacy PFAS in soil and water in select areas.

The detection and quantification of PFASs are the first steps toward eliminating PFASs from polluted areas. Particularly utilized in this context are Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), and High-Performance Liquid Chromatography (HPLC). Using LC-MS/MS, Groffen et al. [16] created a quick, accurate, and dependable analytical technique for identifying a wide variety of PFASs in feathers. They discovered that a 24-hour extraction period was adequate to remove the majority of PFASs from the feathers. The internal standards' extraction recovery ranged, on average, from 68 % (PFBA) to 97 % (PFOS) [16]. Furthermore, passive samplers were first placed along the Cape Fear River in North Carolina in the summer and autumn of 2016 and 2017 for the Dodds et al. [17] investigation. Then, in 2022, these samplers were put into service to assess alterations in the PFAS fingerprint and abundances. Assessment of PFASs revealed that while developing replacement PFAS, such as Nafion byproducts, were primarily localized downstream from the factory, legacy compounds, such as PFHxS, PFOS, and PFHxA, were identified throughout practically all sampling locations over three years [17].

Numerous researchers have carefully studied PFASs in the environment. However, to the best of our knowledge, emerging PFAS compounds are less well-studied in terms of their toxicity and potential health impacts compared to traditional PFASs. For this and other reasons, as adding to knowledge on the state-of-the-art emerging PFASs, we provide this review article. We deliver a comprehensive review of PFAS in the environment, considering classifications, sources, and properties. We further discuss the methods for detecting and quantifying the emerging PFASs in the environment. Moreover, this review article is designed to offer researchers an overview of the current state of research on PFASs and provides an overview of PFASs as an emerging contaminant, acting as a guide for current and future studies.

2. Sources, classification, and properties of PFASs in environments

2.1. Sources of PFAS

Compared to traditional PFASs, there is less data and information accessible on the origins of new PFASs in the environment [18]. However, a small number of studies have been conducted that indicate direct emissions from the production of fluorochemicals are primarily responsible for the presence of these developing pollutants [19] and their alternatives in the environment [3]. Industrial processes are the primary sources of PFAS replacements discharged into the environment [18]. Despite this, research on PFAS substitute emissions is in its infancy [18,19]. Figure 1 illustrates the various sources of PFASs in the environment.

PFASs have been employed in several industrial processes, including manufacturing, electroplating, semiconductor manufacture, and firefighting foam composition [20]. Industrial discharges and emissions can leak PFASs into the air, water, and soil, contaminating the ecosystem. PFASs may be found in wastewater from industrial operations, municipal sewage, and urban runoff. Wastewater treatment plants may fail to adequately remove PFASs from effluent, resulting in the release of PFAS-contaminated water into surface waterways, including rivers, lakes, and oceans [21]. Textiles, paper goods, food packaging, and firefighting foams contain PFASs and may wind up in landfills or waste disposal sites [22]. PFASs may seep from landfills into the soil and groundwater, polluting adjacent water supplies and ecosystems.

PFAS contamination in agricultural soils and discharge into surface waterways can be caused by items containing PFASs, such as fertilizers, insecticides, and biosolids [23]. PFASs from municipal sewage treatment plants may be present in biosolids used as fertilizer. PFASs can travel great distances through the atmosphere in the form of gas-phase molecules or airborne particles [24]. PFAS-containing items are often utilized in urban areas, close to industrial sites, and in other areas where atmospheric deposition of PFASs might occur, contaminating soil and water bodies. Firefighting foam, especially aqueous film-forming foams (AFFFs) that include perfluoroalkyl compounds, is a major source of PFAS pollution [25]. PFAS pollution of soil, groundwater, and surface water has been prevalent because of the use of AFFFs in firefighting and fire control operations at military bases, airports, industrial locations, and firefighting training sites [21]. Because PFAS are extremely persistent in the environment and can build up over time, places where PFAS-containing products were previously used or disposed of may become legacy contaminated. There may be long-term hazards to the environment and public health from PFAS contamination found in old landfills, industrial sites, and military locations.

PFAS pollution is a multifaceted, widespread environmental issue with numerous exposure routes and sources. Comprehensive plans to lower emissions, enhance waste management procedures, and clean up polluted sites are needed to mitigate PFAS pollution and safeguard the environment and public health.



Figure 1. Sources of emerging PFAS in the environment.

2.2. Classifications of PFASs

PFAS refers to a large class of artificial compounds that have carbon-fluorine bonds. Based on their characteristics, functional groups, and chemical structure, these substances can be divided into several groups [26]. Emerging PFASs are classified into several types (as shown in Figure 2), including short-chain PFASs, replacement PFASs, polyfluoroalkyl ether substances (PFAESs), fluorotelomer-based substances, and unregulated PFASs. In their chemical structure, short-chain PFASs generally include fewer than six carbon atoms for perfluoroalkyl carboxylic acids (PFCAs) [27] and fewer than four for perfluoroalkyl sulfonic acids (PFSAAs) [28]. Their shorter carbon chains provide them with more mobility, a lesser propensity for bioaccumulation, high environmental persistence, and challenging removal [29]. The newer PFASs and replacement PFASs were created to take the place of long-chain [30] and conventional PFASs like PFOA and PFOS because of their toxicity, persistence, and bioaccumulation issues [31]. These substitutes frequently consist of short-chain PFASs [29] or other modified compounds intended to address some of the health and environmental issues related to previous forms of PFASs while maintaining the desired qualities of PFASs, such as oil and water repellence [30].

In the chemical structure of PFAESs, one or more ether linkages ($-C-O-C-$) [28] are added, setting them apart from other PFASs and potentially influencing their chemical and physical characteristics [32]. Because of their strong carbon-fluorine linkages, PFES are environmentally persistent like other PFAS [33]; however, their breakdown processes may be slightly altered by the ether groups [28,34]. Because of their ability to resist both water and oil, fluorotelomer-based materials are frequently used in both consumer and industrial items [35]. FTOHs [36], which are the building blocks used to create other fluorinated chemicals, are the sources of these [35,36]. A partially fluorinated alkyl chain, usually short to medium in length, is joined to functional groups like alcohols,

acids, or acrylates [37]. These chains can break down in the environment or inside living things to form persistent PFAAs. These compounds are extremely stable and bioaccumulative and they can contaminate soil, water, and air, adding to the larger problem of PFAS pollution [37]. Besides, there are no governmental limitations or regulatory oversight on unregulated PFASs. Although there are hundreds of PFAS chemicals, the majority are left unregulated since most regulatory frameworks concentrate on a limited fraction [38], such as PFOA and PFOS. Newer short-chain PFAS, PFSA, and fluorotelomer-based chemicals are examples of unregulated PFASs, as are lesser-known and developing molecules.

These categories shed light on the diversity and complexity of emerging PFAS compounds, which may exhibit distinct environmental characteristics, toxicity profiles, and regulatory consequences than historical PFASs. Understanding the origins, fate, and possible dangers of new PFASs is crucial for establishing effective ways to control and reduce PFAS pollution in the environment while protecting human health.

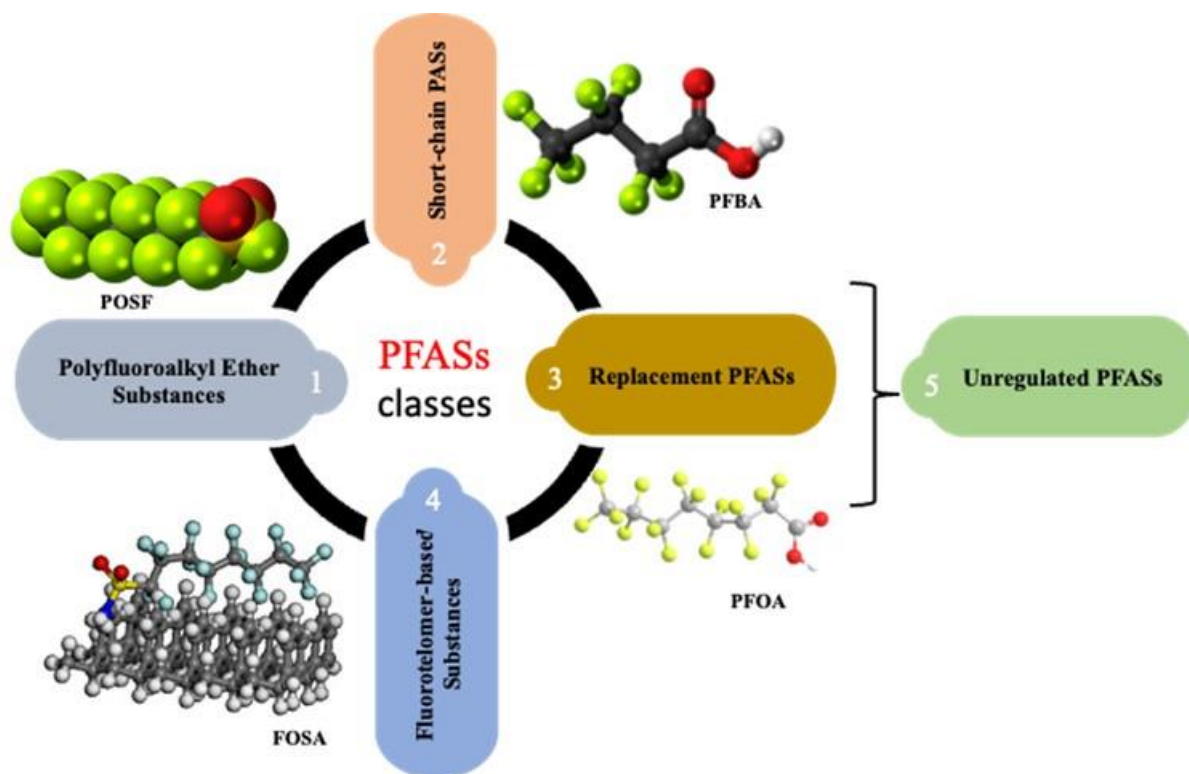


Figure 2. Classes of emerging PFASs.

2.2.1. Short-chain PFAS chemicals

Short-chain PFAS chemicals [39] are a class of newly developed compounds that have a lower carbon content in their molecular structure than heritage compounds like PFOA and PFOS [40]. Because of their simpler molecular structures and reduced reactivity [29], short-chain PFAS are generally more resistant to degradation. They are more challenging to eliminate using traditional treatment methods and frequently last longer in the environment [39,41]. Typically, short-chain PFAS have carbon chains that contain three to six carbon atoms [39], with all hydrogen atoms substituted by

fluorine atoms. Some examples include perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluorobutane sulfonate (PFBS) [42]. They are typically thought to be more prone to degradation in the environment than longer-chain PFASs. They may suffer hydrolysis, photolysis, and microbial degradation, resulting in shorter environmental half-lives and lower persistence in soil, water, and biota [41–43]. Short-chain PFAS compounds may have more mobility and movement in the environment than longer-chain PFASs due to their lower molecular weight and less ability to sorb to soil or sediment particles. This can lead to a broader spread and distribution of short-chain PFAS contamination in groundwater and surface water systems [44]. Furthermore, chemicals have a lesser bioaccumulation potential than longer-chain PFASs due to their shorter carbon chains and rapid removal from organisms. However, certain short-chain PFASs may continue to bioaccumulate in some creatures, particularly aquatic species [45].

While some short-chain PFASs have been shown in laboratory experiments to have comparable toxicological effects as longer-chain PFASs, further study is needed to completely grasp their health consequences. Short-chain PFAS chemicals have received increased attention from regulatory bodies due to their environmental persistence and possible health hazards. Some short-chain PFASs, such as PFBA and PFBS, are subject to regulatory rules and limits, while others are being assessed. Short-chain PFAS compounds have been created to replace legacy PFAS compounds in specific applications, notably in industries where there is rising concern about the environmental and health effects of longer-chain PFASs. However, the usage of short-chain PFASs raises concerns about their possible environmental impact and toxicity. In general, short-chain PFASs are a varied class of chemicals with distinct characteristics and environmental behaviors. Understanding their destiny, transit, and possible dangers is critical for efficiently controlling and reducing PFAS pollution in the environment while safeguarding human health.

2.2.2 Replacement PFASs

Replacement PFASs are a class of PFAS compounds that have been introduced as replacements to legacy PFAS compounds, including PFOA and PFOS. These alternative PFAS were created in response to regulatory constraints, environmental concerns, and public pressure to phase out the usage of longer-chain PFAS chemicals [26]. Replacement PFAS compounds may have chemical structures comparable to legacy PFASs [46], with perfluorinated carbon chains containing functional groups such as carboxylate (COO⁻), sulfonate (SO₃⁻), or phosphate (PO₄⁻) [47]. Examples include PFBS, PFHxS, PFBA, and PFHxA [44]. The development and usage of replacement PFAS have been prompted by regulatory activities targeted at phasing out longer-chain PFAS chemicals due to their persistence, bioaccumulation potential, and negative health impacts [48]. Regulatory bodies, such as the United States Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA) [49], have imposed limitations and recommendations on certain PFAS chemicals, prompting the hunt for alternatives [2].

Replacement PFAS compounds are intended to have similar functional features as legacy PFAS, including water and oil repellence, heat resistance, and chemical stability [2]. This enables them to be utilized as alternatives in a variety of industrial processes and consumer items while maintaining performance. Replacement PFAS compounds may have a different environmental outcome and behavior than legacy PFAS due to differences in chemical structure, chain length, and functional

groups [50]. Some substitute PFAS compounds, such as PFBS and PFHxA, are thought to have shorter environmental half-lives and lower bioaccumulation potential than longer-chain PFAS [51]. Although replacement PFAS chemicals may have some advantages in terms of lower environmental persistence and bioaccumulation [52], there are growing worries regarding their environmental destiny, toxicity, and potential health consequences [53]. Some substitute PFASs, such as PFBS and GenX compounds [50], have been discovered in environmental samples and are being studied and monitored.

The regulatory status of substitute PFAS chemicals varies by country and substance. Some replacement PFASs, such as PFBS and PFHxS, have been included in regulatory recommendations and limits, while others are being assessed for potential environmental and health hazards. Replacement PFAS chemicals are being studied to determine their environmental behavior, toxicity, and possible dangers. This involves research into their prevalence in the environment, fate and transport processes, impacts on ecosystems and human health, and the development of analytical tools for detection and monitoring. Ultimately, replacement PFAS compounds are a complex and developing collection of chemicals that must be thoroughly evaluated to guarantee environmental sustainability and minimize possible negative effects on human health and ecosystems. Regulatory steps, research initiatives, and industry collaboration are required to address developing concerns and discover safer replacements for legacy PFAS chemicals.

2.2.3 Polyfluoroalkyl Ether Substances (PFAESs)

PFAES compounds have perfluorinated carbon chains with one or more ether linkages (-O-) dispersed throughout the molecule [54]. These ether connections improve the chemical stability, lubricity [55], and surfactant characteristics of PFAES compounds [54]. PFAES containing ether linkages are widely employed in some industrial and consumer applications due to their distinct functional characteristics [56]. These include lubricating qualities in automobile and industrial uses [56], surfactant properties in cleaning products and firefighting foams, and anti-icing capabilities in aviation deicing fluids [55]. PFAESs containing ether linkages are widely employed in a variety of industries. Products containing them include lubricants, hydraulic fluids, metal plating agents, surfactants, coatings, adhesives, sealants, textiles, and personal care products [57]. PFAESs containing ether linkages, like other PFAES compounds, are environmentally persistent [58] and resistant to degradation [59]. They can accumulate in the environment over time, raising worries about the possible effects on ecosystems and human health [60]. The usage of PFAES compounds, notably those with ether linkages, has sparked regulatory concerns due to their persistence, bioaccumulative capacity, and possible harm to human health and the environment [61]. Regulatory bodies throughout the world are increasingly studying PFAESs and imposing limits on their usage and emissions [62].

Exposure to PFAES compounds, especially those containing ether linkages, has been linked to a variety of health consequences in people and animals [63]. These can include developmental and reproductive harm, liver damage, immune system impacts, thyroid disturbance, and some forms of cancer [56]. PFAES compounds, notably those with ether linkages, are classified as emerging pollutants of concern due to their extensive occurrence in the environment and potential harm to human health and ecosystems [61]. Concerns about PFAES contamination are being addressed by continual efforts to monitor, analyze, and reduce it. Thus, PFAES compounds with ether linkages are a diversified collection of molecules with significant industrial applications and possible environmental and health

consequences. Understanding their characteristics, uses, and effects is critical for properly controlling and mitigating PFAES pollution and lowering human exposure to these compounds.

2.2.4 Fluorotelomer-based Substances

Fluorotelomer-based compounds are a kind of PFAS that result from telomerization procedures using fluorotelomer alcohols [36]. These compounds have fluorinated carbon chains with terminal functional groups like COO⁻, SO₃⁻, or PO₄⁻ [64]. Fluorotelomer-based compounds are employed in a variety of industrial and consumer applications due to their distinct features, which include water and oil repellency, chemical stability, and surfactant capabilities [64,65]. Fluorotelomer-based compounds are created by telomerization, a chemical process that involves the polymerization of fluorotelomer alcohols to produce oligomers or polymers [66]. Telomerization can provide fluorotelomer-based compounds with a variety of chain lengths and functional groups [67]. Fluorotelomer-based compounds are generally composed of perfluorinated carbon chains that have one or more functional groups connected to the terminal end. Common functional groups include (COO⁻), SO₃⁻, PO₄⁻, and hydroxyl (OH) [68]. These functional groups provide fluorotelomer-based compounds with unique features, such as surfactant qualities or hydrophobicity. Fluorotelomer-based compounds are employed in a variety of industrial and consumer applications due to their versatility [69]. They are frequently used in water and stain repellents for textiles, paper coatings, food packaging, firefighting foams, lubricants, and personal care products. Fluorotelomer-based compounds can degrade in the environment, producing PFCAs or PFSA such as PFOA and PFOS [70]. These products are frequently more stable and bioaccumulative than their precursor chemicals. Fluorotelomer-based compounds and their products are gaining significant attention due to their persistence [71], bioaccumulation potential, and possible toxicity to human health and the environment [69]. Regulatory bodies across the world have imposed limitations and recommendations for fluorotelomer-based compounds.

Research efforts are aimed at better understanding the environmental fate, transport, and toxicity of fluorotelomer-based compounds and their breakdown products. Monitoring programs are also used to determine the presence and distribution of these chemicals in a variety of environmental media, including water, soil, sediment, and biota. Risk management solutions for fluorotelomer-based chemicals seek to reduce human exposure and environmental pollution. These tactics may include product replacement, pollution prevention measures, waste management techniques, and legislative limits on production, use, and disposal. In general, fluorotelomer-based chemicals are an important family of PFAS compounds with numerous uses and environmental consequences. To limit the dangers associated with these substances, scientists, regulators, industry stakeholders, and politicians must work together across disciplines.

2.2.5 Unregulated PFASs

Unregulated PFASs are PFAS chemicals that have not been subjected to regulatory rules or limits in many jurisdictions. These substances might include newly discovered PFAS variations, precursor chemicals, or breakdown products that have not been thoroughly investigated or controlled [72]. Unregulated PFAS are frequently detected as a result of advances in analytical techniques and studies on PFAS contamination. These chemicals may have gone undetected or unquantified due to limitations

in analytical techniques or a lack of awareness of their presence [73]. Unregulated PFAS have a diverse range of chemical structures and characteristics, including different chain lengths, functional groups, and substitution patterns. Some uncontrolled PFASs may have a resemblance to heritage PFAS chemicals, but others may be new chemical entities with distinct features.

Unregulated PFASs are classified as emerging pollutants of concern because of their probable prevalence in the environment, persistence, and dangers to human health and ecosystems [68]. As testing tools improve and scientific understanding develops, more PFAS compounds are discovered, adding to the list of possible pollutants. They can come from a wide range of sources, including industrial discharges, wastewater treatment facilities, landfill leachate, air deposition, and consumer items [74]. Some may also arise as breakdown products or transformation intermediates from other PFAS chemicals. The environmental destiny and behavior of uncontrolled PFASs are determined by characteristics such as chemical structure, hydrophobicity, and reactivity, while others may survive in the environment and travel vast distances via air, water, and biota, resulting in widespread pollution.

There is little information on the toxicological consequences of many unregulated PFAS compounds, making it difficult to assess their potential dangers to human health and the environment. Unregulated PFAS are being studied to determine their toxicity, bioaccumulation, and environmental destiny, with a focus on those that are most concerning. Regulatory bodies are rapidly realizing the need to address uncontrolled PFAS and incorporating them into regulatory frameworks for environmental monitoring, risk assessment, and management [75]. Unregulated PFASs may be regulated by developing analytical methods, setting guideline levels, and imposing manufacturing, use, and disposal limits. Given the unknown underlying uncontrolled PFASs and their potential hazards, a cautious approach is frequently suggested to reduce exposure and prevent environmental contamination. To minimize PFAS emissions, techniques such as pollution prevention, product replacement, and the use of best management practices may be implemented. To summarize, uncontrolled PFASs are a broad range of chemicals with different features and dangers. Continued study, monitoring, and regulatory action are required to address the issues raised by uncontrolled PFASs and safeguard human health and the environment from PFAS pollution.

2.3. Properties of PFASs

PFASs display several unusual properties due to their chemical structure [76]. PFASs are very hydrophobic [77], which means they reject water because fluorine atoms in the carbon chain form a non-polar structure that does not interact well with water molecules. As a result, PFASs are employed in applications that need water repellence, such as waterproof coatings for textiles and nonstick cookware [78]. The combination of PFASs' hydrophobicity and the presence of organic matter promotes PFAS sorption into soil and sediment particles, resulting in their accumulation in terrestrial and aquatic settings.

Furthermore, numerous PFASs are oleophobic [79], which means they resist oils and grease. This feature makes PFASs valuable in applications where oil and grease resistance are required, such as food packaging and stain-resistant coatings [78]. This trait enables PFASs to retain their performance over time, even in demanding conditions [80]. Additionally, PFASs have good thermal stability, which means they can resist high temperatures without disintegrating or deteriorating [81]. This feature makes PFASs valuable in applications requiring heat resistance, such as high-temperature coatings and

electrical insulation. Furthermore, PFASs have low surface tension [82], allowing them to easily spread across surfaces and produce thin, homogenous coatings [83]. Besides, PFASs can accumulate in live organisms [84], particularly fatty tissues, because of their resistance to metabolism and elimination [85]. This property raises concerns regarding the health consequences of PFAS exposure in people and wildlife [86]. This property further adds to their broad prevalence in nature and their ability to accumulate in ecosystems [86]. However, the toxicity of PFASs varies according to the individual chemical, dosage, and route of administration [87,88]. Moreover, because of their dual qualities, PFASs have been utilized in a variety of applications [89,90]. PFAS molecules can be adsorbed onto the surface of solid particles, such as soil minerals or sediment grains, via electrostatic interactions, van der Waals forces [91], or hydrogen bonding (H-bonding) [4,92]; or PFASs are absorbed into porous materials' pore gaps or interstitial locations [93].

PFASs have a complicated sorption process that is impacted by many variables, including the bent material's characteristics, the chemical structure of the surrounding environment, and other considerations. Comprehending these systems is crucial for evaluating the destiny, movement, and ecological influence of PFASs in contaminated locations and creating efficacious remedial tactics.

3 Detection and quantification of PFASs

The persistence of PFASs in the environment and its possible health risks must be analyzed [94] and quantified [95,96]. For PFAS measurement, chromatography methods are routinely used (Table 1) [95]. HPLC can be used to detect PFAS compounds, often in conjunction with ultraviolet (UV) or charged aerosol detection (CAD) [97]. GC-MS is an alternative method for detecting PFASs, especially useful for analyzing volatile compounds like fluorotelomer alcohols (FTOHs). LC-MS/MS is a highly sensitive and selective technique that is widely used for detecting and identifying individual PFAS compounds in various environmental and biological samples [97,98]. However, the most popular method with the most encouraging outcomes is LC-MS/MS. Owing to the remarkable merits, Nassazzi et al. [99] devised and validated a simple sample preparation procedure for PFAS measurement in diverse plant tissues (Figure 3). The instrumental analysis was carried out utilizing LC-MS/MS. The process was optimized based on six distinct extraction circumstances and three different cleaning approaches. Methanol as an extraction solvent, paired with 1 g ENVI carb cartridges, outperformed all other extraction conditions and cleanup approaches evaluated. Validation of the approach demonstrated strong recovery of 90 to 120 %, high within-day and between-day accuracy of <20 % relative standard deviation, and a low detection limit of 0.04 to 4.8 ng g⁻¹ dry weight across several plant matrices. Sixteen out of the twenty-four target PFASs were found in plants, and seventeen in soil throughout the method's testing on soil and various plant tissues of Norway spruce and silver birch at a PFAS-contaminated location. The amount of PFAS in the soil was 43 ng g⁻¹ dry weight. PFAS distribution in Norway spruce tissues was 14 ng g⁻¹ dry weight in roots and 16 ng g⁻¹ dry weight in needles, whereas in silver birch tissues, it varied from 7.1 ng g⁻¹ dry weight in roots to 64 ng g⁻¹ dry weight in leaves. This new technique for PFAS analysis in plants could be useful for risk assessments, remediation, process understanding, and monitoring in the future [99].

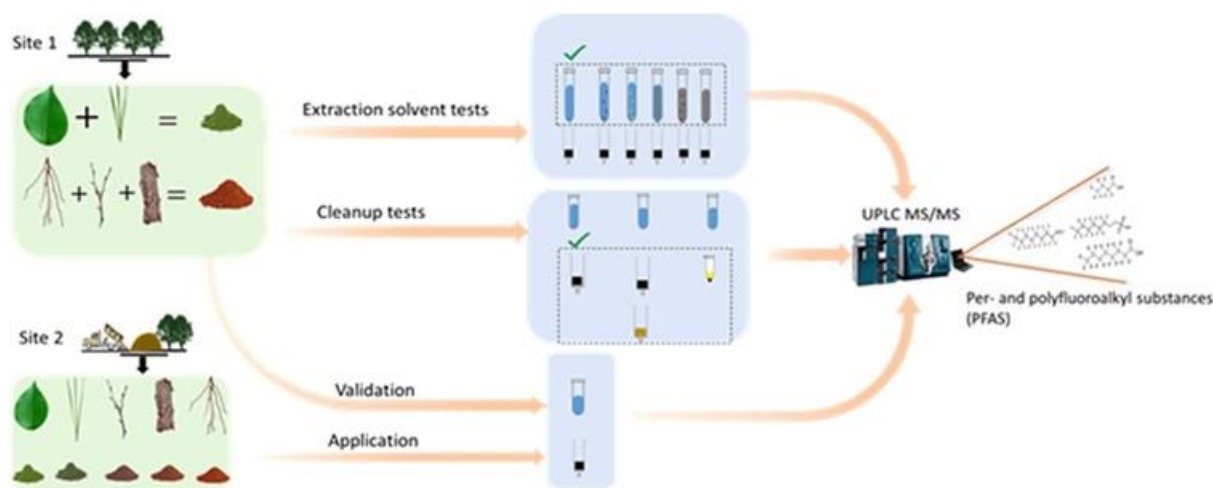


Figure 3. A schematic representation of a unique LC-MS/MS technique for the extraction, cleanup, and analysis of PFASs in various plant matrices [99]. Reproduced with permission under the terms of the Creative Commons CC-BY license.

PFASs are extracted from the sample matrix into a liquid phase by liquid phase extraction (LPE) [100], which is then concentrated and analyzed using methods like LC-MS [101]. PFASs may be extracted from complicated material matrices such as water [102], soil [103], or biological samples with high extraction efficiency using LPE [101]. As a result, target analyte recovery is enhanced, and subsequent analytical tests exhibit increased sensitivity. It has many benefits for the identification of PFAS in environmental samples, such as high extraction efficiency, adaptability, compatibility with analytical methods, decreased matrix interference, affordability, and simplicity of use. Due to these benefits, LPE is the method of choice for many PFAS analysis-related research and environmental monitoring applications. However, the extraction of LPE usually calls for substantial amounts of organic solvents, which may be expensive and harmful to the environment [104]. Concerns about the hazards to laboratory workers' health and safety are also raised when using organic solvents. To get sufficient sensitivity, rather large sample volumes would be needed, particularly when examining samples with low PFAS concentrations [93]. Matrix effects can affect extraction efficiency and analytical readings when complex sample matrices are present, such as in the case of biological or soil samples. Appropriate sample preparation and cleanup techniques are necessary to reduce these impacts.

Before LC-MS analysis, a simple, easy, timesaving, and solvent-free sample pretreatment procedure is required [105,106]. Before chromatographic analysis, sample processing methods such as pressurized-liquid extraction (PLE), solvent extraction, dispersive solid-phase extraction (DSPE)[107], solid-phase extraction (SPE), magnetic solid-phase extraction (MSPE), and others are used to improve tracking PFAS quantities in environmental and biological samples [108]. As a successful pretreatment approach, SPE technology has surpassed classical liquid-liquid extraction (LLE) [108,109]. Because of its dependability, high efficiency, easy operation, and minimal solvent consumption, SPE technology is frequently utilized in culinary, biological, pharmaceutical [108], and environmental studies [108,110]. Deng et al. [96] employed bamboo charcoal as an SPE adsorbent to enrich six perfluoroalkyl acids (PFAAs) in ambient water samples before liquid chromatography-tandem MS analysis [96]. Bamboo charcoal was studied for its porous structure, high specific surface

area, high porosity, and stability. Several experimental factors that have a significant impact on extraction efficiency were thoroughly explored and adjusted. The experimental data showed the limit of detections (LODs) ranging from 1×10^{-2} to $115 \times 10^{-2} \text{ ng L}^{-1}$, a large linear range between 1×10 and $1 \times 10^3 \text{ ng/L}$, and high repeatability and reproducibility. Bamboo charcoal was effectively employed to enrich and determine PPFAAs in real-world environmental water samples [96].

Furthermore, an analytical approach was developed and validated for the detection of sixteen PFAS in seawater, lakes, wastewater, and bi-distilled water by Miserli et al. [97]. After collection, all samples were filtered and extracted/purified/pre-concentrated using a weak-anion exchange SPE method. The quantitative determination of these pollutants was carried out using ultra-performance LC (UPLC) linked to Orbitrap MS with an electrospray ionization (ESI) interface operating in negative mode. The method was validated in all aquatic matrices and showed excellent linearity, recoveries, detection/quantification limits, extended measurement uncertainty, and precision. Following evaluation, the provided completely elaborated workflow was applied to real samples to determine dissolved phase concentrations from secondary influent, secondary, and tertiary effluent of a wastewater treatment plant (WWTP), secondary effluent of a hospital WWTP, and lake samples. PFOSA and PFOA were the most abundant PFASs in all types of water, with concentrations up to 21 ng L^{-1} (secondary influent) and 160 ng L^{-1} (hospital influent). This study found that PFAS with high chains (C8-C13) make up approximately 38.5 % of the overall content of PFASs in the secondary influence of municipal and hospital wastewater. The average elimination efficiency of Σ PFAS was 60 % in secondary treatment, reaching up to 80 % in tertiary treatment. The quantities of PFAS in sea and lake samples were either undetectable or lower than those found in wastewater [97]. Besides, Hassan et al. [111] demonstrated rapid detection of PFAS using paper spray (PS)-based MS methods that use triangular-shaped filter paper for sample loading and ionization (Figure 4a). PS-MS was initially employed in their investigation to directly analyze PFASs in drinking water, tap water, and wastewater. Interestingly, food packaging paper materials may be sliced and analyzed using PS-MS for PFAS contamination (Figure 4b). Desalting paper spray MS (DPS-MS) was demonstrated to be capable of rapidly desalting, ionizing, and identifying PFAS species such as PFOA and PFOS in samples containing salt matrices that would suppress the PFAS ion signal. The retention of PFAS on the paper substrate while salts are rinsed away by water is most likely owing to the hydrophilic interaction between the PFAS polar head and the polar filter paper cellulose surface. The DPS-MS approach is very sensitive and may be used to directly analyze soil extracts and samples. These findings indicate that PS-MS and the associated DPS-MS technology have a strong potential for real-world environmental investigation of PFAS (Figure 4c) [111].

Furthermore, the identification of PFASs in fine airborne particulate matter (PM) presents problems due to the complexity of PM samples and the low amounts of PFASs present. To mitigate this issue, a responsive analytical technique was created and verified by Kourtchev et al. [112] for the identification of sixteen PFASs in fine airborne particulate matter (PM_{2.5}) employing online solid phase extraction (SPE) coupled with LC and negative electrospray ionization high-resolution MS (-) ESI-HRMS. Online SPE enables simultaneous sample clean-up from interfering matrices and reduced LODs by infusing a high volume of sample into the LC system while maintaining chromatographic efficiency and resolution. The approach gives a limit of LODs in the range of 0.08 to 0.5 pg mL^{-1} of sample extract, allowing the detection of chosen PFAS in aerosol particles at low fg m^{-3} levels and demonstrating high tolerance to the PM matrix. The validated technique was used to analyze PFASs

in ambient PM_{2.5} samples obtained from two metropolitan areas in Enniscorthy and Dublin, Ireland. Several PFASs were found to be over the detection limit, including PFBA, PFOA, PFOS, PFBS, and PFNA, as well as fluorotelomer SO³⁻ (4:2, 6:2, and 8:2 FTS). The findings show that certain harmful PFASs, such as PFOS and PFOA, are still present in the environment after being phased out of manufacture and limited in usage in the EU and the United States for more than two decades. The presence of fluorotelomer SO³⁻ (4:2 FTS, 6:2 FTS, and 8:2 FTS, which are used as alternatives to legacy PFOA and PFOS) in ambient PM_{2.5} samples raises concerns about their persistence in the atmosphere and impact on human health, giving emerging evidence that they may have similar health endpoints to PFOA and PFOS [112].

By removing the SPE step from the PFAS detection technique, SPE-free extraction approaches seek to streamline the study of PFASs [103]. This technique makes use of several approaches that enhance analytical sensitivity while using less time and resources for preparation [113]. SPE-free techniques expedite sample preparation by avoiding cartridge conditioning, sample loading, cleaning, and elution [113] needed for traditional SPE procedures. For high testing in clinical or environmental situations, this is very beneficial. Additionally, the cost of SPE cartridges, solvents, and related supplies is high. SPE-free methods use fewer consumables and reagents [114], which results in considerable cost savings. Owing to adsorption losses and ineffective PFAS elution, SPE may result in varied recoveries [115]. Particularly for short-chain PFAS that are more likely to have elution issues, SPE-free techniques reduce such losses and increase recovery rates. Also, SPE techniques contribute to environmental issues by producing large volumes of chemical solvents and plastic trash [116]. SPE-free methods minimize waste and the use of harmful solvents, which are consistent with the principles of green chemistry. SPE-free approaches can help monitor PFASs in water, soil, and air samples, lowering analytical time and increasing throughput for large-scale environmental monitoring programs. Simon et al. [103] proposed an improved, rapid, and easy extraction approach for determining PFASs in soils using high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-CS-GFMS). To eliminate the bias of the SPE step, which is typically utilized in the analysis of extractable organically bound fluorine (EOF), we developed a rapid and easy SPE-free extraction approach (Figure 5a). The extraction technique described is a liquid-solid extraction utilizing acidified methanol with no further SPE. Four extraction processes were used to identify a large percentage of the EOF. A significant underestimate of EOF concentrations was found when comparing the improved procedure with and without an extra SPE clean-up stage (Figure 5b). Variations of up to 94 % were noted, and coextracted inorganic fluoride could not account for them. Thus, an approach (eschewing needless SPE) that was both more economical and environmentally friendly as well as more accurate was devised. The developed method's procedural limit of quantification (LOQ) of 10.30 µg kg⁻¹ was enough to measure the quantities of EOF in all tested samples. The optimal extraction method described here can be very helpful for future PFAS monitoring and any regulatory considerations [103].

The sensitive and selective detection of PFAS in environmental samples is made possible by a combination of sample preparation methods and cutting-edge analytical equipment, such as LC-MS. This enables researchers to evaluate the environmental distribution and possible health risks related to these contaminants.

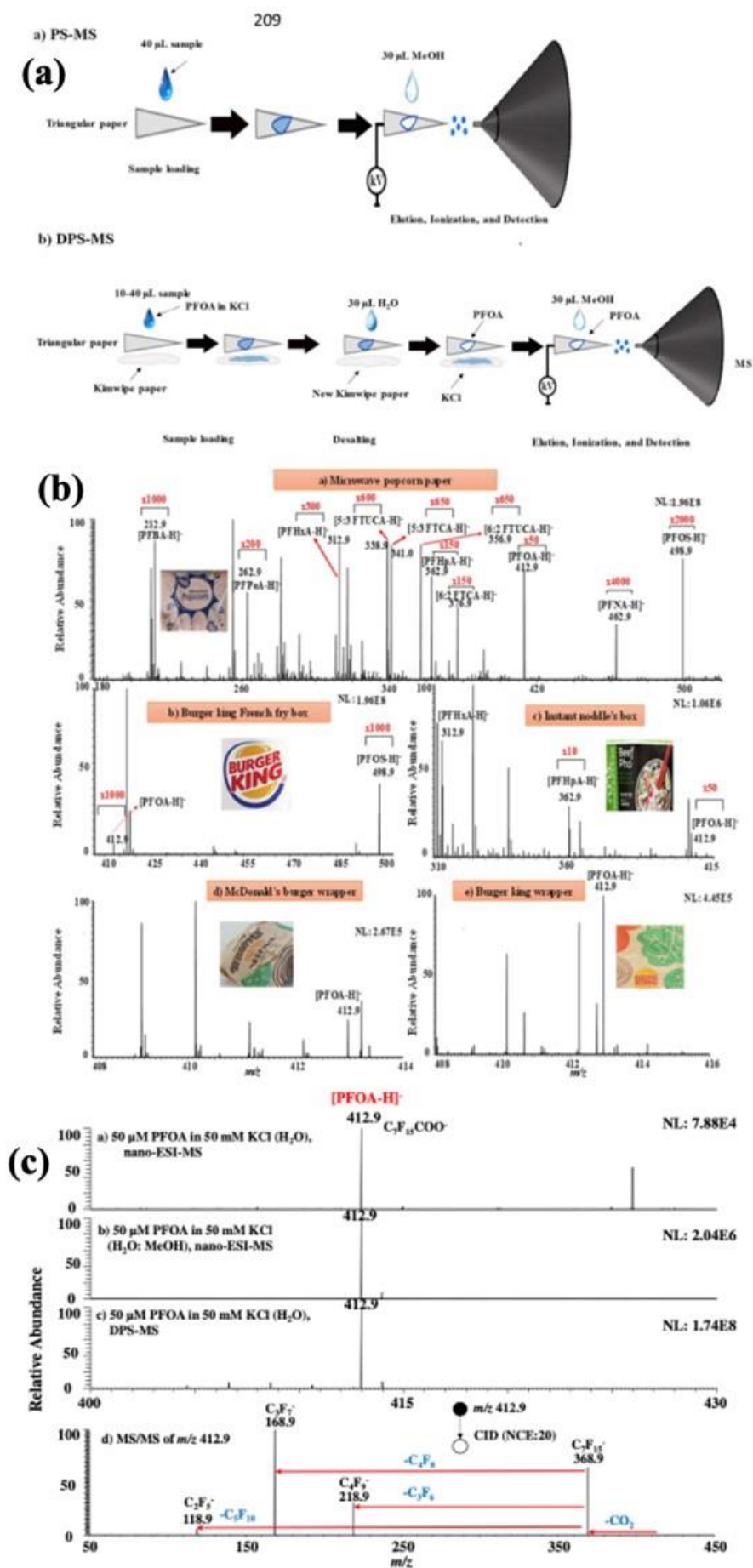


Figure 4. (a) Schematics displaying the equipment and procedures used by the PS-MS and DPS-MS. (b) Mode of negative ions PS-MS spectra of a McDonald's burger wrapper, an instant noodle box (Beef Pro), a Burger King French fry box, a microwave popcorn paper, and an e-burger wrapper. These food packaging materials contained a variety of PFAS, such as PFBA, PFPeA, PFHxA, 5:3 FTCA, 5:3 FTUCA, 6:2 FTUCA, PFHpA, 6:2 FTCA, PFOA, PFNA, and PFOS. (c) The 50 μM PFOA in 50 mM KCl negative ion mode MS spectra were obtained using a) nanoESI-MS (no MeOH added), b) nanoESI-MS (MeOH added), and c) DPS-MS. d) CID MS/MS spectrum at m/z 412.9 of the PFOA ion $\text{C}_7\text{F}_{15}\text{COO}^-$ [111]. Reproduced with permission. License number 5846360316632.

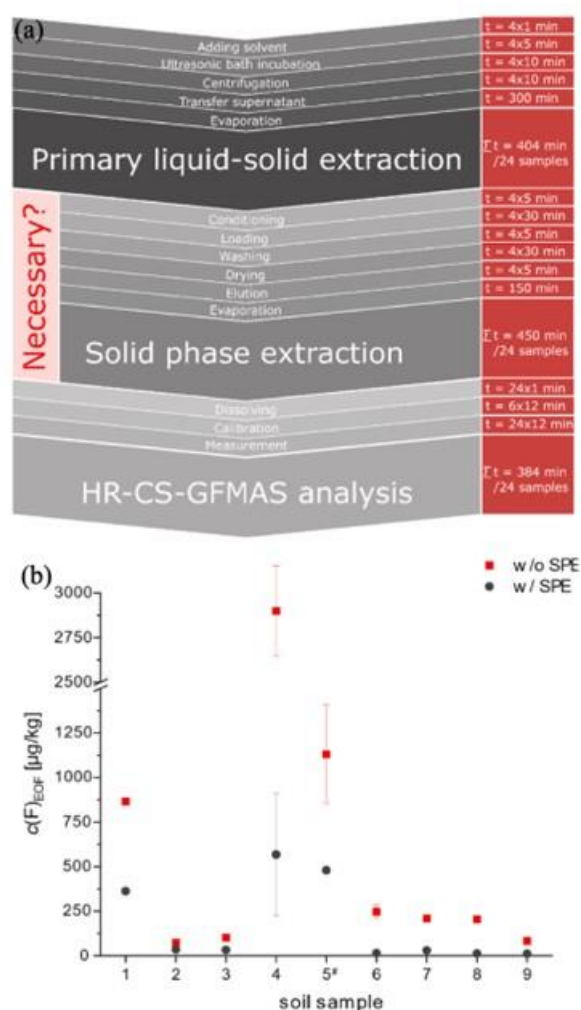


Figure 5. (a) An outline of the HR-CS-GFMS analysis and optimized sample preparation (including an extra SPE stage), along with how each step contributes to the overall execution time (b) The optimized sample extraction with and without SPE (w/o SPE, red squares) and with SPE (w/SPE, black circles) was used to compare the EOF concentrations. #: For SPE samples, $n=2$ [103]. Reproduced with permission. License number 5957070320780.

Table 1. Summary of conventional liquid-phase and solid-phase techniques used to detect and quantify numerous PFAS types in a variety of environmental and health-related samples.

Sample extraction methods	Type of Samples	Types of PFAS	Efficiency and Sensitivity	Detection strategy	LOD	Refs
Solid Phase Extraction	Crop and samples	PFOA, PFOS	Efficiency= 1.3 % to 9.8 %	CX4-COFs-M-SPE@UHPLC-MS/MS	0.1 to 0.28 ng kg ⁻¹	[117]
	Crop and samples	PFOA, PFOS	np	PMPD@HepG2 cell	1 to 4.73 ng L ⁻¹	[118]
	Drinking water	PFOA, PFOS	np	Fluoro-SPE with SDVB-SPE	1 ppb	[119]
	Drinking water	PFHpA, PFOA, PFNA, PFDA, PFHxS, PFOS,	np	HPLC-MS/MS	0.01 to 1.15 ² ng L ⁻¹	[96]
	Seawater, lake and wastewater and bi-distilled water	PFOA, PFOS	Efficiency= 43 % to 119 %	ESI-UPLC@Orbitrap-MS	21 ng L ⁻¹	[97]
	PM _{2.5}	PFOA, PFOS	np	(-) ESI-HRMS-SPE@LC	0.08 to 0.5 pg mL ⁻¹	[112]
	Industrial wastewater, river water, and air samples	PFOA, PFOS	Efficiency= 46 to 112 %	SPE@Combustion ion chromatography (CIC)	0.2 µg L ⁻¹ for a 500 mL sample 0.1 µg L ⁻¹ for 1200 mL sample	[102]
	Human hair	PFBA, PFBS, PFOA, and PFOS	Efficiency= 1 to 16 %	SPE@(LC-QTOF)	0.07 to 0.5 ng g ⁻¹	[120]
	Drinking water	PFOA, PFOS	np	UPLC@MS	23.9 ng L ⁻¹	[101]
	Surface water	PFOA and PFOS	Efficiency= 65.04 %	LC-MS/MS	1.5 ng L ⁻¹	
Liquid-Liquid Extraction	Soil and groundwater	PFOA, PFOS	Efficiency= 90 to 120%	LC-MS/MS	0.04 to 4.8 ng g ⁻¹	[99]
	Non-stick baking papers, Electric rice cooker pots, Frying pans	PFBS, PFHxS, PFOS, PFDS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA	np	LC-MS/MS	0.009 to 0.06.6 ng L ⁻¹	[121]
	Milk	PFOA, PFOS	np	LC-MS/MS	PFOA= 0.057 ng g ⁻¹ PFOS= 0.02.1 ng g ⁻¹	[122]
	Drinking water	PFOA, PFOS, PFHxA, PFODA, PFHpS, PFDS	Efficiency= 94.3 %	LC-MS/MS	0.6 to 8.7 ng L ⁻¹	[123]

M-SPE= Membrane solid-phase extraction; UHPLC-MS/MS= Ultra-high performance liquid chromatography-tandem mass spectrometry; PMPD= Paper mill processing discharge; HepG2= Human hepatocellular carcinoma; LC-MS/MS= Liquid Chromatography-Mass Spectroscopy; Orbitrap-MS= Orbitrap mass spectrometry, ESI= electrospray ionization; PM_{2.5}= fine airborne particulate matter; (-) ESI-HRMS= negative electrospray ionization high-resolution mass spectrometry; LC-QTOF= Liquid-chromatography coupled to accurate mass measurement

4 Toxicological implications of PFASs on human health

PFASs have a variety of toxicological consequences (Figure 6) for human health due to their broad prevalence, persistence, and bioaccumulation [124]. Prenatal exposure to PFAS has been linked to unfavorable developmental outcomes such as low birth weight, shorter gestational age, and delayed fetal growth [124,125]. Some researchers [8,126] have connected PFAS exposure to developmental delays, neurobehavioral problems, and reduced cognitive performance in children [8]. PFASs can impair endocrine function by interfering with hormone production, metabolism, and signaling [126]. This disturbance may hurt reproductive health, including decreased fertility, changed sex hormone levels, and irregular menstrual cycles [8]. Tian et al. [8] studied eighty-two women with preeclampsia and one hundred and sixty-nine healthy volunteers from Hangzhou, China. Fifteen PFASs were found in maternal serum before birth. PFOA and 6:2Cl-PFESA were related to a greater incidence of preeclampsia both linearly and by tertile. Each log-unit rise in serum PFOA and 6:2 Cl-PFESA concentrations was related to higher risks of preeclampsia. These effects were more pronounced among primiparous mothers having female fetuses. Both PFOA and PFUnDA concentrations were strongly related to an increased risk of early-onset preeclampsia, although the relationships were generally nil for late-onset. Furthermore, each logarithmic rise in PFOA concentrations was significantly linked with an increase in systolic and diastolic blood pressures of 0.262 and 0.224 mmHg, respectively. Using Bayesian kernel machine regressions (BKMR), the total impacts of PFAS mixture concentrations on preeclampsia revealed a rising trend, with PFOA contributing the most. In terms of birth weight, the Cox proportional hazards model revealed that preeclampsia was linked with considerably greater chances of low birth weight than normal pregnancy. Higher PFOA exposure was linked to increased LBW risks, both linearly and by tertile. Furthermore, serum PFOA and PFUnDA levels were substantially related to an increased risk of SGA development. Nonetheless, 4:2 FTS and ADONA had an inverse relationship with LBW and SGA occurrences. More poor birth outcomes should be investigated to better understand the health consequences of PFAS exposure and preeclampsia development [8].

PFAS exposure has also been linked to thyroid hormone disturbance, which can impair metabolism, growth, and brain development. PFAS exposure has been related to immunotoxic consequences such as immune function reduction, changes in immune cell populations, and poorer immunological responses to infections and immunizations [127]. PFAS-induced immunosuppression may increase vulnerability to infectious illnesses and decrease vaccination efficacy. PFAS can accumulate in the liver, causing toxicity and damage [128]. PFAS exposure has been linked to higher liver enzyme levels, fatty liver disease, and hepatocellular carcinoma (liver cancer). Pan et al. [32] studied six-week-old female mice subjected to 0, 1, 3, and 10 µg L⁻¹ Cl-PFESA for ten weeks to assess its hepatotoxicity and molecular mechanism. The findings revealed that Cl-PFESA bioaccumulated preferentially in the

liver, causing hepatic cytoplasmic vacuolation and hepatomegaly in mice. Furthermore, serum metabolic profiling revealed that Cl-PFESA exposure resulted in an aberrant rise in amino acids and an abnormal decrease in acyl-carnitine, interfering with fatty acid transport and raising the risk of metabolic disorders. Experiments revealed that Cl-PFESA formed more H-interactions with PPAR- γ than PFOS, Rosi, and GW9662, and had the highest binding affinity to PPAR- γ among the ligands. Cl-PFESA increased PPAR- γ expression, promoting 3T3-L1 cell development. The results we report indicate that Cl-PFESA can cause liver damage and dysfunction in female mice, mediated via PPAR- γ . This was the first study to disclose Cl-PFESA's liver toxicity in female animals, and it gives fresh insights for future research [32].

PFASs have been designated as likely or potential human carcinogens based on animal research and epidemiological data. Certain PFAS chemicals, such as PFOA and PFOS, have been linked to an elevated risk of several cancers, including kidney, testicular, prostate, and pancreatic [129]. Owing to this known knowledge, Temkin et al. [130] examined the available epidemiological, toxicological, and mechanistic data for 26 distinct PFASs. The study showed compelling evidence that various PFASs cause oxidative stress, decrease immunity, and modify receptor-mediated effects. The researchers also discovered intriguing evidence that certain PFAS might cause epigenetic changes and impact cell growth. Experimental findings show that PFAS are not genotoxic and do not typically undergo metabolic activation. Data was inadequate to determine if any PFAS caused chronic inflammation, cellular immortalization, or change in DNA repair. While further study is needed to fill data gaps, there is evidence that some PFASs have one or more of the basic features of carcinogens [130].

Emerging data shows that PFAS exposure may be linked to deleterious cardiovascular consequences such as high blood pressure, dyslipidemia, and an increased risk of cardiovascular disease. PFAS-induced cardiovascular damage may include inflammation, endothelial dysfunction, and disturbance of lipid metabolism [131]. Some researchers have found links between PFAS exposure and renal dysfunction, such as lower kidney function, elevated indicators of kidney damage, and an increased incidence of chronic kidney disease [1]. PFAS-induced renal toxicity might be caused by inflammation, oxidative stress, or direct injury to the renal tubules. PFASs have been linked to neurotoxicity, including changes in neurotransmitter levels [125], poor neurodevelopment, and cognitive deficiencies [28]. Prenatal and early-life exposure to PFAS may raise the likelihood of neurobehavioral disorders, including ADHD and ASD [132]. Persistent Organic Pollutants (POPs) under the Stockholm Convention now include PFOS, PFOA, and their salts as highly restricted substances [133]; PFHxS is also suggested for inclusion in the October 2019 convention [134] and will be taken into consideration by the next Conference in 2021 [135]. In addition to being just as persistent as long-chain PFAAs, short-chain PFAAs also have several very worrying characteristics [136]. As a result, studies are being conducted to raise awareness and control short-chain PFASs [137]. The toxicological effects of PFASs on human health are many and complex, impacting a variety of organ systems and physiological functions. Efforts to decrease human exposure to PFASs and alleviate their negative health consequences are critical for ensuring public health and environmental sustainability.

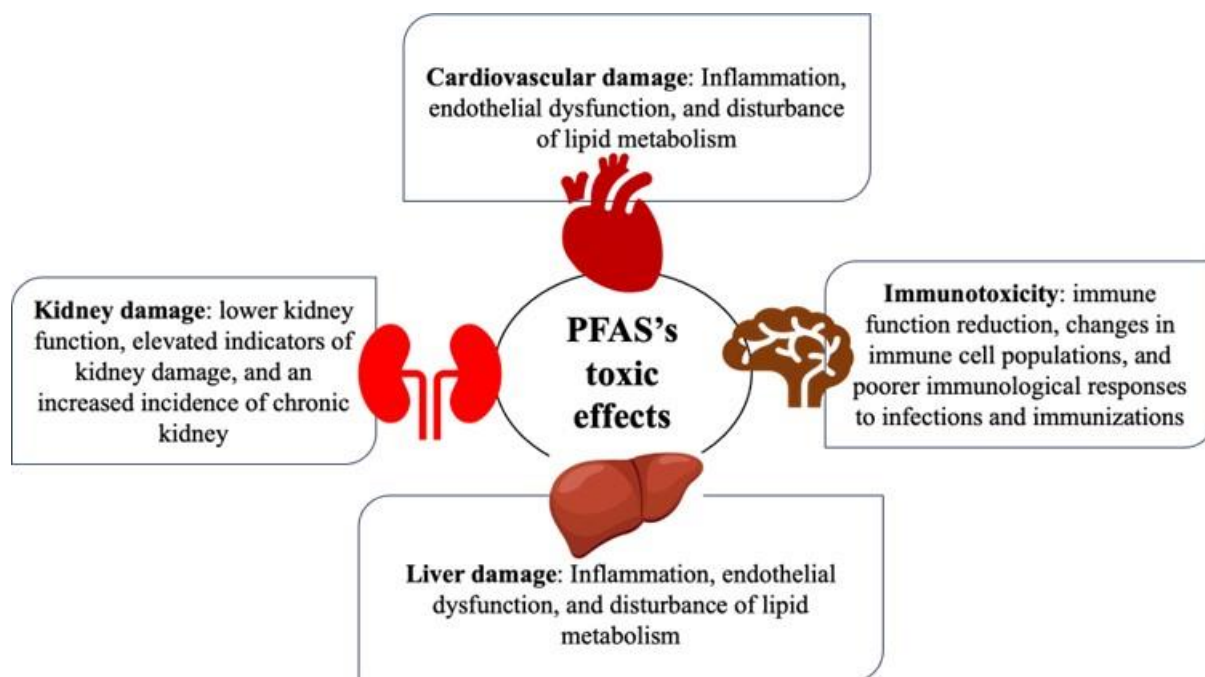


Figure 6. Toxicological effects of PFASs on human health.

5 Conclusion

The origins, physicochemical characteristics, detection and quantification techniques, and toxicological implications of PFASs have been emphasized in this review. PFASs are vital to numerous industries due to their strong chemical stability, surface activity, amphiphilicity, and hydrophobicity. However, these same characteristics also play a role in their environmental persistence and possible hazards to ecological and human health. Targeted, semi-targeted, and non-targeted analysis is now possible with methods like LC-MS/MS, GC-MS, and HPLC. PFAS detection and quantification have come a long way. Researchers can analyze related risks and determine the extent of PFAS exposure using these techniques. Besides, cancer, immune system suppression, liver and thyroid problems, and reproductive damage are associated with PFSS accumulation. To reduce the harmful effects of PFASs on the environment and public health, it is imperative to implement regulations, clean up contaminated locations, and create safer substitutes. Furthermore, comprehending PFAS sources, characteristics, detection techniques, and toxicological effects is integral to the management of PFAS pollution by lowering the dangers of human and ecological exposure through legislation, and scientific innovation.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Eliasu Issaka: Conceptualization, Methodology, Investigation, Data Curation, Resources, Validation, Formal analysis, Writing-Original Draft, Writing-Review and Editing, Supervision, Resources; Mabruk Adams: Investigation, Data Curation, Writing-Review and Editing, Supervision; Enock Adjei Agyekum: Investigation, Data Curation, Writing-Review; Josephine Baffoe: Methodology, Investigation, Data Curation, Writing-Review and Editing; Blessing Tornyeva: Methodology, Investigation, Data Curation

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