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Per- and polyfluoroalkyl substances (PFAS) of possible concern in the aquatic environment

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Contents

Executive summary

For decades per- and polyfluoroalkyl substances (PFAS) have been employed in a variety of products intended for everyday use, such as cosmetics, food packaging, textiles and household products, as well as for specialised applications including mechanical components, electronics, medical devises, fire-fighting foams and biocides, which require unique chemical properties conferred by the extremely stable carbon-fluorine bond. They became a ubiquitous contaminant in different environmental matrices due mainly to intensive industrial production and inappropriate disposal. Chemical stability and mobility of PFAS have lead to their accumulation in soil, sediment, groundwater, surface water and atmosphere worldwide heavily impacting living organisms.

The aim of this report is to increase the awareness of the public, scientific communities and policy makers on PFAS by providing scientific information on these "forever chemicals" and by presenting current state-of-the-art related to their presence in the aquatic environment. The report also describes initiatives under the umbrella of the European Green Deal that have been taken to mitigate the risk from PFAS as well as other actions at policy level to protect water, and indirectly human health, falling among the goals of the Water Framework Directive (WFD).

Perfluoroalkyl acids (PFAA) and their anions constitute part of PFAS for which knowledge and regulatory guidelines are more complete. Among them, some compounds are greatly persistent while others are degraded to highly persistent congeners. Based on the number of carbon atoms in the alkyl chain, long-chain PFAA have been found to bioaccumulate in the environment and biota. Since the presence of PFAS was officially detected in human blood, the most toxic substances such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been gradually substituted by novel and supposedly less harmful PFAS, primarily short-chain PFAA. Further assessment proved their widespread environmental presence due to a high solubility in water and potential to long-term transport in aqueous matrices. Little information is available about perfluoroalkyl ethers which include hexafluoropropylene oxide dimer acid (HFPO-DA) and chlorinated polyfluorinated ether sulfonate (Cl-PFESA) produced as substitutes of the phased out PFOA and PFOS, however emerging evidence indicates that their environmental behaviour and human hazard are similar to the replaced substances. Additional threat is posed by branched isomers of PFAS generated as unwanted by-products when the intended linear isomers are synthesised using electrochemical fluorination (ECF).

Among the wide range of adverse health effects observed in relation to PFAS, liver toxicity, imbalance in lipid metabolism and alterations in the immune system are more frequently described. Although concordant effects of PFAS exposure in humans and toxicological models have been reported for some of the legacy congeners, mode of action (MoA) and adverse outcome pathways (AOP) constitute major drawback for most PFAS in the evaluation of possible effects to organisms and humans. Other knowledge gaps which impede to perform a robust risk analysis, include the toxicity of PFAS in mixture with congeners and substances belonging to different chemical groups, their precursors and degradation products, especially considering sub-lethal and/or chronic effects. Available toxicological data are often noncongruent in terms of methodology and risk assessment approach. Moreover, the exact structure of many novel PFAS developed by the industry is unknown due to trade secret, while ecotoxicological data are still lacking for hundreds of commercially available and novel PFAS which substitute banned compounds.

In the EU legislation, the recast of the Drinking Water Directive (DWD) included for the first time 20 PFAS to be analysed as two chemical parameters: the "sum of PFASs" intended as a sum of PFAS considered in the recast with the parametric (limit) value of 0.10 µg/L, and

"PFASs - total" meaning the "totality of per- and polyfluoroalkyl substances" with the threshold concentration of 0.50 µg/L. The monitoring of environmental waterbodies for PFAS under the WFD is only based on the concentrations of PFOS and its derivatives included among the Priority Substances (PS). The relative safety thresholds for those substances are defined by the environmental quality standards (EQS) established for surface waters and biota. Nevertheless, revision of current technical guidelines for EQS derivation is recommended to include also those endpoints which are based on molecular and genetic biomarkers. The use of specific *in vitro* bioassays complementary to standard PFAS detection methods would constitute an integrated approach taking into account the effects which result from co-occurring substances in realistic samples.

In future perspective, Chemicals Strategy for Sustainability towards toxic-free environments under the EU Green Deal will address PFAS and some of the related knowledge gaps. In particular, revisions of the current regulatory documents are aimed to reduce the emissions of PFAS to the environment and to establish their safety limits from dietary sources. Extending the assessment of PFAS as a group encompassing more substances instead of measuring chemicals one-by-one is expected to provide a better protection of human health and the environment. A coordinated mechanism at European level will be developed in order to simplify and synchronise the safety assessment of chemicals across various pieces of legislation, while further phasing out of PFAS for non-essential uses will be regulated under REACH. At global level, the concerns regarding the presence of PFAS in the environment will be targeted by the Basel and the Stockholm Conventions.

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large group of organofluorine compounds including more than 4.700 synthetic substances (OECD, 2018) that since the mid-20th century have been widely used in commercial and industrial products due to their unique physical and chemical properties (ITRC, 2020), such as resistance to moisture, heat and stains. Recently, increasing body of scientific evidence has raised concerns regarding the threat posed to human and animal health by PFAS residues in the environment. While adverse effects of historically used PFAS are largely known, the toxic action of their novel substitutes is being suggested by the growing amount of data.

Even though PFAS of major concern are well characterised, many others lack the CAS number, information about their physicochemical properties or harmonised data regarding their nomenclature which results in many synonym names. Moreover, the composition of certain PFAS is not clear which hampers performing risk assessment for individual compounds, therefore grouping of PFAS has been proposed to facilitate, in terms of time and costs, studies and determination of these substances in a wide range of matrices (Cousins *et al*., 2020).

1.1 Properties and use of PFAS

While some types of PFAS contain reactive sites, such as sulfonic or carboxylic acid groups (Figure 1), other types are considered chemically inert because they lack chemically active groups. The carbon-fluorine bond - a common feature of all PFAS - is the strongest one in organic chemistry (Natararajan *et al*., 2005) which makes these compounds extremely stable and resistant to degradation, both during their lifetime and in environmental settings. The latter feature has earned them the moniker "forever chemicals", meaning that PFAS and their breakdown products, once discharged to surface waters, accumulate in the environment and may be transported over a long distance from the source of release. Removal of PFAS and their precursors through most conventional wastewater treatment processes is troublesome (Arvaniti and Stasinakis, 2015) and has been associated with increased PFAS concentrations in wastewater treatment plant (WWTP) effluent compared to influent (Post et al., 2012). High aqueous solubility of these compounds is of a serious concern as it facilitates their spread in water ecosystems, and in consequence exposure of aquatic biota and humans. Similarly, their high mobility in soil poses environmental risk due to accidental leakage and application of contaminated water or sludge in agricultural practice. The desirable physicochemical properties of PFAS, such as hydrophobicity and lipophobicity, chemical and thermal stability, surface tension lowering, dielectric properties, radiation and hot-acid resistance, confer them characteristics difficult to replace by surrogate compounds, therefore their extensive application in different fields has progressively increased over the years (OECD, 2013; ECHA, 2018; ITRC, 2020). These characteristics are determined by a variable chemical composition and structure within PFAS category, which may occur together in one compound or, on the other hand, be a singular feature of a specific molecule. In result, many PFAS have a broad spectrum of applications, while others are specifically used in certain types of products. For example, some PFAS are commonly employed as emulsifiers, surfactants and coatings resistant to water, oil, grease, soil and dust, while other PFAS have the ability to create stable foams for fire extinguishers or are employed in the production of electronic equipment and components (KEMI, 2015; Concawe, 2016; OECD, 2018; ITRC, 2020). More examples of the main historical and current applications of PFAS in manufacture and commercial industries are briefly presented in Table 1, while description of their functionality in relation to commercial application is described in Table 2.

Table 1. List of the main applications of PFAS in industrial and consumer products.

Industrial sector	Type of material	Use
Automotive	Raw materials for components and lubricants	Wiring and fuel delivery tubing; low-friction bearings and seals; surface treatment for textiles (seats, carpets, leather and exterior surfaces); lubricants
Aviation, aerospace and defense	Mechanical components and hydraulic fluid additives	Pipelines, seals, gaskets, cables, and insulators
Cable and wiring, semiconductors and electronics	Coating materials and insulators and raw materials for equipment	Surface-treatment to protect from weather, fire and soil; working fluids in mechanical vacuum pumps; component material in cell phones, computers, speakers, etc.
Building and Construction	Coating materials and paint additives	Additives in paint, ink, varnish, polish and coatings; film to cover solar collectors; surface-treatment protection on fabrics, metals, stone, concrete, etc.; metal and plastic coating; adhesives and surface treatment agent
Cosmetics and Personal Care Products	Cleaning fluids, cosmetic and hygiene products	Shampoos, hair conditioner, hand creams, nail polish, eye makeup, denture cleaners, dental floss and micro powders used in creams and lotions; oil and water repellent in sun creams and body lotions
Fire-fighting	Raw materials for components and equipment	Fuel repellents, foam stabilizers and fire-fighting foams; coating for fire-fighting equipment
Food processing	Food packaging materials and coating materials	Oil /grease repellent on paper, cardboards and food packaging; Fast Food packaging; coating material in trays, ovens, grills
Household products	Nonstick coating materials and wetting agent in cleaning products	Teflon production; floor polishing and cleaning agents
Medical articles	Raw materials and stain- and water-repellents	Surface-treatment protection on surgical textile; surgical patches; cardiovascular synthetic grafts and medical implants; video endoscopes; X-ray film
Plant Protection Products	Manufacture of biocides	Active ingredients in plant growth regulators, herbicides and ants and termites baits; inert ingredients in pesticide formulations

Textiles and leather	Raw materials for highly porous fabrics	Surface-treatment oil, water and stain repellent on carpets, furniture, outdoor clothing, textile-related articles and leather

Data from CONCAWE, 2016, Appendix 1 in pp. 88-89; ITRC, 2020, table 2-4 in pp. 32-34; OECD, 2018, Table 1 and 2 in pp. 12-13.

Table 2. Functionalities related to fluoropolymer commercial application.

Commercial application	Mechanical strenght	Resistance to chemicals	Nontoxic, biocompatible, biological degradation resistant	Flexibility	Low dielectric constant	Resistance to photolysis, oxidation, hydrolysis
Aerospace	X	X	-	X	X	X
Automotive industry	X	X	-	X	X	X
Medical devices	X		X	X	-	X
Pharmaceutical manufacture	X	X	X	X	-	-
Consumer outdoor apparel	X		X	X	-	-
Technical clothing (military, firefighters, first responders, medical personnel)	X	X	X	X	-	X
Consumer electronics	X	X	-	X	X	X
Wireless communications	X	X	-	X	X	X
Satellite navigation systems	X	X	-	X	X	X
Semiconductor industry	-	X	-	-	X	-
Building construction	X	-	X	X	X	X
Energy production and storage	X	-	-	-	X	X
Food and beverage production	X	X	X	X	-	X
Food protection and packaging	X	X	X	X	-	
Drinking water filtration	-	X	X	-	-	X
Environmental protection	-	X	X	-	-	X

Data adapted from Henri *et al*., 2018

1.2 Chemical structure and classification of PFAS

The common feature of PFAS is the presence of perfluoroalkyl moiety C_nF_{2n+1}- with a variable number of repeats forming an aliphatic fluorinated carbon chain, in which all the hydrogen (H) atoms bound to the carbon (C) atom have been substituted by fluorine (F) atoms (Buck *et al.*, 2011). This definition implies that at least one CH_3- must be present in the molecule. More recently, OECD/UNEP (2018) described chemicals belonging to this class as every molecule containing the -C_nF_{2n}- group. Considering a rising concern related to the presence of PFAS in the environment, Buck *et al.* (2011) proposed an everyday-use terminology and classification of these compounds as a simplified alternative to standardized IUPAC or CAS nomenclature. Presently, PFAS are grouped in two main families encompassing:

- **perfluoroalkyl substances** ("perfluoro" and "perfluorinated" substances as previously described by Banks *et al.*, 1994): aliphatic compounds with all H atoms of non-fluorinated precursor replaced by F atoms, with the exception of those H atoms whose replacement would change the nature of any functional groups present (Figure 1A);

- **polyfluoroalkyl substances**: aliphatic compounds in which not all H atoms bound to C atoms have been substituted by F atoms to form perfluoroalkyl moiety (Figure 1B).
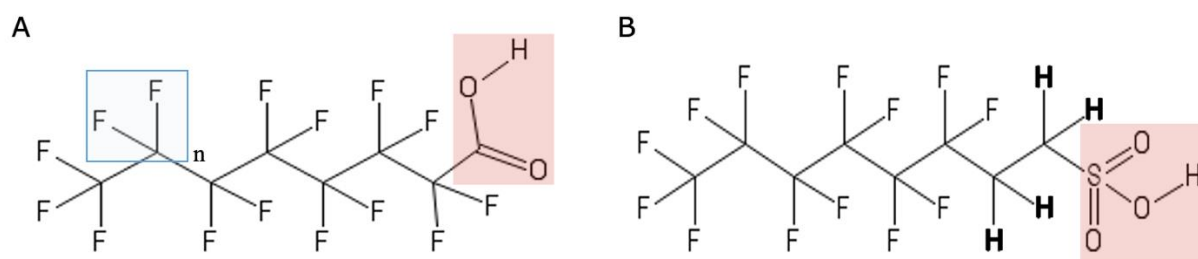

Figure 1. Skeletal structure of PFAS. Perfluoroalkyl substances (A), example of perfluorooctanoic acid (PFOA) exhibiting a full perfluoroalkyl moiety (blue frame) which may be present in other PFAS in a variable number. Polyfluoroalkyl substances (B), example of 6:2 fluorotelomer sulfonate (6:2 FTS) presenting hydrogen atoms (highlighted in bold) bound to the alkyl backbone. Besides the number of perfluoroalkyl moieties, different physicochemical properties may be conferred by the main variable functional groups (carboxylic or sulfonic, shaded fields) and by side groups or chains bound to selected perfluoroalkyl moieties. n: variable number of perfluoroalkyl moieties in PFAS molecules.

Various ways of grouping PFAS exist following different aspects of their structure and properties. One of the most relevant ones is based on the presence or absence of repeated molecular units, according to which PFAS are classified in two respective sub-groups:

- **polymeric**, composed of very long alkyl chains (e.g., reaching 180 000 C atoms in PTFE resins) including *(i)* fluoropolymers, *(ii)* side-chain fluorinated polymers and *(iii)* perfluoropolyethers. They are represented mainly by polytetrafluoroethylene (PTFE), fluorinated ethylene propylene (FEP), perfluoroalkoxy alkanes (PFA), ethylene tetrafluoroethylene (ETFE) (Henry *et al.*, 2018). A more detailed overview of other different types of fluoropolymers is given by Gardinier, 2015.

- **non-polymeric**, usually containing up to 13 C atoms in the alkyl chain and possible side chains, including *(i)* perfluoroalkane sulfonyl fluoride (PASF) and derivatives, *(ii)* perfluoroalkyl iodides (PFAI), fluorotelomer (FT) and based compounds, *(iii)* per- and polyfluoroalkyl ether (PFPE) and derivatives, and *(iv)* perfluoroalkyl acids (PFAA) including perfluoroalkyl carboxylic acids (PFCA), perfluoroalkane sulfonic acids (PFSA),

perfluoroalkyl phosphonic acids (PFPA) and perfluoroalkyl phosphinic acids (PFPIA) (Buck *et al*., 2011).

The present report will briefly describe four distinct groups which are abundant in the environment: perfluoroalkyl acids (PFAA), PFAA precursors, perfluoroalkyl ethers and branched PFAS.

1.2.1 Perfluoroalkyl acids (PFAA)

Perfluoroalkyl acids PFAAs and their anions are those for which knowledge and regulatory guidelines are more complete. They are divided in perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulphonic acids (PFSA) and can also be classified as "long-chain" and "short-chain" compounds depending on the number of C atoms in the fluorinated carbon chain (Table 3). OECD (2013) defined non-polymeric long-chain PFAS as PFCA with ≥7 perfluoroalkyl carbons (or ≥8 total carbons), PFSA with ≥6 perfluorinated carbons (i.e. ≥6 total carbons) and also precursors of long-chain PFCAs or PFSAs. Short-chain PFAS include PFCA with seven or fewer perfluorinated carbons and PFSA - five or fewer perfluorinated carbons (Buck *et al*., 2011; OECD, 2013; ITRC, 2020).

Table 3. Examples of perfluoroalkyl acids (PFAA) divided into perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulfonic acids (PFSA). Within each group, PFAA are divided in short-chain and long-chain substances (adapted from ITRC, 2020 and EFSA et al., 2020).

PFCAs (perfluoroalkyl carboxylic acids)					
Chain type	**Acronym**	**Chemical name**	**CAS–number**	**N. C atoms**	
				Tot.	**Perfl.**
Short-chain	PFBA	Perfluorobutanoic acid	375-22-4	4	3
	PFPeA	Perfluoropentanoic acid	2706-90-3	5	4
	PFHxA	Perfluorohexanoic acid	307-24-4	6	5
	PFHpA	Perfluoroheptanoic acid	375-85-9	7	6
Long-chain	PFOA	Perfluorooctanoic acid	335-67-1	8	7
	PFNA	Perfluorononanoic acid	375-95-1	9	8
	PFDA	Perfluorodecanoic acid	335-76-2	10	9
	PFUnDA	Perfluoroundecanoic acid	2058-94-8	11	10
	PFDoA	Perfluorododecanoic acid	307-55-1	12	11
	PFTrDA	Perfluorotridecanoic acid	72629-94-8	13	12

PFSAs (perfluoroalkane sulfonic acids)					
Chain type	**Acronym**	**Chemical name**	**CAS-number**	**N. C atoms**	
				Tot.	**Perfl.**
Short-chain	PFBS	Perfluorobutane sulfonic acid	375-73-5	4	4
	PFPeS	Perfluoropentane sulfonic acid	2706-91-4	5	5
Long-chain	PFHxS	Perfluorohexane sulfonic acid	355-46-4	6	6
	PFHpS	Perfluoroheptane sulfonic acid	375-92-8	7	7
	PFOS	Perfluorooctane sulfonic acid	2795-39-3 (potassium salt); 1763-23-1 (acid)	8	8
	PFNS	Perfluorononane sulfonic acid	68259-12-1	9	9
	PFDS	Perfluorodecane sulfonic acid	335-77-3	10	10
	PFUnS	Perfluoroundecane sulfonic acid	749786-16-1	11	11
	PFDoS	Perfluorododecane sulfonic acid	79780-39-5	12	12
	PFTrDS	Perfluorotridecane sulfonic acid	N/A	13	13

Tot.: total number of C atoms. Perfl.: number of perfluorinated C atoms.

1.2.2 PFAA precursors

PFAA precursors are polyfluoroalkyl substances with a perfluoroalkyl moiety and a nonfluorinated one, which degrade when released in the environment, leading to the formation of PFCA and PFSA (Houtz and Sedlak, 2012). Examples of PFAA precursors are reported in Figure 2. Among PFCA precursors, fluorotelomers are a group of molecules artificially synthesised through telomerisation which is a polymerisation reaction leading to a telomer, an oligomer of low molecular weight. Examples of fluorotelomers are fluorotelomer alcohol (FTOH), fluorotelomer acrylate (FTAC), fluorotelomer methacrylate (FTMAC), fluorotelomer sulfonates acid (FTSA), fluorotelomer carboxylic acid (FTCA), fluorotelomer unsaturated carboxylic acid (FTUCA), fluorotelomer mercapto dimethylamido sulfonate (FTSAS), fluorotelomer ethoxylate (FTEO), etc. Among PFSA precursors, the ones that can be transformed to PFOS are the most abundant and are represented by the class perfluorooctyl sulfonamides $C_8F_{17}SO_2NRR'$ (Zhang *et al*., 2021). Examples of those compounds are N-methyl perfluorooctane sulfonamide (MeFOSA), N-ethyl perfluorooctane sulfonamide (N-EtFOSA), N-methyl perfluorooctane sulfonamido ethanol (MeFOSE), N-ethyl perfluorooctane sulfonamido ethanol (EtFOSE), N-ethyl perfluorooctane sulfonamido acetate (EtFOSAA) and phosphate diesters EtFOSE-based (DiSAmPAP) (Zhang *et al*., 2021).
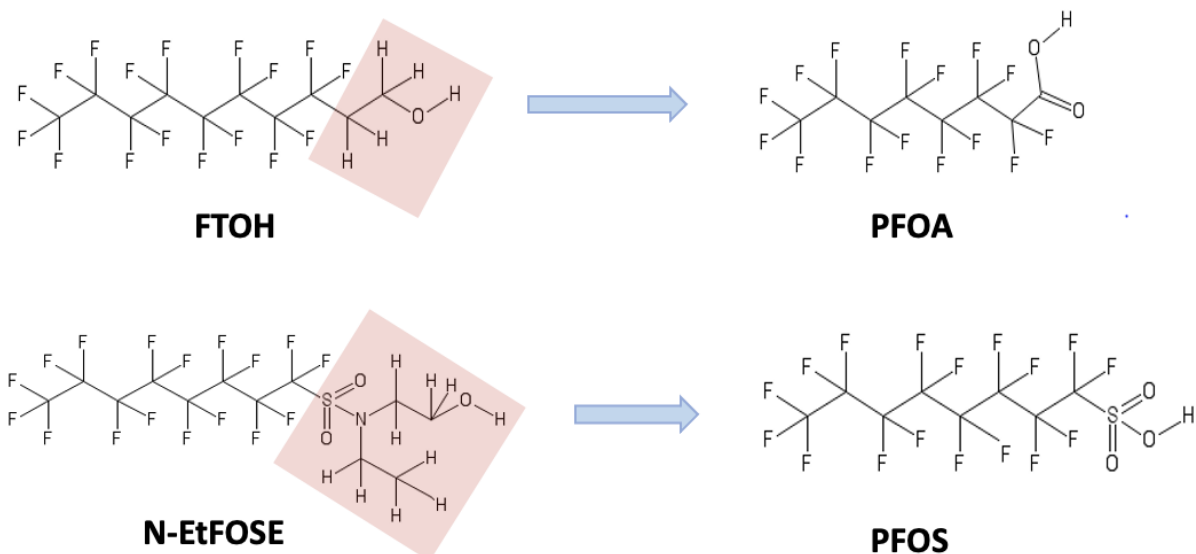
Figure 2. *Fuorotelomers as PFAS precursors. 8:2 fluorotelomer alcohol (FTOH) is a precursor of pefluorooctanoic acid (PFOA), while N-ethyl perfluorooctanosulfonamidoeoethanol (N-EtFOSE) is a precursor of perfluorooctane sulfonic acid (PFOS). Each precursor is composed of fluorinated chain and nonfluorinated moiety (highlighted in red).*

1.2.3 Per- and polyfluoroalkyl acid substitute: perfluoroalkyl ethers as novel PFAS

Per- and polyfluoroalkyether (PFAE) is a diverse group of PFAS exhibiting two perfluorocarbon chains combined by an ether-linkage which have been produced as substitutes of toxic phased out PFAS like PFOA and PFOS (Buck *et al*., 2012; Wang Z *et al*., 2013; FEON, 2015). Since the industry considers the information relative to new replacement compounds as confidential, little information is available on those molecules but emerging evidence indicates that their environmental behaviour and human hazard are similar to the substance they were produced to substitute. A recent working paper produced for the Nordic Council of Minister by Wang Z *et al*. (2020) discerns five groups of PFAE, the chemical structures of which are represented in Table 4:

- **Group 1:** Perfluoropolyethers (PFPE). 127 CAS have been described belonging to this group (Wang Z *et al.,* 2020). They are used as lubricants, emulsifiers in cosmetics and grease/waterproof paper.

- **Group 2:** Perfluoropolymers made of perfluoroether and tetrafluoroethylene (TFE) or hexafluoropropylene (HPF). They can be distinguished in 4 categories: Pefluoroalkoxy (PFA) resins, fluoroelastomers, perfluoroelastomers, and perfluorosulphonic acids. Wang Z *et al.* (2020), identified 57 CAS number for this group.

- **Group 3:** Perfluoroethers non polymers with unsaturated bonds. Those are monomers used to produce polymers. Wang Z *et al*. (2020) listed 51 CAS numbers for this group, with uses among automobile industry, non-stick coatings for cookware, water resistant fabrics, flame retardant, fire-fighting foam, membrane in fuell cells.

- **Group 4:** Perfluoroalkyl ether non polymers with saturated bonds. 152 CAS numbers were found by Wang Z *et al.,* 2020. Some of the most known PFAE belonging to this group are HFPO-DA also known by its trade name Gen X (CAS 62037-80-3) a replacement of PFOA, F53 acid (CAS 754925-54-7), 9Cl-PF3ONS also known as 6:2 Cl-PFESA or its trade name F53B acid (CAS Number 756426-58-1), F53B potassium salt (CAS 73606-19-6) and ADONA (CAS Number 958445-44-8), C604 ammonium

difluoro (CAS Number 1190931-27-1) and potassium difluoro{[2,2,4,5-tretrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy}acetate (CAS 1190931-39-5). These substances are employed for food contact materials, solar cells, cable and internet infrastructure, electrolyte for fuel cells and high-tech garment.

- **Group 5:** Side-chain perfluoroether polymers. 7 CAS numbers for polymers without known chemical formula have been described so far (Wang Z *et al.,* 2020). These substances are mainly used for coating applications in food packaging, photovoltaic panels, ceramic surfaces and textiles. Almost no information is available for this family of compounds.

Some of the best known perfluoroalkyl ethers and their trade names are (Figure 3):

- Hexafluoropropylene oxide dimer acid, ammonium salt (HFPO-DA) known as GenX, replacing PFOA in the production of PTFE;
- Chlorinated polyfluorinated ether sulfonate (Cl-PFESA) commercialised under the name of F-53B;

- Perfluoropolyether (PFPE) described by Wang Z *et al.,* 2013 with undefined formula presenting ethyl and propyl groups variable in number between 0-2 and 1-4, respectively, which replaces PFNA;

- Perfluoro{acetic acid, 2-[(5-methoxy-1,3-dioxolan-4-yl)oxy]} under the commercial name of cC604;

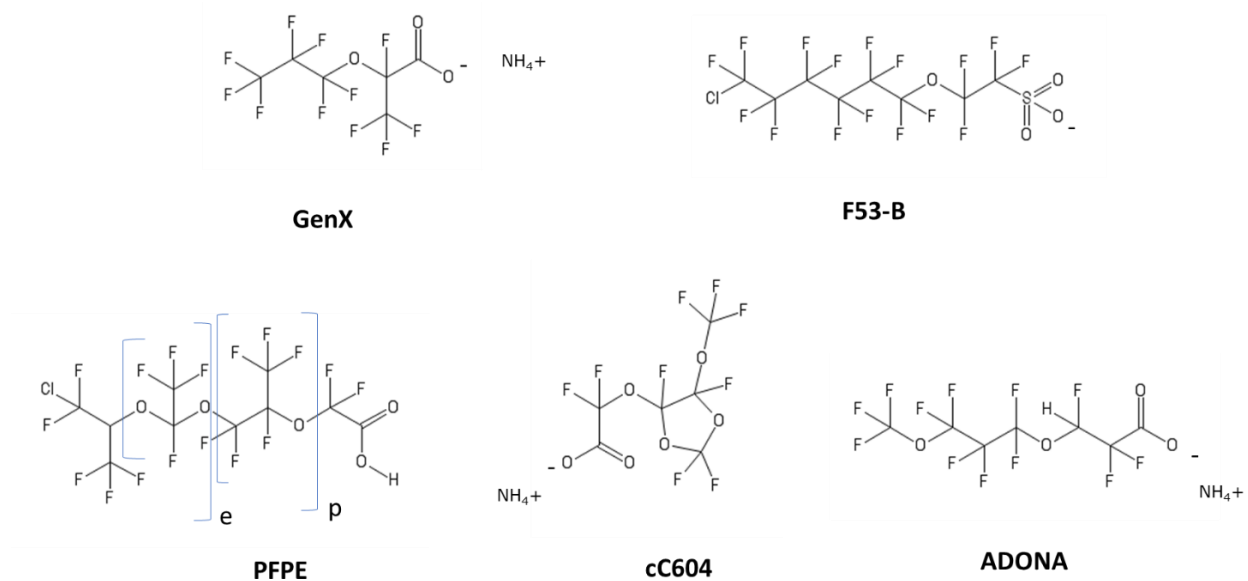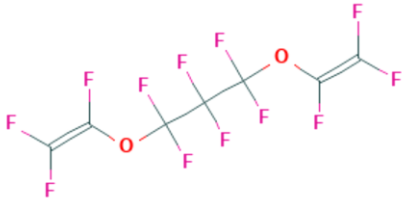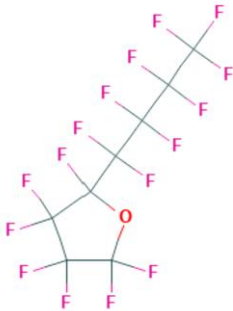- Ammonium 4,8-dioxa-3H-perfluorononanoate (ADONA).

GenX

F53-B

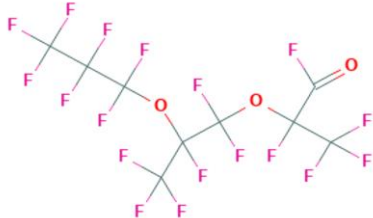**PFPE**

cC604

ADONA

Figure 3. Chemical structures of the most common PFECAs used as replacement PFAS. HFPO-DA, GenX, Cl-PFESA (F-53B), PFPE with a variable number of ethyl (e) and propyl (p) groups[1], cC604 and ADONA. Based on Lohman *et al*., 2020.

[1] According to Wang Z *et al*., 2020.

Table 4. CAS number, chemical name and chemical structure of PFAE from Group 1 to Group 4 as reported for the Nordic Council of Minister by Wang Z et al., 2020. No chemical structure is available for Group 5 substances.

CAS	Chemical name	Chemical structure
Group 1: Perfluoropolyethers (PFPE)		
60164-51-4	Poly[oxy[trifluoro(trifluoromethyl)-1,2-ethanediyl]], α-(1,1,2,2,2-pentafluoroethyl)-ω-[tetrafluoro(trifluoromethyl)ethoxy]-	
69991-67-9	1-Propene, 1,1,2,3,3,3-hexafluoro-, oxidised, polymd.	
Group 2: Fluoropolymers made of perfluoroether monomer (Group 3) and tetrafluoroethylene (TFE)		
26425-79-6	Ethene, tetrafluoro-, polymer with trifluoro(trifluoromethoxy)ethene	
Group 3: Perfluoroether non polymers used to produce Group 2 substances		
1187-93-5	Trifluoro(trifluoromethoxy)-ethylene	

CAS	Name	Structure
13846-22-5	1,1,2,2,3,3-Hexafluoro-1,3-bis[(trifluorovinyl)oxy]propane	
Group 4: Perfluoroether non-polymers with saturated carbon bond		
335-36-4	2,2,3,3,4,4,5-Heptafluorotetrahydro-5-(nonafluorobutyl)furan	
2641-34-1	2,3,3,3-Tetrafluoro-2-[1,1,2,3,3,3-hexafluoro-2-(heptafluoropropoxy)propoxy]propionyl fluoride	

1.2.4 Branched PFAS

Branched PFAS have been produced as unwanted by-products when the intended linear isomers are synthesised using electrochemical fluorination (ECF). The telomerisation method adopted more recently assures nearly 100% of linear form compared to 70-80% for PFOS, 80-85% for PFOA and 95% for PFHxS obtained with ECF (Schulz *et al.*, 2020; Benskin *et al.*, 2010). While branched isomers of PFOS are widely studied and some information is available for PFOA and PFHxS (Schulz *et al.*, 2020), other branched isomers also exist in the environment, such as FOSA (or PFOSA) (Chen X *et al.*, 2015), EtFOSA (Zabaleta *et al.*, 2018), EtFOSE, EtFOSAA (Liu J *et al.*, 2019), etc. An example of linear and branched PFOS is shown in Figure 4.

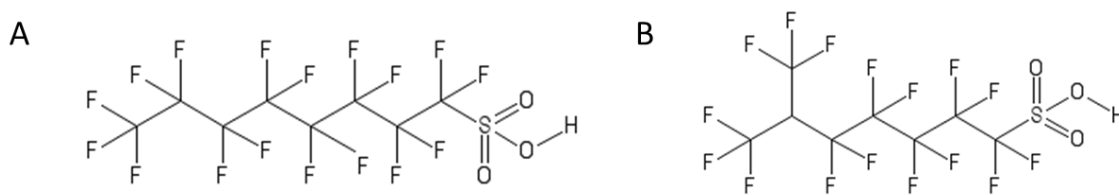**Figure 4.** Linear structure of PFOS (A) and its branched isomer (B).

2. PFAS in the environment

PFAS can be released to the environment directly as residues owing to products' life cycle, residual raw materials or impurities from manufacturing, or from indirect sources by degradation of PFAS and their precursors. As pointed out in the recent EFSA report (EFSA *et al*., 2020), some PFAS are greatly persistent, others are degraded to highly persistent PFAS and other PFAS are easily degraded and assimilated in the environment and in humans. In particular, it regards perfluoroalkyl acids (PFAA) considered final degradation products of the most commonly used PFAS and their precursors alongside their direct emission from production sites and life cycle of certain fluoropolymers (Brendel *et al*., 2018; OECD, 2013).

The short-chain replacement PFAS and the subclass of per- and polyfluoroalkylether carboxylic acids (PFECAs), initially considered less toxic, have been commonly found in environmental samples due to their properties of solubility and a higher potential for long-range transport in water. On the other hand, long-chain PFAS have a higher tendency to adsorb to particles and to bioaccumulate in animals (Martin *et al*., 2009; Ahrens *et al*., 2010; Brendel *et al*., 2018; Hoisaeter *et al*., 2019; Li F *et al*., 2020). On this basis, biota and abiotic compartments such as sediments may act as a putative sink for PFAS. Table 5 describes differences between long- and short-chain PFAS revealed in aquatic environments.

Table 5. Behaviour of short-chain vs. long-chain PFAS in the environment and organisms based on physicochemical properties.

Physicochemical properties	Short-chain PFAS	Long-chain PFAS
Water solubility	Higher	Lower
Water/soil mobility	Higher	Lower
Adsorption to soil and sediment	Lower	Higher
Bioaccumulation potential in animals	Lower	Higher
Bioaccumulation potential in plants	Higher	Lower
Overall expected toxicity	Lower	Higher

Adapted from AECOM, 2019.

Based on the OECD data, it has been estimated that most PFAS present in the environment and biota can be classified as linear isomers and non-polymers, and constitute potential precursors to PFAA (Goodrum *et al*., 2020). The same study concluded that the majority of PFAS with putative biological activity are fluorotelomer-related substances, followed by PFAAs and PFAA precursors. Such precursors degrade biotically or abiotically to PFAS constituting their indirect source.

Some precursors are not necessarily introduced to waters, as is the case for HFO-1234yf – a replacement of the phased-out HFC-134a, both fluorinated compounds used as refrigerants. Even though not classified as PFAS, these substances degrade to trifluoroacetic acid (TFA) in the atmosphere which further precipitates at increasing rates to soil and surface waters in rainfall (Pickard *et al*., 2020).

2.1 PFAS in soil and aqueous environment

The widespread use of PFAS in concert with their high persistence have led to the ubiquitous occurrence of this group of chemicals in the environment. Being highly soluble in water, PFAS are distributed worldwide in the ambient mainly by waste streams of industrial production sites (e.g., sewage sludge or wastewater), fire-fighting foam training sites (Dauchy *et al*., 2017), due to inefficient wastewater treatment (OECD, 2013; ITRC, 2020; EFSA *et al*., 2020) or to landfill leachate leading to contamination of the surrounding groundwater (Hepburn *et al*., 2019) ([Figure 5](#)). The latter shows an abundant presence of PFOA, other short-chain PFAA and PFAA precursors reflecting the shift from long-chain PFAS after restrictions in their industrial production and use (Hamid *et al*., 2018). Estimations of the USEPA within the National Sewage Sludge Survey shown that in the USA the sum of PFAS in biosolids was 2749-3450 kg/year, of which approximately 1375-2070 kg were applied on agricultural land (Venkatesan and Halden, 2013). Contaminated soil due to the application of sewage sludge has been indicated as a significant reservoir of PFAS from which the substances migrate to surface waters, surface sediments and groundwaters as recently concluded based on the global analysis of environmental PFAS concentrations (Brusseau *et al*., 2020). Especially, long-term retention of PFAS was observed in the vadose zone where leaching to groundwater occurs frequently (Brusseau *et al*., 2020). The analysis of the US EPA monitoring data revealed that PFAS concentrations in groundwaters are often higher than those determined in surface waters with increasing trends over time (Guelfo and Adamson, 2018).

Even though the industrial production of PFOA and PFOS have been halted, the release of long-chain PFAS to waterbodies will continue in future due to the degradation of PFAS precursors from sediment, soil and ice (Ahrens 2011). It means that novel PFAS currently in production, for example per- and polyfluoroalkyl ethers, may constitute a future source of pollution if not contained upon disposal, due to their decade-scale half-lives (Washington *et al*., 2019). PFOS, a C8 fluorocarbon, is one of the long-chain PFAS present to the major extent in the environment with a high potential of biomagnification, reason for which its detection in biota is significantly higher than PFOA, a C7 fluorinated substance. While they are found at similar concentration in invertebrates, fish, birds, reptiles and mammals, PFOS can be up to three orders of magnitude higher than PFOA (Ahrens and Bundshun, 2014). Long-chain PFAS (up to C13) are also present in the environment and their concentrations increase depending on geographic area and trophic level (Sturm and Ahrens, 2010). Similar to other anthropogenic pollutants, PFAS have been detected in remote areas, such as the Arctic and Africa (EU-strategy, 2019; EFSA *et al*., 2020). C9 to C12 perfluorinated acids have been found in the Arctic and North Atlantic Ocean at concentrations below 1 ng/L, presumably being the products of volatile precursors following oxidation (Joerss *et al*., 2020). In Africa, contamination of water bodies happens mainly through WWTP, solid waste dumpsites and urban centres (Arinaitwe *et al*., 2016; Groffen *et al*., 2018; Ibor *et al*., 2020) facilitated by tropical temperature and high humidity favouring PFAS volatilisation and consequent deposition (Rankin *et al*., 2016; Ssebugere *et al*., 2019). The presence of C8-C12 PFAS has been assessed in rivers and lakes in Kenya, Ethiopia, Uganda, Nigeria, South Africa and Ghana (Ssebugere *et al*., 2020).

The short-chain PFAS, including perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA) and pefluorobutanoic acid (PFBA), are giving evidence of environmental and health threats (Conder *et al*., 2008; Giesy *et al*., 2012; Houtz *et al*., 2013; Du *et al*., 2015) and result to be more persistent than the long-chain ones they substitute such as PFOA and PFOS (Brendel *et al*., 2018). They adsorb less to soil and sediment compared to the long-chain molecules which results in decreased mobility in groundwater (Brendel *et al*., 2018; Hoisaeter *et al*., 2019). Concerning linear and branched isomers, the first ones sorb easily to soil and sediment, while their branched forms tend to be more mobile in water (Schulz *et al*., 2020). Surface sediments, based on their characteristics, showed a more variable spatial distribution

of overall PFAS compared to water (Joerss *et al*., 2019). Sedimentation areas proved to contain emerging PFAS such as 6:6 and 6:8 perfluoroalkyl phosphinic acids (PFPIA) (Joerss *et al*., 2019).

Polyfluorinated substances containing 4, 5, 6, and 7 carbon atoms are the most abundantly found in the environment, with PFBS and PFBA accounting for more than 50% of the total. The short-chain molecules are already present in the air (Ahrens *et al*. 2011), land and waste (O'Connel *et al*., 2012; Yan *et al*., 2012), surface water and groundwater (Backe *et al*., 2013; Yao *et al*., 2014; Banzhaf *et al*., 2017) and ocean (Kwok *et al*., 2015). Worldwide, their presence has been detected in the Arctic (Cai *et al*., 2012), China (So *et al*., 2007; Zhou *et al*., 2013), India (Yeung *et al*., 209), Canada and US (Keller *et al*., 2005; Washington *et al*., 2010; Ahrens *et al*., 2011) and in Europe in the Rhine river (Moller *et al*., 2010). As summarised by Li *et al*., 2020, the concentrations of the most represented short-chain molecules in drinking water are quite uniform, varying from 10-104 ng/L for PFBA, 10-80 ng/L for PFBS, 10-324 ng/L for PFHpA, 10-318 ng/L for PFHxA and 10-191 ng/L for PFPeA. For river water, maximum concentrations are of 335 ng/L for PFBA and 153 ng/L for PFBS, while the levels of PFHpA, PFHxA and PFPeA are one order of magnitude lower. In sea water, all short-chain PFAS are three orders of magnitude less concentrated since they reach a maximum of few hundreds of pg/L, while in the atmosphere their presence ranges from few to less than 200 ng/L.

In the aquatic environment, PFAS cross the cell membrane of algae through a mechanism that is more pronounced for long-chain compounds (Latala *et al*., 2009). Consequently, they impact filtering and grazing invertebrates that feed on those autotrophic organisms and successively elicit effects in all vertebrates. Biomagnification through the food web depends not only on the chain length but also on the functional group; sulphonate for example has a higher accumulation tendency and uptake than the carboxylate group (Ahrens *et al*., 2016; Verhaert *et al*., 2017) which makes molecules such as PFOS much more abundant than PFOA in fish and higher predators. Biomagnification of long-chain PFAS has been proven in freshwater in France (Munoz *et al*., 2017; Simmonet-Laprade *et al*., 2019), Hong Kong (Loi *et al*., 2011) and China (Fang *et al*., 2014).

Fate and transport of PFAS after their release to the environment and their potential risk on living organisms are extremely influenced by physicochemical properties of these compounds. Moreover, surfactant characteristics favour the concentration of PFAS on air-water or non-aqueous phase liquid (NAPL)-water interfaces (Sima and Jaffé, 2021). In addition to PFAS chemical composition and structure (e.g., length of chain), factors such as natural characteristics of the site to which PFAS have been released (e.g., soil properties, type of water body, atmospheric conditions) highly influence partitioning, transport pathway, transformation and assimilation by biota, plants and humans (ITRC, 2020).

2.2 PFAS in the atmosphere

PFAS residues can also be spread via the atmosphere (exhaust gases, volatilisation, dust) and contaminate soil and water environments following precipitation ([Figure 5](#)). Besides PFAS production sites, landfill ambient air has been recently indicated as a potential source of atmospheric PFAS due to high concentrations of PFAA, particularly their semi-volatile precursors (Hamid *et al*., 2018). The presence of trifluoroacetic acid (TFA) in rainwater and fogwater has been reported over last decades at concentrations reaching 8.8×10^3 ng/L (Chen H *et al*., 2019; Tanivasu *et al*., 2008; Römpp *et al*., 2001; Wujcik *et al*., 1999). A recent modelling of PFAS air emission and atmospheric transport showed that 5% of total emitted PFAS and 2.5% of GenX may deposit within about 150 km from the production facility and reach nearly 0.1 and 10 ng/m^3 of respective concentrations at 35 km downwind (D'Ambro *et al*., 2021). A range of other new PFAS has been reported in precipitations (Scott *et al*., 2006).

Being extremely volatile, very short fluorinated chain cannot be removed by filters commonly used in industrial plants, as is the case for trifluoromethane (CHF_3), a gas 12400 times more dangerous than CO_2 for global warming potential (Mihre et al., 2013).
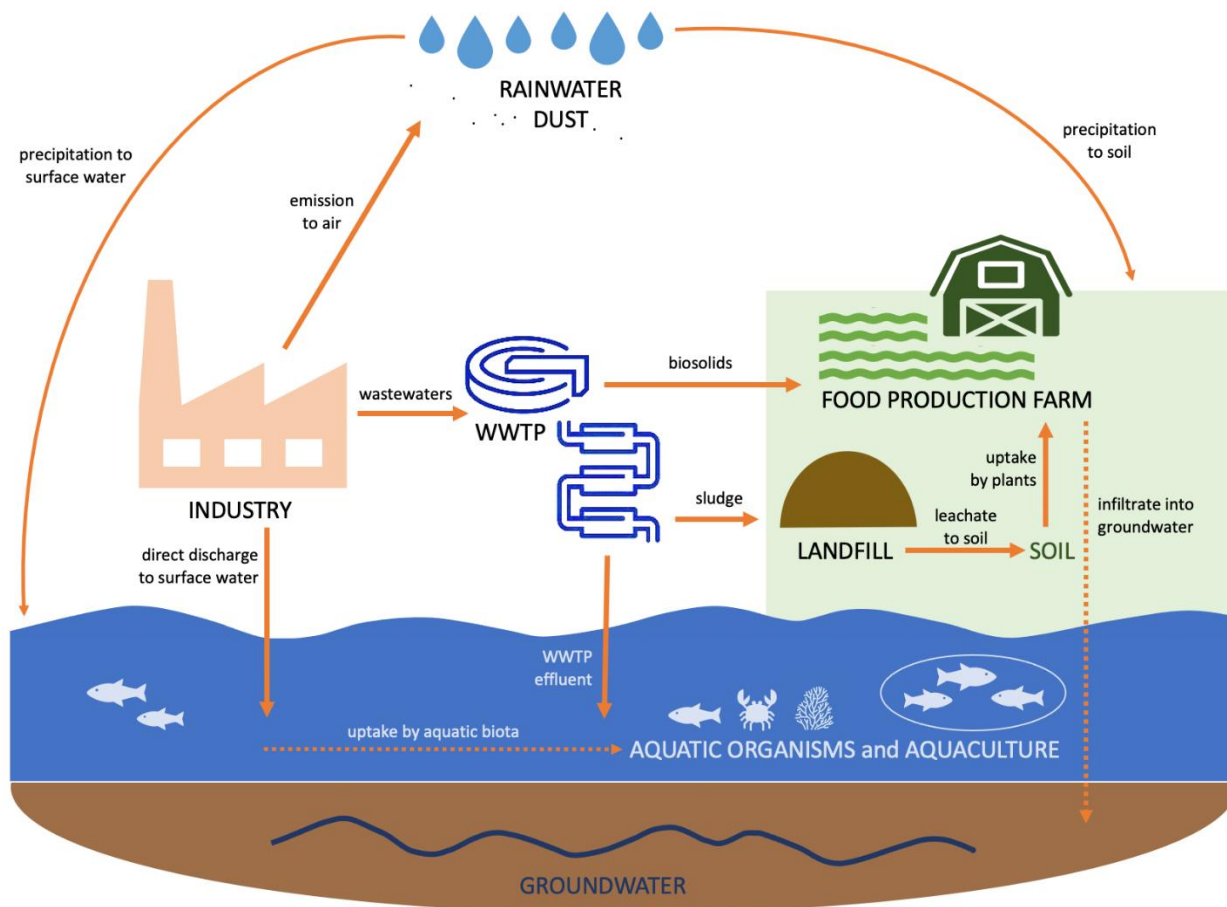

Figure 5. PFAS circulation in the environment. PFAS generated in industrial setting generally follow three environmental pathways: i) emission to air with exhaust gasses, volatilisation and dust from where they precipitate to surface waters and soil, and further infiltrate to groundwater; ii) direct discharge to surface waters being the source of exposure for aquatic wildlife and aquaculture species; iii) incomplete removal during treatment process in wastewater treatment plants (WWTP) with resulting discharge to surface water as WWTP effluent and leachate from contaminated sludge disposed in landfill. The latter source, alongside direct application of PFAS-containing biosolids, may be uptaken by plants including those intended for food production and/or infiltrate to surface water ecosystems and groundwater.

2.3 Environmental distribution and behaviour of branched versus linear PFAS

Branched PFAS are produced as an undesirable outcome when linear PFAS are synthesised using electrochemical fluorination (ECF) instead of telomerisation. Even though the different isomers have diverse behaviour, their mixtures effects have been considered as cumulative assuming equal health risk (Schulz et al., 2020).

In the environment, linear isomers bind preferentially to sediment and soil compared to the branched ones (Yu et al., 2013) probably due to a reduced hydrophobicity respect their

branched counterparts (Benskin *et al*., 2007, Gao *et al*., 2019) (Figure 6). This could explain the lower percentage of linear PFOS in lakes than in rivers as water currents may limit its potential to sorb to sediments (Ma *et al*., 2018). In a similar study in Swedish rivers, Ahrens *et al*., 2018, found that where both linear and branched PFOS were present, their concentrations in water were similar even though ECF produces up to 80% of linear PFOS.

A study on groundwater by Gao *et al*., 2019, showed that also linear PFOA and PFHxA sorb preferentially to soil compared to the branched form. In the ocean, branched isomers tend to be more present in the bulk water than in the surface microlayer (28% and 18%, respectively) possibly because of a lower surface activity caused by branching (Johansson *et al*., 2019).

For what concerns bioaccumulation in organisms, the majority of studies in humans show a preference in bioaccumulation of branched isomers, while animal studies show the opposite trend (Beeson and Martin, 2015) (Figure 6). In particular, human serum seems to bioaccumulate slightly more branched PFOS isomers (Karrman *et al*., 2007). In chickens, linear PFOS accumulation was higher in liver and yolk from chicken grown closed to a chemical facility (Briels *et al*., 2018), indicating facilitated concentration of linear PFOA, PFOS and PFHxA (Wang F *et al*., 2019). The same trend has been observed in nestling of white-tailed eagles (Løseth *et al*., 2019) and in carps from French rivers (Zhong *et al*., 2019). Moreover, branched PFAS were associated with decreased serum globulin and amplified beta-cell function, positively linked to TSH level, negatively correlated with non-HDL cholesterol and maintained the ratio between mother and infant (Liu HS *et al*., 2018; Reardon *et al*., 2019; Jain and Ducataman, 2018; Gyllenhmmar *et al*., 2018). A more exhaustive summary of the presence of branched PFAS in humans is given by Schulz *et al*., 2020.
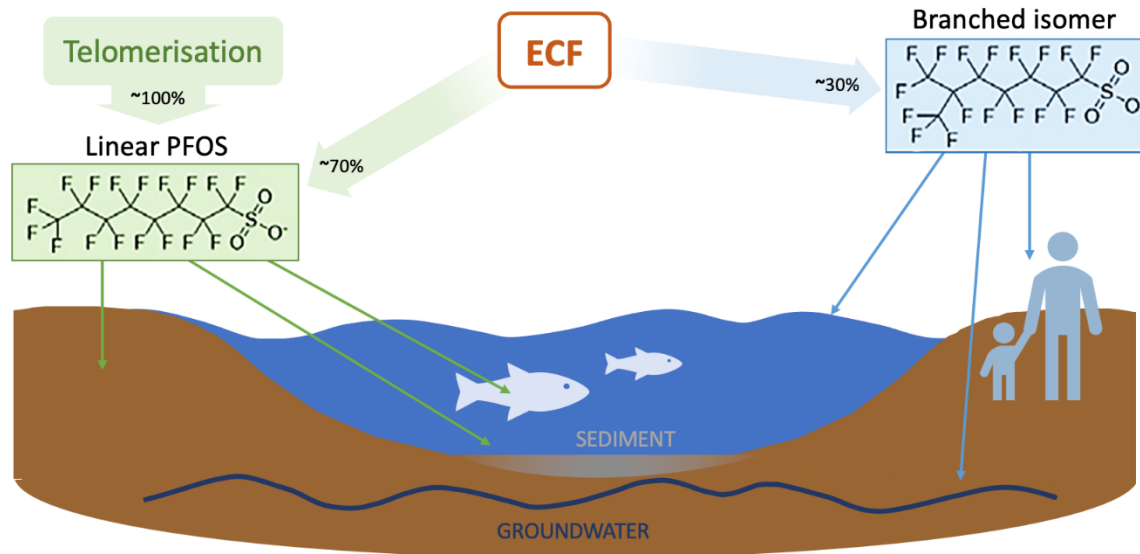

Figure 6. Schematic fate of linear and branched PFOS in the environment. The synthesis of linear PFAS by electrochemical fluorination (ECF) generates 30% of branched by-products which affect humans and biota. Adapted from Schultz et al. 2020.

3. Analytical methods and techniques for PFAS determination in environmental samples

Even though analytical methods for PFAS detection in multiple environmental media are being developed worldwide (Nakayama *et al*., 2019; Lorenzo *et al*., 2018), few of them have been validated. The regulatory frameworks of the European Union (EU) still lack standardised protocols and guidelines for monitoring PFAS and related substances in environmental samples (ECHA, 2018; EU-Strategy, 2019) which calls for urgent advancements in adopting or developing suitable strategies. In a draft document on the monitoring of PFAS in groundwater across the EU, Rüdiger *et al*. (WFD CIS, 2020) highlight the need for each Member State (MS) to adopt a state-of-the-art detection methodology to reduce variability among limits of quantification (LOQ) which differ greatly based on the employed technique. The authors also stress on the importance of designing standardised methods for a much wider number of PFAS.

In the USA, two standardised quantitative methods for determination of PFAS in drinking water have been established by the United States Environmental Protection Agency (USEPA): Method 537.1 (Shoemaker and Tettenhorst, 2020) and Method 533 (USEPA, 2019b). The method 537.1 can determine 12 PFAA and 6 precursors, while method 533 can detect 16 PFAA and 9 precursors including PFHxA, PFHpA, PFOA, PFOS, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA or PFUnDA), perfluorododecanoic acid (PFDoA or PFDoDA), perfluorobutane sulfonic acid (PFBS) and perfluorohexane sulfonic acid (PFHxS). However, the latter method requires twice as long for the extraction protocol as the 537.1 method (ITRC, 2020; USEPA, 2020; USEPA, 2019b;). A third test method, SW-846 Method 8327, which minimizes sample transfer and extraction, has been validated for detection of 24 PFAS compounds in aqueous samples (groundwater, surface water and wastewater) (ITRC, 2020; USEPA, 2020a). Furthermore, the SW-846 Method 8328, based on isotope dilution is under validation as a robust approach for complex non-drinking aqueous matrices such as wastewater influents, biosolids, fish tissues, soils and sediments (USEPA, 2020b).

Due to major concerns regarding PFOS and PFOA, the ISO 25101:2009 method has been established for determining the concentrations of these compounds in unfiltered water samples (drinking water, groundwater and surface fresh and marine water) employing high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (ITRC, 2020). At national level, the German Institute for Standardisation (*Deutsches Institut für Normung* DIN) established standard methods DIN 38407-42 and DIN 38414-14 (2011) for quantitative determination of selected perfluorinated compounds (PFC) by HPLC/MS-MS following solid phase extraction (SPE), in unfiltered water samples (drinking water, groundwater, surface waters and sewage) and in soil samples (sludge, compost and soil), respectively.

The above-mentioned protocols are based on quantitative methods which permit to measure the concentrations of specific PFAS. Such methods may employ particular technologies targeting different groups of organofluorine compounds (Table 6). However, their application is problematic due to the lack of reference materials for many PFAS and unavailability of suitable standards. Furthermore, they require equipped laboratories and highly qualified professionals to quantify PFAS at lower detection limit (Concawe, 2016).

The overall content of PFAS in environmental matrices may be determined through qualitative techniques, such as Total Oxidisable Precursor assay (TOP or TOPA), Total Organofluorine (TOF) assay and Extractable or Adsorbable Organic Fluorine (EOF/AOF) method (Concawe, 2016; Cousins *et al*., 2020; ITRC, 2020). The TOP assay, applicable to both aqueous and soil matrices (ITRC, 2020), is a method that determines a difference between the concentrations of perfluoroalkyl acid precursors and PFAS resulting from the degradation of precursors,

showing the presence or absence of total PFAS in a sample (Cousins et al., 2020), while the TOF and EOF/AOF assays determine the content of all organofluorine substances. These methods can be performed using Combustion Ion Chromatography (CIC) (Concawe, 2016; Cousins et al., 2020; ITRC, 2020), Particle-Induced Gamma-ray Emission (PIGE) spectroscopy or through X-ray Photoelectron Spectroscopy (XPS) (Cousins et al., 2020). Unlike quantitative methods, they allow to detect a wide range of fluorine compounds, among which PFAS, providing relatively less specific results (Table 6). This approach can be used considering the precautionary principle in environmental monitoring by detecting the presence or absence of PFAS (Concawe, 2016; Bokkers *et al*., 2018; EU strategy, 2019; Cousins *et al*., 2020).

At present, analytical methodology capable of analysing branched and linear PFAS at the same time is not available as well as commercial calibration standards. Benskin *et al.* (2010) first summarised methods applied for branched PFAS quantification while more recently Pellizzaro *et al.* (2018) quantified 14 linear PFAA and 10 branched isomers of PFOA and PFOS employing LC-MS/MS coupled to the solid-phase extraction (SPE).

The important issue regarding either quantitative or qualitative methods is a high possibility of false positive results due to the presence of PFAS in glassware and plasticware used during analytical procedure and to the cross-contamination by contact or/and adsorption, especially from Teflon (degassers, SPE cartridges) waterproof or polytetrafluoroethylene (PTFE) coatings, etc.[2] The official protocol 8327 from USEPA states that "careful selection of reagents and consumable is necessary"[3] since trace levels of PFAS would alter the analytical result. For a good quality assurance, the use of PFAS-free sampling materials and laboratory equipment is recommended in order to avoid accidental enrichment of PFAS fraction in a sample (ITRC, 2020; Rodowa *et al*., 2020).

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[2] https://www.aphl.org/aboutAPHL/publications/lab-matters/Pages/ABCs-of-PFAS.aspx
[3] https://www.epa.gov/sites/production/files/2019-06/documents/proposed_method_8327_procedure.pdf

Table 6. Quantitative and qualitative methods for determination of PFAS in environmental and biological samples. Examples of recent studies describing the application of selected analytical methods are provided for each sample type.

	Sample	Compounds	Analytical method	Matrix (country)	Reference
Quantitative methods	Air	Small volatile molecules, e.g., FTOH, FASA, FASE	GC-MS	Indoor air (Norway)	Padilla-Sanchez *et al.*, 2017
		Ionic PFAS, e.g., PFCA and PFSA	HPLC-MS/MS	Outdoor air (Czech Republic)	Paragot *et al.*, 2020
	Water	PFCAs, PFSAs, precursors, emerging PFAS	UHPLC-MS/MS	Wastewater, surface water, groundwater and drinking water (Italy)	Ciofi *et al.*, 2018
			HRMS (Orbitrap, TOF-MS)	Drinking water (UK)	Harrad *et al.*, 2019
		Larger polar molecules, e.g., PFCA and PFSA	LC-MS/MS	River water (Spain)	Navarro *et al.*, 2020
		Small volatile molecules	GC-MS or MS/MS	River water and wastewater (Spain)	Portolés *et al.*, 2015
	Abiotic solids	PFCA, PFSA, and precursors	GC-MS or MS/MS	Wastewater, soil and sludge (France)	Dauchy *et al.*, 2017
			UHPLC-MS/MS	Dust (Greece)	Besis *et al.*, 2019
			HPLC-MS/MS or	Sediment (Italy)	Pignotti and Dinelli, 2018
			HRMS (Orbitrap, TOF-MS)	Dust (UK)	Harrad *et al.*, 2019
	Biological	PFCA, PFSA, precursors, emerging PFAS	UHPLC-MS/MS	River water and fish (Finland)	Junttila *et al.*, 2019
				Breast milk (Spain)	Beser *et al.*, 2020
			HPLC-MS/MS	Fish (Italy)	Mazzoni *et al.*, 2019
		Unknown PFAS	UHPLC-HRMS	Soil (Germany)	Kotthoff *et al.*, 2020
Qualitative methods	Various environmental and biological	PFAA precursors as result of PFAS degradation	TOP assay	Wastewater (USA)	Houtz et al., 2016
				Soil (Germany)	Janda *et al.*, 2019
				Water, soil, biota (France)	Simonnet-Laprade *et al.*, 2019a
		Organofluorine compounds, e.g., PFAS	AOF assay	River water, groundwater, wastewater (Germany)	Willach *et al.*, 2016
			EOF assay	River water, sediments, biota (Norway)	Langberg *et al.*, 2020
			TF assay	Serum, (Sweden)	Miaz *et al.*, 2020

TOP: Total Oxidisable Precursor; AOF: Adsorbable Organic Fluorine; EOF: Extractable Organic Fluorine; TF: Total Fluorine

4. PFAS-related concerns

4.1 Health and environmental risk

PFAS are considered high-priority hazardous substances due to their environmental persistence, bioconcentration[4], bioaccumulation[5] and biomagnification[6] in aquatic and terrestrial food chains, which poses a high risk to the ecosystems and in turn may cause adverse health effects in humans (OECD, 2013; EU-strategy, 2019). In case of perfluorinated acids (PFCAs and PFSAs), the rate of bioconcentration/bioaccumulation seems to directly correlate with the length of the fluorinated carbon chain (Conder *et al*., 2008). Functional groups may further enhance this relationship as described for PFSAs which appear more bioaccumulative than PFCAs with the same number of C atoms. However, non-polymeric PFAS are of greatest concern due to major mobility compared to their polymeric counterparts and to major diversity of properties resulting in a wide range of effects in organisms (Henry *et al*., 2018).

PFAS have been detected globally in humans and wildlife as reported in a large number of epidemiological studies. One of the most striking examples is the presence of PFOS and other PFAS in the human blood serum of 99% samples collected across the USA over the period 1999-2012 (USEPA, 2019). Associations of PFAS concentrations in biological liquids with suggested effects of exposure in humans has been listed in a recent review (Chohan et al., 2020). Also recently, juvenile seabirds from American coasts showed traces of PFOS in liver even after years from its ban, along with the presence of novel PFAS (Robuck *et al*., 2020). Even though knowledge regarding the uptake of PFAS by organisms is increasing, research is still needed for determination of sensitive species.

Exposure-related effects of PFAS in several species include liver toxicity, imbalance in the lipid metabolism, immune system dysfunctions and developmental toxicity (EU-strategy, 2019; EFSA *et al*., 2020; Fenton *et al*. 2020). Among PFAS, PFOS and PFOA are classified in Europe as suspected carcinogens (EU-strategy, 2019), however evidence on the causal association between exposure to PFAS and cancer is insufficient (ITRC, 2020). Major and less frequent putative effects of PFAS on human health are shown in Figure 7.

Recently, EFSA *et al*. (2020) indicated diet as the primary source of PFAS exposure, in particular fish, fruits, eggs and processed products derived from these ingredients as well as drinking water. Importantly, tap water samples collected worldwide showed the presence of newly-identified PFAS, among which cyclic and ultrashort-chain substances (Kaboré *et al*., 2018; Mak *et al*., 2009). Dust ingestion and indoor air inhalation were identified among the main non-dietary PFAS exposure sources (Sunderland *et al*., 2019). Primary exposure pathways and sources of PFAS are shown in Figure 8.

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[4] Concentration of a substance in an organism due to the environmental exposure.
[5] Concentration of a substance in certain tissues of an organism through intake, absorption *via* the oral/contact route or by environmental exposure.
[6] Concentration of a substance in an organism in relation to the chemical concentration in its diet.

Health effects in adults

Thyroid disease
Increased cholesterol levels
Liver damage
Kidney cancer
Testicular cancer
Breast cancer
Inflammatory bowel disease (ulcerative colitis)

Developmental effects in children

Reduced response to vaccines
Lower birth weight
Delayed mammary gland development
Growing, learning and behavioral issues
Early puberty onset
Low sperm count and mobility

Effects on pregnancy

Increased time to pregnancy
Increased miscarriage risk (i.e. pregnancy loss)
Pregnancy induced hypertension/ pre-eclampsia (increased blood pressure)

Figure 7. PFAS toxicity endpoints in humans. In addition to adverse health effects which can be elicited in adults, PFAS can have negative impact on pregnancy and alter the development of children with consequences in adulthood. Effects with high certainty of evidence are indicated by bold characters. Adapted from EEA, 2019.
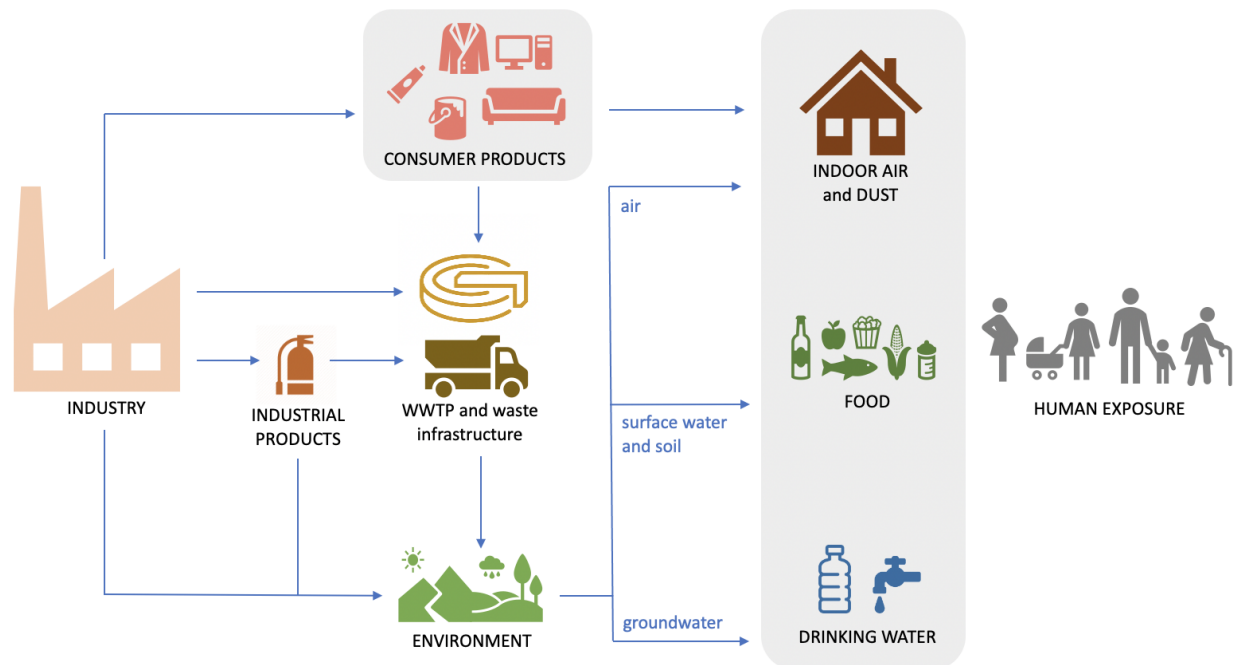
Figure 8. Main pathways of human exposure to PFAS. From manufacture settings, PFAS may spread in the environment through the application of industrial products, incomplete removal during waste treatment processes or through direct discharge. Environmental contamination may become a source of PFAS through the main three ways: the systems exploiting groundwater to provide drinking water, surface water and soil used for agri-food production as well as air and dust inhaled indoor. Consumer products constitute a source of PFAS exposure through contact and absorption, and contribute to worsening the quality of domestic air due to their half-life.

It is hard to estimate the range of PFAS spread in the environment because influenced by characteristics of individual compounds. Especially persistence, solubility and mobility, may determine whether PFAS spread in multiple environmental settings impacting very large areas or follow specific transport pathways which can form complex routes of diffusion between interconnected ecosystems. Predicting long-term effects of PFAS in organisms due to bioaccumulation is troublesome even with standardised methods which might show no acute toxicity while chronic effects may occur upon prolonged exposure to low doses (von der Trenck *et al*., 2018).

4.2 Gaps in the scientific evidence and monitoring data

Current frameworks regulating organofluorine compounds are based on studies limited to a subset of PFAS for which more complete scientific data are available, while effects of less-studied PFAS co-occurring in environmental matrices remain generally unaddressed. Likewise, most human biomonitoring data are relative to the levels of PFOS and PFOA in biological liquids, mostly blood, breast milk and umbilical cord blood (ECHA, 2018; EU-strategy, 2019; EFSA *et al*., 2020). Moreover, studies describing effects to human health are based on cohorts composed of chemical workers and communities near to industrial sites which employ PFAS or to areas with contaminated municipal drinking water, where exposure is increased due to extremely high PFAS concentrations (EFSA *et al*., 2019; ITRC, 2020). Animal studies are

usually based on one-species data accounting for direct effects in one generation, while fewer studies exist on PFAS transfer to further generations and to other species in the food chain.

Missing ecotoxicological data for the vast majority of PFAS and the heterogeneity of organofluorine compounds as a group is linked to insufficient information on real environmental concentrations of PFAS and background levels. Even less is known about combined effects of PFAS in chemical mixtures and the occurrence of PFAS substitutes (Brandsma *et al*., 2019). Environmental monitoring of PFAS and determination of their routes in the ecosystems are complicated because of many possible PFAS sources, many of which have multiple mechanisms of release. The monitoring relying on PFOS and PFOA, once the most commonly used PFAS, became inadequate since their ban and introduction of novel compounds as alternative substitutes which may pose risk to humans and wildlife. Among such substitutes, short-chain PFAS have been considered less bioaccumulative, however scientific evidence regarding their safety is limited to a small number of compounds. Their degradation into extremely persistent forms, increased solubility and mobility in water compared to long-chain PFAS may favour contamination of groundwater (Wang Z *et al*., 2015; Rankin *et al*., 2016) and the uptake by plants, including food crops (Ghisi *et al*., 2019; Kim H *et al*., 2019; Liu Z. *et al*., 2019). It has been observed that low PFOS concentrations in soil (0.23-4 mg/kg soil) may increase the bioaccumulation factor (BCF) for vegetable and wetland plants, which then tends to decrease at 40 mg PFOS/kg soil concentration (Lal *et al*., 2020; Qiao *et al*., 2021). For PFAA, a positive relationship between initial concentrations and bioaccumulation in plants was observed (Zhang D *et al*., 2019). Once in plant roots, the transfer of PFAS to shoots is not entirely reversible (Müller *et al*., 2016; Wang TT *et al*., 2020) and occurs more easily for short-chain PFAS (Zhang L *et al*., 2019), while long-chain PFAS bioaccumulate in roots rather than being transported to other plant tissues (Gredelj et al., 2020; Sharma *et al*., 2020; Zhang L *et al*.,2019). Recently, the ubiquitous presence of TFA in plants has been highlighted, pointing at the same time at limited data available for the uptake of PFAA precursors, ultrashort-chain and emerging PFAS, such as GenX or fluorinated ethers, by agricultural plants (Lesmeister *et al*., 2020).

Since the implication of short-chain PFAS in harmful effects on human health and environment are uncertain, concerns about their employment as replacement for long-chain PFAS have been raised (Wang Z *et al*., 2013; Scheringer *et al*., 2014; Blum *et al*., 2015). It has been recently pointed out that despite hazard quotients (HQ) calculated for the majority of PFAS detected in the environment (i.e., below their predicted no-effect concentrations) could reach values <1, knowledge gaps related to new PFAS along with their mixture toxicity, precursors and degradation products make risk analysis incomplete, especially considering sub-lethal and/or chronic effects (Sinclair *et al*., 2020). Available toxicological data are often noncongruent in terms of methodology and risk assessment approach. Moreover, the exact structure of many replacement PFAS is covered by trade secret. Mode of action (MoA) and adverse outcome pathways (AOP) constitute major knowledge gaps for most PFAS that impede robust assessment of PFAS-related effects in realistic samples (i.e., mixture effects) (Goodrum *et al*., 2020).

In addition to knowledge gaps, current technical guidelines for EQS derivation require revision to cover endpoints targeted by PFAS which involve molecular and genetic biomarkers. Although the extent to which biomarkers are able to provide unambiguous and ecologically relevant indicators of exposure to toxicants or their effects is still under debate, and interpretation of the biomarkers response requires full understanding of environmental, physiological, and toxicological factors (Forbes *et al.*, 2016), ecologically relevant biomarkers such as behaviour, reproduction, growth, energy metabolism, lysosomal integrity, immunotoxicity, along with genotoxicity biomarkers appear as promising candidates to improve ecological risk assessment and to support regulatory decisions (Mouneyrac and Amiard-Triquet, 2013). In particular, biomarkers' responses may help in understanding the

mechanisms underlying effects observed at higher levels, provide important insights regarding the mechanisms of toxicity and how organisms deal with toxic chemicals, and generate testable hypotheses in carefully controlled studies that lead to unravelling the mechanistic bases of chemical effects on whole organisms (Forbes *et al*., 2006).

4.2.1 PFAS-related modes of action and adverse outcome pathways

Information on the mode of action (MoA) is particularly useful in the assessment of PFAS safety based on intermediate effects, including those substances for which toxicological data are incomplete, and their combined action in mixtures, especially when considering a group of molecules with a wide range of possible effects. The MoA indicates a causal chain of events at different levels of biological complexity, from exposure to the final outcome.

Hitherto, the unique MoAs among PFAS have been established for PFOS and PFOA providing a causal explanation of liver tumour development in rodents through activation of peroxisome proliferator-activated receptor α (PPARα) upon exposure to these compounds, with a possible relevance for effects in humans (Fenton et al., 2020). Nonetheless, other effects independent from PPARα or the same effect elicited through different pathways cannot be precluded. The MoA associated with the majority of health effects of PFAS have not been fully characterised in animal models and humans (ATSDR, 2021).

Except PFOS and PFOA, studies aimed at determining the MoA of PFAS have been performed for only a few compounds. Among a range of molecular targets shown to be activated by PFAS as initiating events are the nuclear receptors, mainly peroxisome proliferator-activated receptors (PPARα, PPARγ and PPARβ/δ), pregnane X receptor (PXR), liver X receptor (LXR), constitutive androstane receptor (CAR) and estrogen receptor subunit alpha (ERα) (Behr *et al*., 2020; Bijland *et al*., 2011; Bjork *et al*., 2011; Li C-H *et al*., 2019; Rosen *et al*., 2017, Rosenmai *et al*., 2017). Established receptor-mediated MoAs are limited to hyperplasia and carcinogenic effects in liver specific to animal models (Butenhoff *et al*., 2012; Corton *et al*., 2018; Elcombe *et al*., 2012), while few studies addressed other critical effects such as developmental toxicity, immune suppression or lipid metabolism (Andersen *et al*. 2007; Jones *et al.* 2003; Pouwer *et al.* 2019; Tan *et al*. 2013; Xu HE *et al*. 1999; Temkin *et al*. 2020).

Concentration addition of PFAS in mixture suggests similar MoAs with shared molecular targets (Godfrey *et al*., 2017; Kar *et al*., 2018) but with different potency between PFAS and their analogues, partly explained by unequal excretion and kinetics of protein interaction (Wolf *et al*., 2008; Zeilmaker *et al*., 2018). When assessing cytotoxicity of novel PFAS in human liver HL-7702 cell line, Cl-PFESA and HFPO homologues bound to the human liver fatty acid binding protein (hL-FABP) with unique modes and higher binding energy than PFOS and PFOA (Sheng *et al*., 2017).

Currently used standard detection methods to detect PFAS are independent of their MoAs, thereby unable to detect related effects. Specific effect-based methods (EBMs) are being developed to overcome this gap, such as *in vitro* bioassays which are complementary to the analytical methods and permit to avoid more complex *in vivo* testing. Besides EBMs based on PPAR-mediated signaling, PFAS-CALUX bioassay is able to detect cumulative effects of PFAS by assessing thyroid hormone competitive binding to transthyretin receptor (TTR) using human U2OS cell line stably transfected to express thyroid receptor β (TRβ) (Collet *et al*., 2019). Additionally, it was possible to establish relative potency factors (RPF) for 23 PFAS in water and food samples expressed as PFOA equivalence (Bil *et al*., 2021) (as further described in section 4.2.2.1).

4.2.2 Combined effects of PFAS in mixture with other substances

Despite differences in solubility and mobility among PFAS, shared distribution patterns have been observed in the environment (Bil *et al*., 2021) leading to co-occurrence in various ecosystems (surface water, groundwater, soil), drinking water and food (Brandsma *et al*. 2019; Ghisi *et al*., 2019; Hopkins *et al*., 2018; Joerss *et al.,* 2019; Li P *et al.,* 2019) which constitutes conditions for exposure to multiple PFAS simultaneously. Nevertheless, a handful of literature is available on combined effects elicited by PFAS and even less studies provide approaches for the risk assessment of PFAS mixtures. Moreover, complex PFAS mixtures have been included in few studies.

Different effects depending on the composition and concentrations of PFAS and other compounds in mixtures have been reported in model organisms (Figure 9). Recently, liver injury along with increased risk of cardiovascular disease were observed in mice exposed to a binary mixture of PFOS and polychlorinated biphenyl PCB126 but not to single compounds (Deng *et al*., 2020). Additive effects of PFOS and PFOA were observed in acute toxicity tests on American bullfrog (*Rana catesbeiana*), although some effects on frog development were elicited by single compounds (Flynn *et al*., 2019). Recent studies found correlations between levels of selected neurotransmitters in Northern leopard frog brains and exposure to a mixture of PFAS commonly found in sites contaminated by AFFF and major PFAS bioaccumulation when compared to PFOS exposure (Foguth *et al*., 2020). Contrarily, a mixture of six PFAS was less potent in inducing behavioural toxicity in zebrafish (*Danio rerio*) embryos than individual compounds, with the greatest toxic potential attributed to long-chain PFAS and sulfonate active group and major bioaccumulation of short-chain PFAS (Menger *et al*., 2019). Similarly, prediction based on computational modelling showed the following trends in toxicity of theoretical halogenated mixtures containing PFAS on zebrafish embryos: single chemical > binary mixture > tertiary mixture (Kar *et al*., 2018). Upon a 78-hour *in vivo* exposure to the mixture containing PFOS, PFHxA and PCB126, zebrafish embryos showed modified expression of genes involved in the PCB126 toxicity-related pathway (Blanc *et al*., 2017). Alterations in dopaminergic signaling and endocrine function (i.e. 17β-estradiol level) were induced by exposure of Atlantic cod (*Gadus morhua*) juveniles to a mixture of PFAS and polycyclic aromatic hydrocarbons (PAHs) (Khan *et al*., 2019). *In vitro* testing coupled to *in silico* modelling based on the generated data to predict the effects of theoretical mixtures showed increased cytotoxicity of amphibian fibroblast cell line when exposed to binary mixtures of common PFAS (PFOS, PFOA, PFHxS and PFHxA) with additive effects except for PFOS and PFOA (Hoover *et al*., 2019).

In humans, the Health Outcomes and Measures of Environment (HOME) study provided outcomes on prenatal and early life exposure to relevant environmental chemicals, among which PFAS, in an American prospective cohort. Among others, the effect of PFAS on the endocrine system quantified in blood and urine of pregnant women along with other endocrine disrupting chemicals (EDCs) (PCBs, polybrominated diphenyl ethers and organochlorine pesticides) was associated with lower birth weight (Woods *et al*., 2017). A mixture approach adopted to assess combined PFAS effects on maternal and neonatal thyroid function by measuring thyroid hormones and PFAS concentrations in plasma supported the hypothesis of PFAS mixture effects due to prenatal exposure, however exposure concentrations and long-term impact were not evaluated (Lebeaux *et al*., 2020; Preston *et al.,* 2020). In other studies, mixture effects of six PFAS, including PFOA substitute (F-53B), were positively associated with levels of estrogen hormones in Chinese newborns (Liu Z. *et al*., 2020). A cumulative risk assessment of 17 PFAS employing the hazard index approach showed the association of blood/serum PFAS levels with hepatotoxicity or reproductive toxicity in a subgroup of general Swedish population exposed to PFOS through diet (contaminated fish) (Borg *et al*., 2013). The same endpoints assessed in a professionally exposed population toxic effects due to PFOA and/or combination of its congeners (Borg *et al*., 2013). After assessing the concentrations

of 17 PFAS in maternal plasma and serum during gestation, no association with spontaneous preterm birth was detected, however link with biochemical pathways of inflammation was suggested due to altered monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) levels (Liu X. *et al*., 2020).
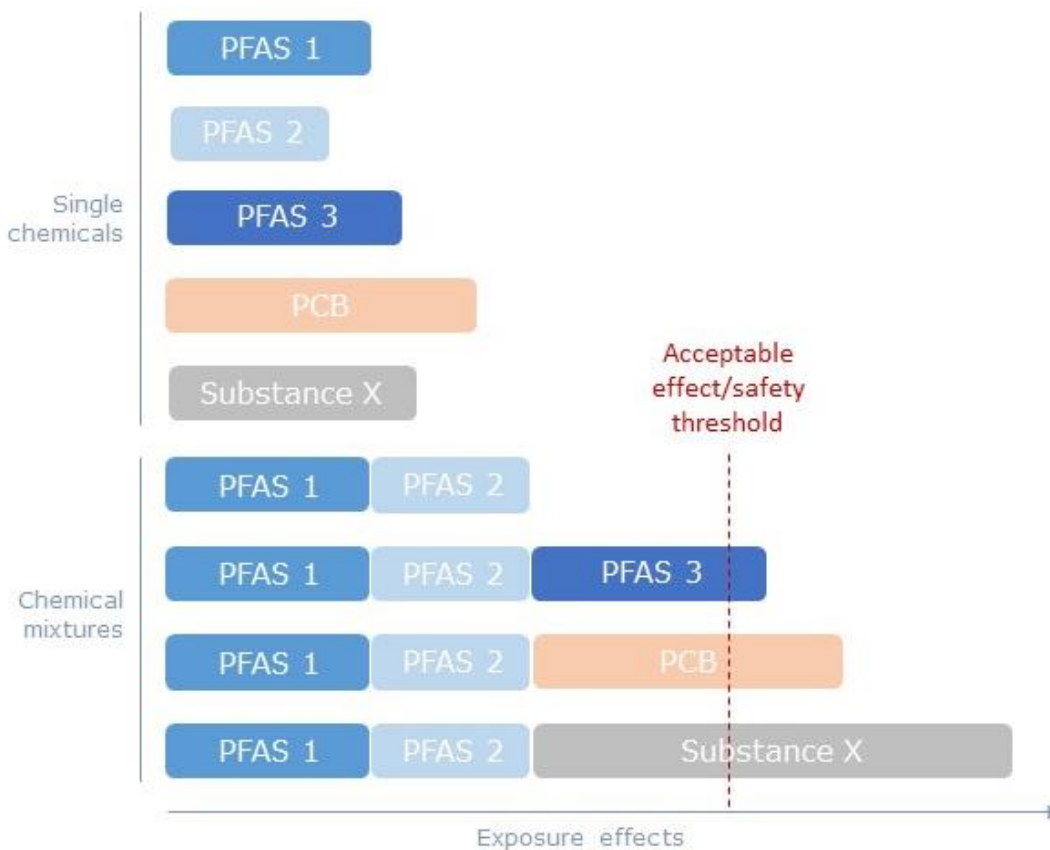

Figure 9. Effects of PFAS as single substances and in mixture. When present individually, various chemicals may elicit acceptable effects without exceeding the safety threshold. In combination with other substances of the same class, such as mixture of PFAS, or with chemicals eliciting similar effects through a different mode of action (MoA), such as polychlorinated biphenyls (PCB), may to the exceedance of the safety value. In some cases, the effects of certain substances (substance X) may be enhanced by co-occurring compounds.

4.2.2.1   Establishing safety thresholds for cumulative effects of PFAS

In the scientific evaluation of the risks to human health related to the presence of PFAS in food, EFSA (2020) established a threshold of 4.4 ng/kg body weight as tolerable weekly intake (TWI) referred to the sum of PFOA, PFNA, PFHxS and PFOS, which takes into account possible mother-to-child bioaccumulation upon long-term exposure (Figure 10). The substances were selected due to similar toxicokinetic properties, accumulation and long half-lives in humans, as well as the contribution of ~50% to the overall exposure to 17 PFAS included in the risk assessment. The estimate was based on the decreased response of the immune system to vaccination considered the most critical effect observed in animals and humans. The key study (Abraham *et al*., 2020) showed that only for PFOA there was a significant association with antibody titres against three different vaccines. For PFOS, PFHxS and PFNA this was not observed. However, the association was also significant for the sum of the four PFASs.

Therefore, assuming equal potencies for the four PFAS, the health based guidance value (HBGV) was derived considering the lowest benchmark dose level (BMDL$_{10}$) of 17.5 ng/mL for the sum of the four PFASs in serum identified for 1-year-old children in a human study (Abraham *et al.*, 2020). Using physiologically-based pharmacokinetic (PBPK) modelling, this serum level of 17.5 ng/mL in children was estimated to correspond to long-term maternal exposure of 0.63 ng/kg bw per day. Since a decreased vaccination response is regarded as a risk factor for disease rather than a disease itself, and since the study was based on infants which appear to be a vulnerable population group, no additional assessment factors for potential intraindividual differences in toxicokinetics and toxicodynamics were deemed necessary. Therefore, EFSA established a group tolerable weekly intake (TWI) of 7 x 0.63 = 4.4 ng/kg bw per week, in order to take into account the long half-lives of these PFASs (EFSA, 2020).
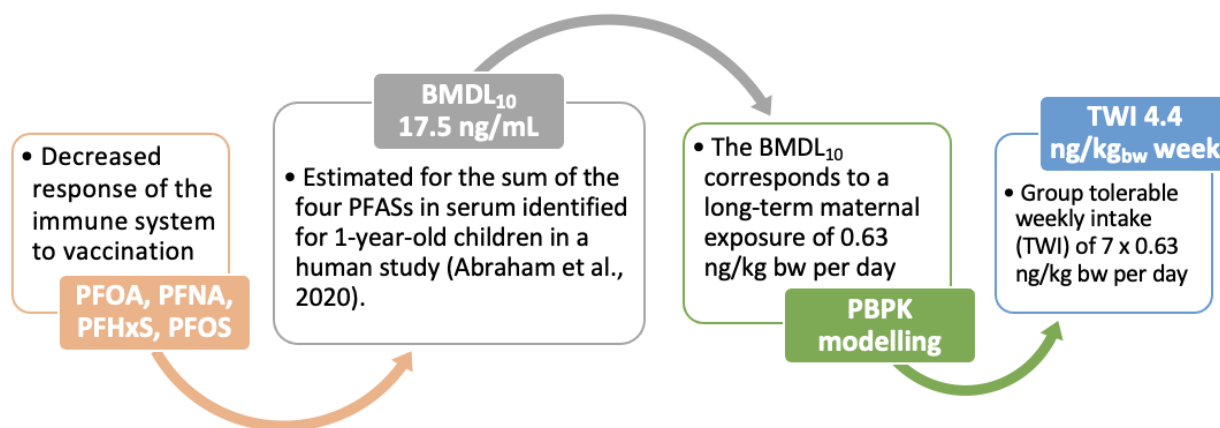
Figure 10. Schematic representation of the derivation of the EFSA's tolerable weekly intake (TWI). The established value of 4.4 ng/kg body weight (bw) per week is referred to the sum of PFOA, PFNA, PFHxS and PFOS (EFSA, 2020).

A relative potency factor (RPF) approach previously applied to other classes of chemicals, including dioxins and dioxin-like polychlorinated biphenyls (PCB) (Zeilmaker *et al.*, 2018; Bosgra *et al.*, 2009, Boon *et al.*, 2008; Van den Berg *et al.*, 2006), has been proposed as a screening tool for the assessment of dietary cumulative exposure to PFAS (Bil *et al.*, 2021). This approach does not rely on grouping substances depending on their mode of action (MoA) but rather on their target organ. The evaluation was based on literature data reporting liver toxicity in male rat after oral exposure to 14 PFAA and 2 PFAA precursors, with reference to PFOA as the most toxic PFAS (PFOA equivalence). For each PFAS, benchmark dose (BMD) was first established according to the EFSA guidance on the use of the benchmark dose approach in risk assessment (EFSA, 2017) and then applied to derive RPF with 90% confidence interval. When considering chain length, the study showed that the potency of PFCA and PFSA with 7 to 12 perfluorinated carbon atoms is equal to or higher than the potency of PFOA. Among the assessed PFAS, perfluoroalkyl ether carboxylic acids (HFPO-DA and ADONA) and fluorotelomer alcohols (6:2 FTOH and 8:2 FTOH) resulted less potent than the reference compound. Moreover, the RPF approach allowed to determine the exceedance of safety limits by PFOA equivalents in dietary exposure to PFAS through a specific food category (fish). In this way, the RPF approach becomes useful also in estimating the risk associated with cumulative exposure to PFAS resulting from food or drinking water intake (Bil *et al.*, 2021). A schematic representation of the RPF methodology is shown in Figure 11.

$$RPF_i = BMD_{PFOA}/BMD_i$$

$$C_{sumPFAS} = {}^n\Sigma_{i=1} \, (C_i * RPF_i)$$

Sum of PFOA equivalents based on the RPF

Estimation of the cumulative exposure

Concentration or consumption limit values
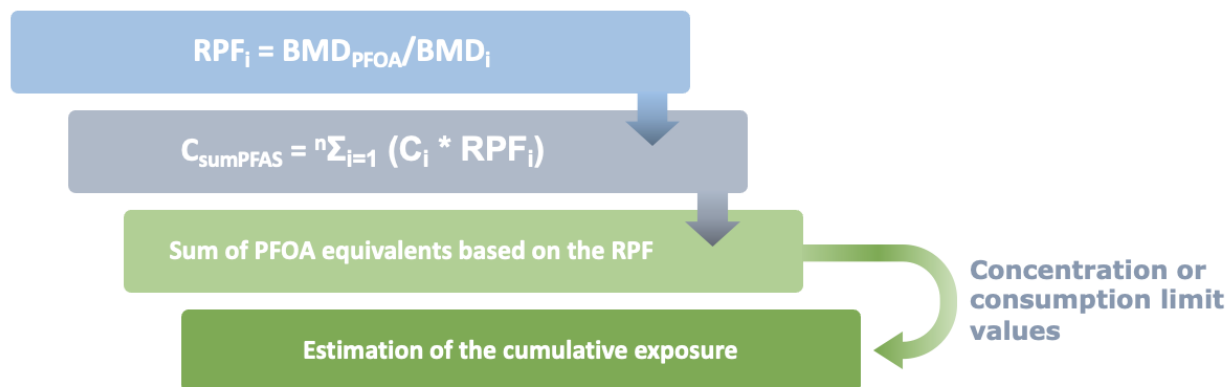
Figure 11. Schematic representation of the relative potency factor (RPF) methodology. The RPF is defined as the ratio of the benchmark dose (BMD) of the PFAS index compound (i.e., PFOA) and the BMD of any other PFAS ($PFAS_i$). The individual PFAS concentrations (C_i) per sample are then multiplied by their corresponding RPF to obtain the concentration in PFOA equivalents. The sum of all PFOA equivalents can then be compared to an available drinking water concentration limit or fish consumption limit (Bil et al., 2021).

Recently, an approach for prioritisation and characterisation of PFAS mixtures from surface water sites with a history of aqueous film-forming foams (AFFF) use was developed for realistic scenarios based on heterogeneous ecotoxicological data quality (East *et al*., 2020).

4.2.3 Environmental presence and effects of novel PFAS and PFOS/PFOA substitutes

While the identity of few hundreds of new compounds developed and introduced to substitute the PFAS of major concern (Table 9 listing the PFAS proposed as chemical parameter for the revised Drinking Water Directive) is now known, very few information on their toxicity and behaviour in the environment is available. Thus far, a range of newly-identified anionic, cationic, zwitterionic, non-ionic and partially fluorinated PFAS with different chain lengths and cyclic/linear structures have been reported in environmental samples and aqueous film-forming foams (AFFF) (Place and Field, 2012; Xiao, 2017). A summary of novel PFAS detected in the environment is presented in Table 7. For the purposes of this review, the reliability of the reported studies was not further assessed according to guidance recommendations (EC, 2018).

It has been reported that some of these compounds may be transformed to PFAA and/or fluorotelomer sulfonates during biological and photolytic degradation (D'Agostino and Mabury, 2017; Mejia-Avendaño *et al*., 2016; Moe *et al*., 2012). In landfills, the release of PFAS to leachate is mainly due to biodegradation processes, especially the methanogenic phase, however little is known about PFAA precursors including semi-volatile compounds and their degradation products (Hamid *et al*., 2018). PFCA precursors constitute up to 75% of all PFAS identified in sludge samples from the European Nordic territories and their presence, alongside ultrashort-chain PFAS and a mix of PFAS classes, was detected in wastewater treatment plant (WWTP) effluent samples (Kärrman *et al*., 2019). Moreover, the aquatic environment has been suggested as a possible ultimate sink of newly-identified PFAS given their solubility in water and moderate hydrophobicity coupled to ionic or ionizable properties and nonvolatile characteristics (Xiao, 2017). Among them, ultrashort-chain PFAS have been detected in a variety of water samples with up to 40% of total PFAS in rainwater (Yeung *et al*., 2017), even though general literature on their environmental presence is scarce which might be due to analytical limitations and consideration of these compounds as less bioaccumulative than

long-chain PFAS. Bioaccumulation factor (BAF) estimated in fish for perfluoro-4-ethylcyclohexane-sulfonate (PFECHS), an emerging cyclic PFAS detected in 86% of samples from the Baltic Sea (Joerss *et al*., 2019), exceeded BAF value of PFOA (2.8 vs. 2.1, respectively) (De Silva *et al*., 2011), while BAF calculated for F-53B was almost equal to that of linear PFOS (Wang Y *et al.*, 2016). Although environmental concentrations of F-53B have been determined in various water samples, aquatic organisms and human biological liquids (Munoz *et al*., 2019), very few biomonitoring reports are available for other trademarked PFAS.

It is still uncertain to which extent the novel PFAS affect human health, however their adverse effects have been reported in organisms. Growth inhibition and antioxidant responses to PFECHS, F-53B and GenX were observed in *Chlorella* sp. at environmentally relevant concentrations (Niu *et al*., 2019). In northern pike (*Esox lucius*) collected upstream and downstream a wastewater treatment plant (WWTP), altered tissue-specific expression of toxicity response-related genes was associated with PFECHS concentrations (Houde *et al*., 2013). The potential of PFECHS as endocrine disrupting chemical was suggested by the outcomes in *Daphnia magna* based on immune-specific assay for vitellogenin (Vtg) content and relative gene expression (Houde *et al*., 2016). Chronic exposure of zebrafish to F-53B resulted in the PFAS accumulation in liver resulting in hepatotoxicity and altered gene expression involving organism development in male sex and metabolic processes in female fish through PPAR signalling pathway (Shi G *et al*., 2019c). Mice orally exposed to 0.02-0.5 mg/kg/d of hexafluoropropylene oxide trimer acid (HFPO-TA) showed altered expression of genes involved in PPAR and chemical carcinogenesis pathways which proved higher potential to elicit hepatotoxic effects and to bioaccumulate than PFOS (Sheng *et al*., 2018). Other studies on animal models and humans estimated the bioaccumulation potential of HFPO-TA to be higher than that of PFOA but lower compared to BAF calculated for PFOS and F-53B (Pan *et al*., 2017; Cui *et al*., 2018).

When considering ultrashort-chain PFAS, former combined algal toxicity tests upon exposure to trifluoroacetic acid (TFA) showed growth inhibition, however a threshold of 0.1 mg/L could be established as safe for aquatic ecosystems (Berends *et al*., 1999). Other studies showed no risks due to lower TFA concentrations (Russell *et al*., 2012; Solomon *et al*., 2016; Wiegand *et al*., 2000) even in mixture with trichloroacetic acid (TCA) assessed in aquatic microcosms (Hanson *et al*., 2002), although a low increase of glutathione-S transaminase (GST) was observed. Even though C2-C4 PFAS are frequently detected in surface waters and WWTP effluents, their long-term effects on humans and biota are unknown (Kärrman *et al*., 2019).

The effects in model organisms due to degradation products of short-chain PFAS with relative lethal and effect concentrations have been recently reviewed by Peshoria *et al*., 2020. Other effects of novel and non-legacy PFAS assessed in recent years are reported in Table 7.

Table 7. Effects of emerging PFAS on aquatic biota based on recent scientific literature.

Compound	Species	Effect	Reference
6:2 FTOH	Zebrafish embryos (*Danio rerio*)	Mortality: $LC_{50}=830\mu M$ 2-fold increased expression of *tgfb1a* and *bdnf* genes. Behavioural assay: increase in distance travelled and velocity.	Annunziato *et al.*, 2019
6:2 FTAA	Zebrafish (*Danio rerio*)	Not detected in adult tissue and offspring. Mixture effects in chronic toxicity test with 6:2 FTAB: decreased average number of egg production, increased malformation and mortality in offspring. Changes in endocrine hormones, sex-bias.	Shi G *et al.*, 2019a
6:2 FTAB (major component of Forafac®1157)	Zebrafish (*Danio rerio*)	Detected in adult tissue and offspring. Mixture effects with in chronic toxicity test with 6:2 FTAA: decreased average number of egg production, increased malformation and mortality in offspring. Changes in endocrine hormones, sex-bias. Extensively metabolised in fish, whereas 6:2 FTAB and 6:2 FTAA co-exposure disrupted the adult endocrine system and impaired offspring development.	Shi G *et al.*, 2019a
		Acute toxicity test: $LC_{50}=43.73\pm3.24$ mg/L (6:2 FTAB appeared to be less toxic than PFOS). No effect on hatching percentage and live embryos hatched. The malformation percentage increased at higher concentrations. Up-regulated transcriptional levels of certain genes related to apoptosis and immunotoxicity. Accumulation of ROS and changes in antioxidant enzymes.	Shi G *et al.*, 2018
6:2 FTCA	Zebrafish embryos (*Danio rerio*)	Survival: 72 h-$LC_{50}=25.1\pm1.5$ mg/L, 120 h-$LC_{50}=7.33\pm0.50$ mg/L (lower compared to PFOA, thus indicating higher toxicity for zebrafish). Reduced hatching success, survival and erythroid	Shi G *et al.*, 2017

		cell numbers at both the transcriptional and protein levels. Increased embryo malformations.	
6:2 FTSA	Zebrafish embryos (*Danio rerio*)	Survival: 72 h-LC_{50}=25.1±1.5 mg/L, 120 h-LC_{50}=7.33±0.50 mg/L Reduced hatching success and erythroid cell numbers at transcriptional and protein levels. Increased embryo malformations rate.	Shi G *et al.*, 2017
6:2 Cl-PFESA (F-53B)	Aquatic biota (seawater samples): six benthic invertebrates, ten fish species, one seabird and one marine mammal	Increased BAF in gastropod compared to PFOS but the difference was not observed in other species. The trophic magnification factor (TMF)=3.37 in the marine food web, indicating biomagnification potential along the marine food chain.	Chen H *et al.*, 2018
	Zebrafish embryos (*Danio rerio*)	BCF=125−358 (113−193 for PFOS). Exposure did not significantly affect hatching rate, mortality, larval body weight and body length. The yolk sac area was significantly reduced in the high exposure. Affected both metabolic transcript level and organismal metabolic phenotype.	Tu *et al.*, 2019
		No significant acute toxicity on mortality, hatch and malformation to zebrafish larvae. Observed hepatic steatosis. Changes in lipids profile: increased total cholesterol and triglycerid levels, and decreased LDL level. Abnormal regulation on gene expressions. 6:2 Cl-PFESA mediated the lipid metabolism in a similar mode and to a comparable extent as PFOS.	Yi *et al.*, 2019a
		No significant acute toxicity on mortality, morphology or body length to zebrafish embryos, only decrease in body weight at higher dose. Concentration in whole-body burdens increased in a concentration-dependent manner. Increased reactive oxygen and decreased antioxidant response. Induced oxidative stress in early	Wu Y *et al.*, 2019

		developing zebrafish (changes at the levels of activity, mRNA and protein of antioxidant genes).	
	Zebrafish adults (*Danio rerio*)	In adult zebrafish LC$_{50}$=15 mg/L (at 96 h), similar to that of PFOS (17 mg/L), in zebrafish larvae LC$_{50}$=13.77 mg/L, lower compared to PFOS (54 mg/L). Induced decrease in growth and reproductive capability in adult F0 zebrafish. Altered TH levels and gene expression in the F1 and F2 generations during different developmental periods which reflected the transgenerational thyroid-disrupting capacity of 6:2 Cl-PFESA in zebrafish.	Shi G *et al.*, 2019b
	Rainbow trout (*Oncorhynchus mykiss*)	Comparable bioaccumulative potencies and similar distribution tendencies to PFOS.	Yi *et al.*, 2019b
	Green algae (*Scenedesmus obliquus*)	Total log BAF=4.66. Algal growth IC$_{50}$=40.3 mg/L (as reference per PFOS was 112 mg/L). Changes in algal growth rate and chlorophyll a/b contents were observed at 11.6 mg/L and 13.4 mg/L, respectively. Exposure inhibited the algal growth in a concentration-dependent manner and induced adverse effects on the membrane property and oxidative stress.	Liu W *et al.*, 2018
8:2 diPAP	Gilt-head bream (*Sparus aurata*)	Distribution: accumulates in liver, plasma and gills, and to a lesser extent in muscle, bile and brain. Analysis of metabolites in tissues and biofluids: the major metabolite in all tissues/biofluids except for bile where PFOS was dominating.	Zabaleta *et al.*, 2017
Cl-PFOS	Zebrafish larvae (*Danio rerio*)	No significant acute toxicity (7 days) on mortality and hatching or malformations. Observed hepatic steatosis and changes in lipids profile: increased total cholesterol and triglycerid levels, decreased LDL level. Abnormal regulation on gene expressions. Lipid metabolism mediated in a	Yi S *et al.*, 2019a

		similar mode and to a comparable extent as PFOS.	
	Rainbow trout (*Oncorhynchus mykiss*)	Comparable bioaccumulative potencies and similar distribution tendencies as PFOS.	Yi S *et al.*, 2019b
HPFO-DA (GenX)	Blue spot goby (*Pseudogobius* sp.)	Dietary bioaccumulation (21 days uptake period + 42 days depuration period) not detected indicating either a lack of uptake or rapid elimination (<24 h). GenX did not appear to bioaccumulate in benthic, sediment-associated estuarine fish species.	Hassell *et al.*, 2020
OBS	Zebrafish embryos (*Danio rerio*)	BCF=20−48 (113−193 for PFOS). Exposure did not significantly affect hatching rate, mortality, larval body weight and body length. Several metabolic endpoints were differentially affected at the organismal level. Metabolic disruption elicited only at higher concentrations.	Tu W *et al.*, 2019
	Zebrafish adults (*Danio rerio*)	No dysbiosis in gut microbiota or disturbance of hepatic metabolism in adult male zebrafish at low-dose exposure (7-21 days). [7]	Wang C *et al.*, 2020
	Crucian carp (*Carassius carassius*)	Distribution in fish tissues was similar to PFOS. Log BAF=3.70 (lower bioaccumulation than PFOS). Bioaccumulation potential and binding affinity with specific proteins.	Shi Y *et al.*, 2020
PFECHS	Invertebrates (*Daphnia magna*)	Acute LC_{50}=186.61 mg/L. Under-regulation of Vtg-related genes (VTG1) and upregulation of genes related to cuticle. No effects on survival, molting frequency, growth or reproduction at the individual/population level. Endocrine disruption effect only at concentrations higher than levels reported in the aquatic	Houde *et al.*, 2016

[7] OBS LC_{50}=25.5 mg/L for zebrafish, indicating low toxicity [United Nations, 2011. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). (ST/SG/AC.10/30/rev.4)].

		environment (concentrations detected in surface water samples 1.04-1.38 ng/L).	
PFO3OA, PFO4DA, PFO5DoDA	Zebrafish embryos (*Danio rerio*)	Endpoint: uninflated swim bladders (5 dpf) $EC_{50}=1227.4\pm66.8$ mg/L (PFOA=251.5±24.2 mg/L). Toxicity increased in the order PFO5-DoDA>PFO4DA>PFOA>PFO3OA. Log Kow=3.94 for PFO3OA (4.81 for PFOA). Results suggested thyroid disruption effect that may cause swim bladder malformation.	Wang J *et al.*, 2020

BCF: bioconcentration factor. Vtg: vitellogenin.

5. Legal frameworks for PFAS restriction

5.1 EU directives and regulations

Several EU legislations provide a means to control PFAS pollution as protection measures for the environment and human health ([Figure 12](#)). The presence of PFAS in aquatic environments is regulated by the Water Framework Directive (WFD, 2000/60/EC) and related legislation. The EU Industrial Emissions Directive (IED) sets the management of the PFAS emissions from industrial sources (Directive 2010/75/EU), while Waste Treatment Plants (WTP) are regulated by the Waste Framework Directive (Directive 2008/98/EC) and the Sewage Sludge Directive (Council Directive 86/278/EEC).

In the European Union (EU), regulation No 850/2004 on POPs implemented the Stockholm Convention as for their use, reduction, elimination and related actions, however PFAS were not specifically addressed. Following the OECD studies (OECD, 2002) and risk assessment by the Scientific Committee on Health and Environmental Risks (SCHER, 2005) classifying PFOS as very persistent, very bioaccumulative and toxic, but agreed on their use in critical fields which appear not to pose significant environmental risk when release to the environment is minimised. PFOS ban in the majority of finished and semi-finished products was then specifically addressed by the EU Directive 2006/122/EC, with the threshold of 50 mg/kg for exempted products (i.e., wetting agents for electroplating systems, substances employed in photolithography processes, photographic coatings, mist suppressants for hard chromium plating and hydraulic fluids for aviation). The threshold was subsequently lowered by regulation (EC) No 757/2010 amending the regulation on POPs, including restriction of PFOS content to the threshold of 10 mg/kg in substances and preparation for industrial products exempted from the phase-out.

In 2009, after including PFOS in annex B of the Stockholm Convention, the EU further restricted the industrial use and manufacturing of PFOS and then PFOA under REACH regulation (No 1907/2006, No 522/2009, Commission Regulation 2017/1000). The REACH regulation includes the Candidate List of Substances of Very High Concern (SVHC) for authorisation which contain chemical substances attaining at least one criterium for the following adverse effects in organisms/environments or properties: carcinogenic, mutagenic and toxic for reproduction (CMRs); persistent, bioaccumulative and toxic (PBT) or very Persistent and very Bioaccumulative (vPvBs). On 4 July 2020, based on the SVHC Candidate List, restrictions on the use, import and production of PFOA, its salts and derivatives came into effect with thresholds specific to their applications (No 2019/1021, No 2020/784). The Candidate List includes other PFAS (PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTDA) due to their vPvB or PBT properties (EU-strategy, 2019; ECHA, 2020; ECHA 2020b) which might also be considered candidates for including in the Stockholm Convention (ECHA, 2020).

Other EU frameworks have been established to limit the exposure to PFAS from dietary sources due to migration from Food Contact Materials (FCMs) (EC, No 1935/2004 and No 10/2011). The Annex I of the regulation includes a list of substances allowed for use as plastic additives for packaging materials and articles in contact with food. Although such applications are banned for PFOS, PFNA and PFHxS, other PFAS can be employed as additives, polymer production aid (PPA) agents, monomers or polymers (Bokkers et al., 2018). An Action Plan within the "EU-strategy" (2019) in collaboration with the European Food Safety Authority (EFSA) recommended to "Phase out the use of all PFASs in all types of FCMs" through the REACH regulation and to remove PFAS from the positive list (i.e., list of substances allowed for use in FCMs) of the Framework Regulation No 1935/2004 (EU-strategy, 2019).
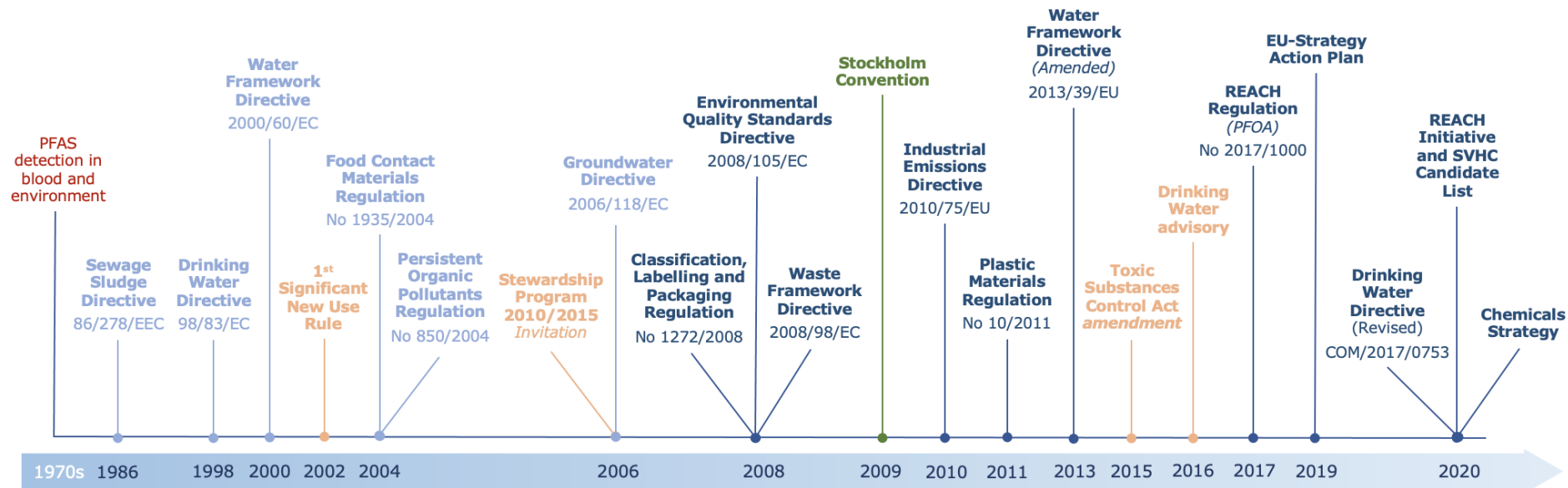
Figure 12. Main legislative documents posing restrictions on PFAS in the EU, USA and at global level. Since the detection of PFAS in human blood and environmental samples, European directives and regulations ensured indirect protective measures by establishing general rules for a range of substances (light blue). Further, the EU legislative restrictions including amendments of previous frameworks as well as future strategies specifically mentioned PFAS (dark blue). The USEPA included several PFAS already in the first Significant New Use Rule (SNUR) in 2002 and in subsequent amendments until 2020 (orange). At global level, the Stockholm Convention remains the main international provision restricting PFAS after the first review in 2009 (green). SVHC: Substance of Very High Concern

PFHxA and PFHxS, the latter used as alternative to PFOA, have been proposed as candidates for the list under the REACH restrictions. Two more PFAS were proposed for eventual inclusion in the restriction Annex XIV of REACH within the SVHC Candidate List: PFBS and 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, its salts and acyl halides (HFPO-DA), which have been used as substitutes of PFOS and PFOA, respectively (ECHA, 2020; ECHA, 2020b). Recently, a REACH initiative called for evidence and information on the use and production of PFAS by companies in order to evaluate harmful effects in a broad PFAS restriction scenario[8]. Further safety measures to protect from hazardous substances, including PFOA, PFNA, PFDA and ammonium pentadecafluorooctanoate (APFO), have been established by the Classification, Labelling and Packaging (CLP) regulation (No 1272/2008) that aligns the European system of classification and labelling rules to the Globally Harmonised System (GHS).

In a future perspective, the Chemicals Strategy for Sustainability towards toxic-free environments under the European Green Deal dedicated special attention to PFAS considering numerous cases of environmental contamination, among which drinking water, large impact on human health with a full spectrum of illnesses having high economic and social footprint (COM(2020) 667 final) (Figure 13). Therefore, the strategy adopted a PFAS group approach under relevant legislation for water as well as for ban in fire-fighting foams and other uses not essential for the society. Further aims include enhanced policy dialogues and international fora on a global scale, support for development of innovative remediation methodologies and safe PFAS substitutes.

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[8] https://eurovent.eu/?q=articles/reach-initiative-call-evidence-broad-pfas-restriction-gen-113700
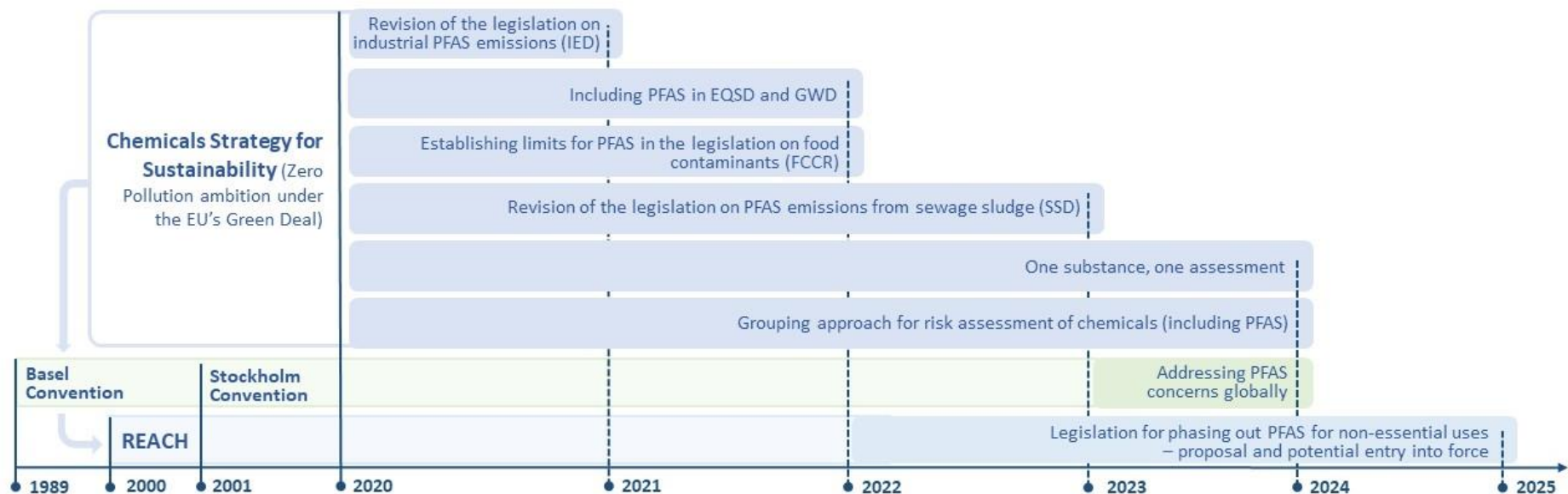
Figure 13. Timelines of ongoing and future actions addressing PFAS under the Chemicals Strategy for Sustainability. Several revisions of the current European legislation documents are foreseen to reduce the emissions of PFAS to the environment and to establish their safety thresholds from dietary sources. The assessment of PFAS as a group is expected to encompass more substances instead of measuring chemicals one-by-one in order to better protect human health and the environment. "One substance, one assessment" approach will simplify and synchronise the safety assessment of chemicals across various pieces of legislation through a EU coordinated mechanism, an expert group and the Public Administration Coordination Tool. Additionally, the REACH regulation will provide legislative basis for phasing out PFAS for non-essential uses until 2024 with predicted entry into force in 2025. At global level, the concerns regarding the presence of PFAS in the environment will be targeted by the Basel and the Stockholm Conventions. IED: Industrial Emissions Directive. EQSD: Environmental Quality Standards Directive. GWD: Groundwater Directive. FCCR: Food contaminants Commission Regulation. SSD: Sewage Sludge Directive. REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals.

5.2 PFAS under the Water Framework Directive, Drinking Water Directive and Groundwater Directive

The European Union Water Framework Directive (WFD) (2000/60/EC) was established with the aim to protect and improve the quality of EU water bodies (including surface, inland, rivers, lakes, transitional and underground waters as well as coastal waters out to one nautical mile) based on quality standards defined in terms of chemical, hydrological and biological status. To achieve a good chemical status of water bodies and to define levels above which actions reducing the emissions, discharges and losses of harmful compounds as well as measures determining phase out of the most hazardous chemicals, the Environmental Quality Standard Directive (EQSD, 2008/105/EC) established safety thresholds for 33 priority substances that may pose a potential risk to the water environment and aquatic organisms. Among those substances, perfluorooctane sulfonic acid (PFOS) and its derivatives were included as PFAS representatives. The Directive 2013/39/EU, which amended the WFD and the EQSD, provided harmonised guidelines for risk assessment and monitoring of 45 priority substances and other substances of concern. However, no other PFAS have been considered among priority hazardous substances.

The EQS values for PFOS and its derivatives, derived from data on ecotoxicological effects, high-quality monitoring data and risk assessments, are summarised in Table 8. The EQS were derived following the methodology described in the *Technical Guidance for Deriving Environmental Quality Standards* (EC, 2011a). The EQS parameter for long-term exposure to PFOS expressed as the annual average value (AA-EQS) was proposed as 6.5×10^{-4} µg/L for surface inland surface water and 1.3×10^{-4} µg/L for other surface waters (rivers, lakes, and artificial or heavily modified water bodies). The maximum allowable concentration (MAC-EQS), as acceptable concentration for acute exposure, was established at 36 µg/L for inland surface water and 7.2 µg/L for other surface waters. In relation to human consumption of fishery products, the EQS biota considered as safe for aquatic organisms was set up at 9.1 µg/kg wet weight. At the time of writing, the above-described EQS values for PFOS and its derivatives are under revision.

Table 8. Environmental Quality Standards (EQS) of PFOS as Priority Substance under the amended Water Framework Directive (2013/39/EU).

Substance	AA-EQS Inland surface waters [µg/L]	AA-EQS Other surface waters [µg/L]	MAC-EQS Inland surface waters [µg/L]	MAC-EQS Other surface waters [µg/L]	EQS Biota [µg/kg wet weight]
Perfluorooctane sulfonic acid (PFOS) and its derivatives	6.5×10^{-4}	1.3×10^{-4}	36	7.2	9.1

AA: annual average; MAC: maximum allowable concentration

Other EU legislations complementing the WFD are the Groundwater Directive (GWD) (2006/118/EC), which supplements on the procedures for assessing the chemical status of groundwater, and the Drinking Water Directive (DWD) (98/83/EC) that specifies criteria to ensure the quality of water intended for human consumption. Additionally, EU Member States have set up national regulations to further restrict PFAS concentration limits in water (Concawe, 2016; OECD, 2013).

The European Commission is currently reviewing the annexes of the Groundwater Directive, and PFAS is one of the three main groups of substances proposed to be included[9]. The process builds on the voluntary groundwater watch list mechanism, which supported a study with monitoring data from 11 European countries. The review process also considers the DWD recast.

Regarding the DWD (98/83/EC), the revised directive of 16 December 2020 established a list of 20 PFAS (Table 9) with limit values for two chemical parameters: the "sum of PFASs" intended as a sum of 20 PFAS considered in the recast with the parametric value of 0.10 µg/L, and "PFASs - total" meaning the "totality of per- and polyfluoroalkyl substances" with the threshold concentration of 0.50 µg/L (Directive (EU) 2020/2184). Decision to use one or both parameters for monitoring in drinking water is left to the Member States (MS), however, technical guidelines regarding the analytical methods, in particular detection limits and the frequency of sampling, are not defined so far and are to be established by the Commission by 2024.

Table 9. List of PFAS proposed as a chemical parameter in the recast of the Drinking Water Directive (DWD) (EU) 2020/2184. The number of total carbon (C) atoms and classification according to the length of alkyl chain are shown.

Name	Acronym	Total C atoms	Chain*	CAS	Other acronyms
Perfluorobutanoic acid	PFBA	4	Short-chain	375-22-4	
Perfluorobutanesulfonic acid	PFBS	4	Short-chain	375-73-5	
Perfluoropentanoic acid	PFPA	5	Short-chain	2706-90-3	PFPeA
Perfluoropentane sulfonic acid	PFPS	5	Short-chain	2706-91-4	PFPeS
Perfluorohexanoic acid	PFHxA	6	Short-chain	307-24-4	
Perfluorohexanesulfonic acid	PFHxS	6	Long-chain	355-46-4	
Perfluoroheptanoic acid	PFHpA	7	Short-chain	375-85-9	
Perfluoroheptane sulfonic acid	PFHpS	7	Long-chain	357-92-8	
Perfluorooctanoic acid	PFOA	8	Long-chain	335-67-1	
Perfluorooctanesulfonic acid	PFOS	8	Long-chain	1763-23-1	
Perfluorononanoic acid	PFNA	9	Long-chain	375-95-1	
Perfluorononane sulfonic acid	PFNS	9	Long-chain	68259-12-1	
Perfluorodecanoic acid	PFDA	10	Long-chain	335-76-2	
Perfluorodecane sulfonic acid	PFDS	10	Long-chain	335-77-3	
Perfluoroundecanoic acid	PFUnDA	11	Long-chain	2058-94-8	
Perfluoroundecane sulfonic acid	PFUnDS	11	Long-chain	749786-16-1	PFUnS
Perfluorododecanoic acid	PFDoDA	12	Long-chain	307-55-1	PFDoA
Perfluorododecane sulfonic acid	PFDoDS	12	Long-chain	79780-39-5	PFDoS
Perfluorotridecanoic acid	PFTrDA	13	Long-chain	72629-94-8	
Perfluorotridecane sulfonic acid	PFTrDS	13	Long-chain	NA	

NA: not available. * OECD, 2013.

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[9] https://ec.europa.eu/environment/water/fitness_check_of_the_eu_water_legislation/index_en.htm

5.3 Historical steps towards regulatory frameworks for PFAS at global level

The early production and use of PFAS was not restricted by any legal regulation. Over the years, the number of studies evaluating the potential risk posed by PFAS exposure to environmental and human health increased (OECD, 2015). In the 1970s, PFAS have already been detected in the blood in chemical company workers and in various environmental media (Figure 8). However, nothing changed in the regulatory context because scientific results were mostly generated as internal reports of producing companies undisclosed for the public and based on the analytical technology unable to reliably determine PFAS compounds resulting in the lack of robust evidence on PFAS toxicity (Lindstrom *et al.*, 2011; OEDC, 2015).

Only in the 2000s, the USA paved the way towards progressive removal of the most toxic PFAS from production and use (Figure 10). The USEPA coordinated a voluntary phase-out of PFOS by world leading manufacturer of PFOS-based compounds and issued a series of Significant New Use Role (SNUR) documents that limited the employment of 183 PFAS to highly specific technical applications to which no alternatives were available (USEPA, 2002a and 2002b; ITRC, 2020). In 2006, eight major PFAS industries were invited to participate in the Stewardship Program 2010/2015 aiming at a 95% reduction and ultimately the elimination of PFOA and related chemicals from emissions and products in the USA by 2015 (USEPA, 2006). Further measures, including the amendment of the Toxic Substances Control Act (TSCA) led to the requirement of reporting planned new uses, such as surface coatings, of PFOA and related chemicals as well as long-chain PFAS that have been phased out (USEPA 2013, 2015 and 2020c). For drinking water, USEPA issued lifetime health advisory recommending the notification of PFOS and PFOA levels exceeding 70 ppt (combined) in community water supplies (USEPA, 2016). However, in order to better protect against cancer and non-cancer effects including the impact on the immune system and liver toxicity, single States established lower notification levels, which for the State of California equal 5.1 ppt for PFOA and 6.5 ppt for PFOS when detected separately, being far lower than response levels of 10 ppt for PFOA and 40 ppt for PFOS (OEHHA, 2019).

At global level, the Stockholm Convention on Persistent Organic Pollutants (POPs) imposed in 2009 restrictions on the use of PFOS, its salts and perfluorooctane sulfonyl fluoride (PFOSF) in specific products (decision SC-4/17), and in 2019 included PFOA, its salts and related compounds for elimination from specific uses, except fire-fighting foams for which at present there are no valid alternatives (IISD, 2019). Perfluorohexane sulfonic acid (PFHxS), its salts and related compounds have been proposed for listing in the Annex A to the Convention without specific exemptions (Stockholm Convention, 2019). Potential toxicity of PFAS precursors has also been highlighted by the OECD (OECD, 2007).

5.4 Measured Environmental Concentrations (MEC) in European inland surface water

In the EU, the presence of several PFAS in inland surface water, groundwater and drinking water is confirmed by measurements from the Member States (MS). As shown in Figure 14, some of the substances are common to all three compartments.
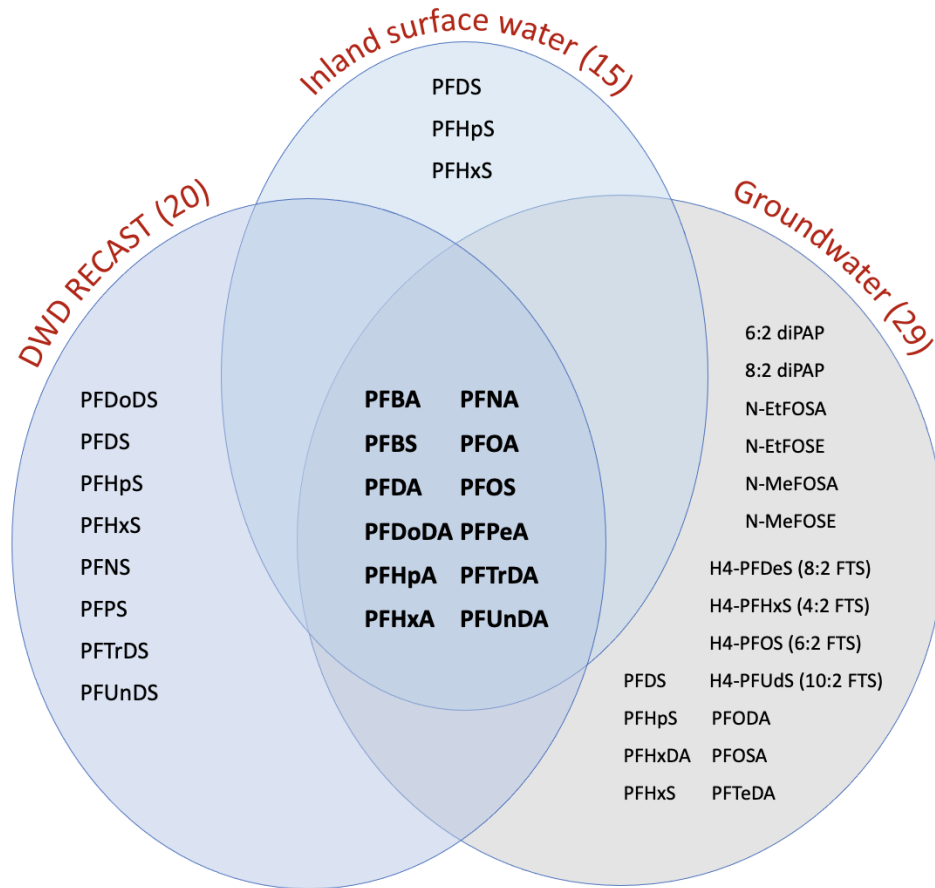
Figure 14. PFAS monitored in inland surface water, groundwater and drinking water in the EU. The number of monitored substances is indicated in brackets for each water compartments. For drinking water, 20 PFAS are determined according to the recast of the Drinking Water Directive (DWD) (EU) 2020/2184. Disaggregated monitoring data are available only for inland surface water from the prioritisation exercise undertaken in 2014-2016 (Carvalho et al., 2016). Three of listed compounds (PFDS, PFHpS and PFHxS), although having the same acronyms, refer to either sulfonic acid or sulfonate.

To evaluate PFAS exposure in EU inland surface waters, the JRC performed an analysis of measured environmental concentrations (MEC) of PFAS based on data from the prioritisation dataset collected by the JRC in 2014 (Carvalho *et al*., 2016)[10]. This dataset combines records from five datasets (SoE of the European Environmental Agency, MSDAT, EMPODAT, IPCHEM of the JRC, JDS3) and includes quantified and non-quantified measurements (estimated concentrations are below limit of quantification, LOQ) from the EU MS gathered during 2006-2014 period. Each of non-quantified samples in the prioritisation dataset is set to half LOQ[11]

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[10] The report is available in CIRCABC https://circabc.europa.eu/w/browse/52c8d8d3-906c-48b5-a75e-53013702b20a.

[11] Under the QA/QC Directive and EQS Directive, MS are required to replace the non-quantified samples by half LOQ to assess compliance with the EQS for individual substances, however the amended EQSD mentions that "when the calculated mean value of a measurement, when carried out using the best available technique not entailing excessive costs, is referred to as "less than limit of quantification", and the limit of quantification of that technique is above the EQS, the result for the substance being measured shall not be considered for the purposes of assessing the overall chemical status of that water body".

of applied analytical method as stipulated in Directive 2009/90/EC. The quality of monitoring data in this dataset is checked and verified according criteria described in Carvalho *et al.*, 2016.

Data availability

In the prioritisation dataset compiled by the JRC in 2014, totally 53595 samples for 15 compounds of PFAS family, including PFOS (a priority substance), are available from 13 MS. The observations were collected at 1191 sites across the EU. Detailed information about the number of reporting MS, number of monitoring sites, overall number of samples and number of quantified samples (given as % of the total) for each of the considered 15 compounds is shown in Table 10. In case of perfluorotridecanoic acid (PFTrDA), only one sample from one MS is available in the dataset, hence this substance was excluded from the analysis, although it is shown for completeness in the tables and figures below. The number of reported samples for the other substances vary from 296 (PFTrDA) to 8822 (PFOA). Abundant data (8580 samples) are available for perfluorononanoic acid (PFNA). Six substances (PFOA, PFNA, PFHpA, PFDA, PFHxA and PFBS) were monitored in at least 8 MS, with PFOA being the most intensely monitored PFAS among MS.

Table 10. List of PFAS for which monitoring data are available in the prioritisation dataset. Statistical information about the number of reporting Member States (MS), number of monitoring sites, overall number of samples and number of quantified samples (% of the total for each substance) is provided for each PFAS.

CAS	Substance	Acronym	MS	Sites	Samples	Quantified samples (% of the total)
#1763-23-1	Perfluorooctanesulfonic acid	PFOS	5	500	4926	42.20
#2058-94-8	Perfluoroundecanoic acid	PFUnDA	5	443	1763	23.65
#2706-90-3	Perfluoropentanoic acid	PFPeA	2	303	3720	29.25
#307-24-4	Perfluorohexanoic acid	PFHxA	8	386	3848	40.46
#307-55-1	Perfluorododecanoic acid	PFDoDA	4	323	1320	15.91
#335-67-1	Perfluorooctanoic acid	PFOA	13	945	8822	40.65
#335-76-2	Perfluorodecanoic acid	PFDA	8	564	4712	12.03
#335-77-3	Perfluorodecanesulfonic acid	PFDS	1	86	510	0.00
#355-46-4	Perfluorohexanesulfonic acid	PFHxS	3	317	3794	19.87
#375-22-4	Perfluorobutanoic acid	PFBA	2	300	3562	32.85
#375-73-5	Perfluorobutanesulfonic acid	PFBS	8	349	3714	41.76
#375-85-9	Perfluoroheptanoic acid	PFHpA	10	465	4027	23.79
#375-92-8	Perfluoroheptanesulfonic acid	PFHpS	1	69	296	0.00
#375-95-1	Perfluorononanoic acid	PFNA	11	452	8580	16.78
#72629-94-8	Perfluorotridecanoic acid	PFTrDA	1	1	1	0.00

Data quality

The quality of MEC is essential for making a proper risk assessment analysis. The quality of PFAS data is verified according to the rate of quantification (percentage of quantified samples from all for a given substance) and range of LOQs for non-quantified samples (for which estimated concentrations are below LOQ).

For instance, considering data from all MS together, the quantification rates of individual PFAS (see Table 10) vary from zero (PFDS and PFHpS; these two substances were monitored only in one MS and have lower numbers of samples) up to 42.2% (PFOS) with an average of 24.2% for the considered 14 substances.

In addition, Figure 15 shows the range of LOQs for reported non-quantified samples for each monitored PFAS. The majority of non-quantified samples were taken with LOQs in the range of 0.001 – 0.1 µg/L. In this context, the quality of available monitoring data for PFAS in the prioritisation dataset of the JRC is acceptable, except for PFTrDA, and questionable for PFDS and PFHp.
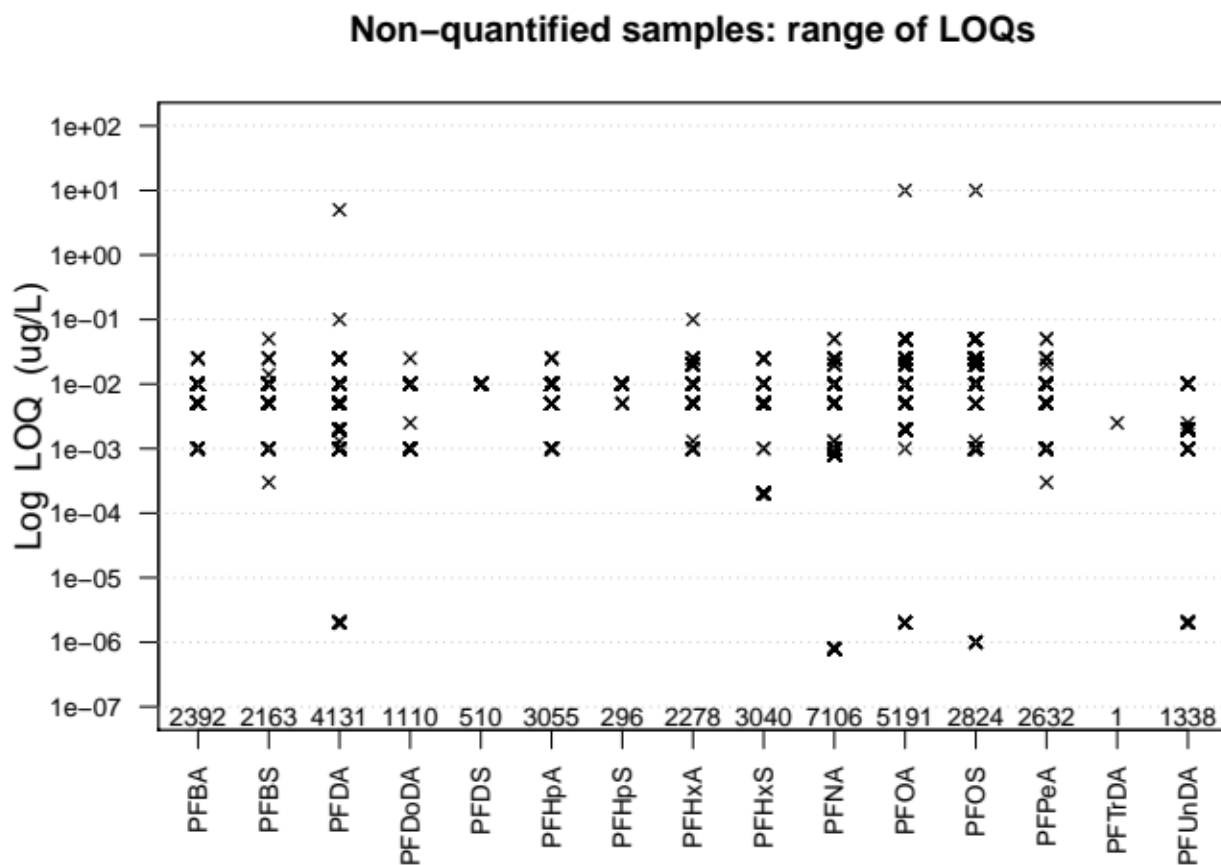
Figure 15. Range of limits of quantification (LOQs) for reported non-quantified samples (for which estimated concentrations are below LOQ) for every PFAS in the prioritisation dataset (JRC 2014) considering together data from all reporting MS. The overall number of non-quantified samples per substance are indicated at the bottom of the plot.

Measured environmental concentrations (MEC)

The summary statistics of measured environmental concentrations (MEC) across Europe, including minimum, average, median, 90th percentile (P90), 95th percentile (P95) and maximum, considering together the data from all MS is presented in Table 11. Regarding the nine substances which have been monitored in 4 and more MS, the analysis showed median concentrations from 0.001 µg/L to 0.012 µg/L (average ~0.0056 µg/L), mean concentrations from 0.0024 µg/L to 0.053 µg/L (average ~0.018 µg/L), while the 95th percentiles (P95) of MEC ranged from 0.005 µg/L to 0.14 µg/L (average ~0.059 µg/L).

Table 11. Basic statistics of measured environmental concentrations (µg/L) across Europe (jointly data from all countries) for PFAS in the prioritisation dataset of the JRC, including minimum, average, median, 90th percentile (P90), 95th percentile (P95) and maximum values.

CAS	Substance	Acronym	Min	Mean	Median	P90	P95	Max
#1763-23-1	Perfluorooctanesulfonic acid	PFOS	5.00E-07	2.42E-02	1.16E-02	3.60E-02	6.30E-02	5.00E+00
#2058-94-8	Perfluoroundecanoic acid	PFUnDA	1.00E-06	1.51E-02	1.00E-03	2.52E-02	1.00E-01	1.18E+00
#2706-90-3	Perfluoropentanoic acid	PFPeA	1.50E-04	1.12E-02	5.00E-03	1.80E-02	3.80E-02	9.74E-01
#307-24-4	Perfluorohexanoic acid	PFHxA	2.00E-04	1.76E-02	5.00E-03	2.80E-02	6.50E-02	8.92E-01
#307-55-1	Perfluorododecanoic acid	PFDoDA	2.00E-05	2.43E-03	1.00E-03	5.00E-03	5.00E-03	1.00E-01
#335-67-1	Perfluorooctanoic acid	PFOA	1.00E-06	5.34E-02	1.20E-02	6.80E-02	1.40E-01	1.20E+01
#335-76-2	Perfluorodecanoic acid	PFDA	1.00E-06	8.18E-03	5.00E-03	5.00E-03	1.25E-02	2.50E+00
#335-77-3	Perfluorodecanesulfonic acid	PFDS	5.00E-03	5.00E-03	5.00E-03	5.00E-03	5.00E-03	5.00E-03
#355-46-4	Perfluorohexanesulfonic acid	PFHxS	2.00E-07	7.58E-03	5.00E-03	1.00E-02	2.10E-02	9.80E-01
#375-22-4	Perfluorobutanoic acid	PFBA	5.00E-04	2.08E-01	5.00E-03	2.30E-02	5.23E-02	2.35E+02
#375-73-5	Perfluorobutanesulfonic acid	PFBS	1.50E-04	2.26E-02	5.00E-03	2.99E-02	5.73E-02	4.33E+00
#375-85-9	Perfluoroheptanoic acid	PFHpA	2.50E-04	1.17E-02	5.00E-03	1.60E-02	8.24E-02	1.00E+00
#375-92-8	Perfluoroheptanesulfonic acid	PFHpS	2.50E-03	4.97E-03	5.00E-03	5.00E-03	5.00E-03	5.00E-03
#375-95-1	Perfluorononanoic acid	PFNA	4.00E-07	4.91E-03	5.00E-03	5.00E-03	8.00E-03	3.20E-01
#72629-94-8	Perfluorotridecanoic acid	PFTrDA	1.25E-03	1.25E-03	1.25E-03	1.25E-03	1.25E-03	1.25E-03

In addition, Figure 15 gives more detailed picture (box-plots) of MEC for each of the PFAS with available measurements in the prioritisation dataset of the JRC when considering together data from all reporting MS. Regarding PFBA, there are several very high concentrations in the range of outliers. Higher MEC have been observed for PFBA, PFBS, PFHpA, PFHxA, PFOA, PFOS, PFPeA and PFUnDA (for all substances the mean concentration ≥0.01 µg/L).

Finally, the 95th percentiles (P95) of monitored concentrations of the considered 15 PFAS, for which measurements were available in the prioritisation dataset of the JRC, are presented in Figure 17. Higher P95 (P95≥0.04 µg/L) have been observed for the aforementioned eight PFAS (PFBA, PFBS, PFHpA, PFHxA, PFOA, PFOS, PFPeA and PFUnDA).

PFAS: Box-plots of measured concentrations
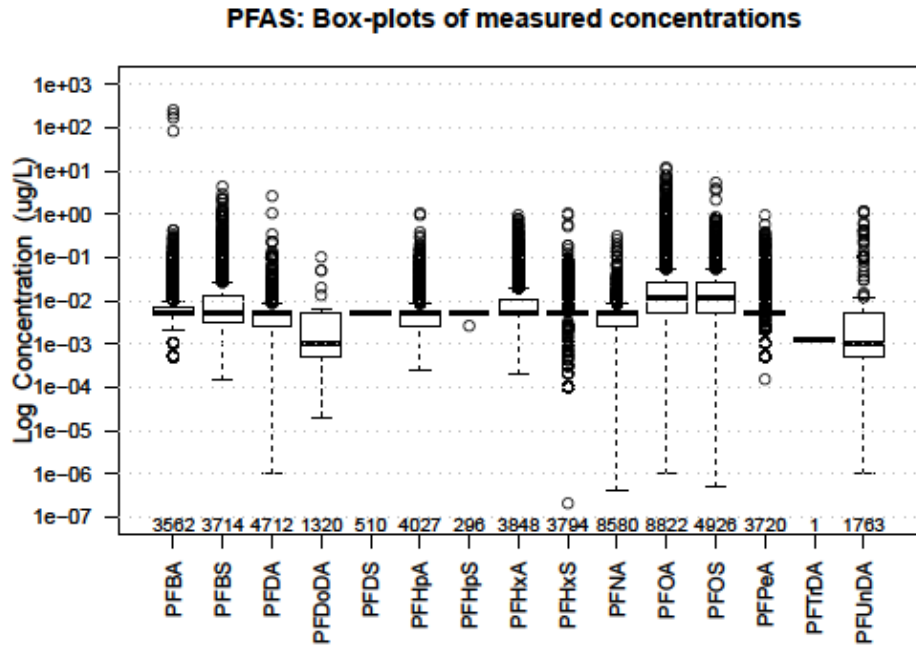
Figure 16. Box-plots of measured environmental concentrations (MEC), considering together data from all reporting MS, for each of the PFAS with available measurements in the prioritisation dataset of the JRC. The lowest line of the figure shows the overall numbers of samples.

PFAS: 95th percentile of concentrations
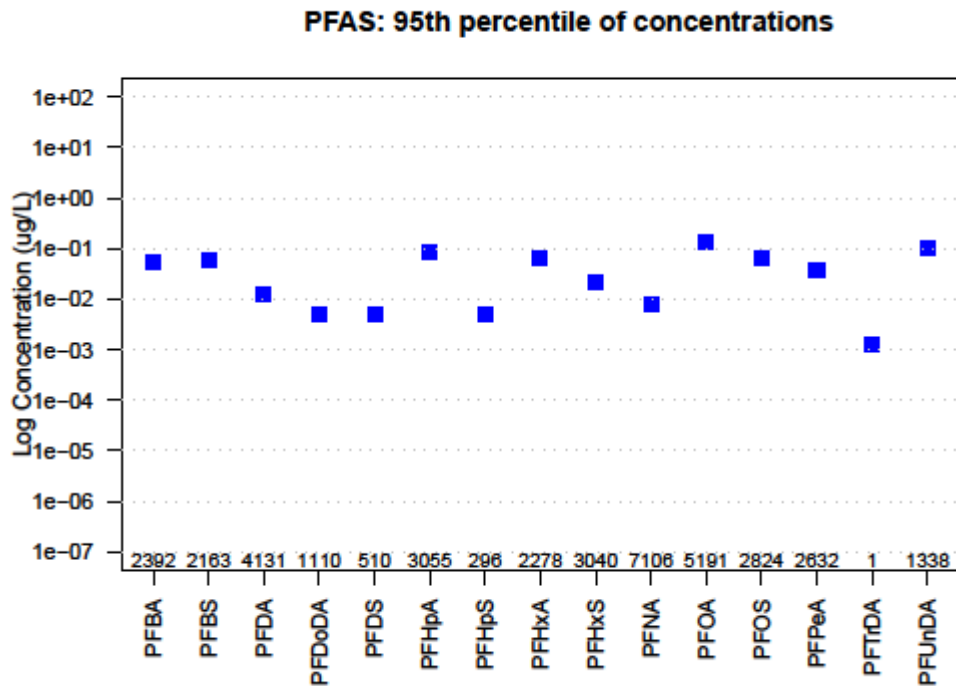
Figure 17. 95th percentile (P95) of measured concentrations, considering together data from all reporting Member States (MS), for each of the PFAS with available measurements in the prioritisation dataset. The lowest line of the figure shows the overall numbers of samples for each substance.

6. Conclusions

The growing global concern over PFAS urges the need of more (eco)toxicological data to investigate risks of PFAS exposure and to better protect health, environment and natural resources from persistent and mobile chemicals. Moreover, there is no legislation covering all PFAS, therefore a new approach which would consider the PFAS as a group, would envisage to target classes of PFAS. Figure 18 shows the knowledge gaps which have been identified to improve their detection and risk assessment in the environment.
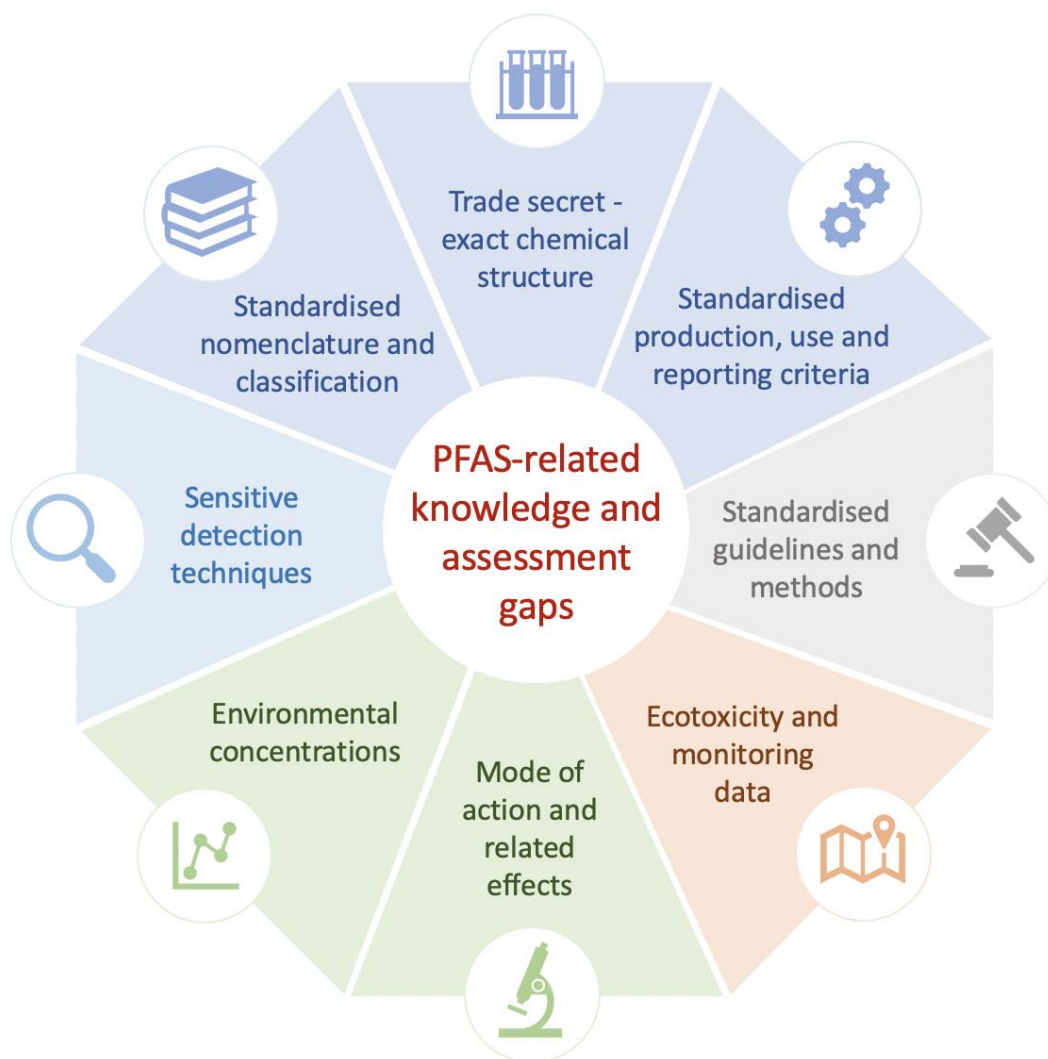
Figure 18. Knowledge and assessment gaps relative to PFAS in the environment.

The steps towards filling the knowledge gaps regarding PFAS have been undertaken by the Chemicals Strategy for Sustainability (COM(2020) 667 final) under the European Green Deal. Scientific evidence points at the need to include a comprehensive set of PFAS compounds and classes, their precursors and possibly degradation products in

environmental assessments based on clear selection criteria. The grouping approach and "One substance, one assessment" for synchronised legislation regulating their monitoring during production process, in final products and in various environmental compartments will partly cover this demand. However, defining how to report the sum of PFAS as a group in relation to the environmental quality standards (EQS) remains the main challenge.

Increased efforts to reduce PFAS emissions and to obtain more monitoring data are necessary to assess the effective concentrations and profiles of these compounds in the environment. In long-term perspective, phasing out and substitution with environmentally-friendly alternative substances as part of eco-innovation and bioeconomy is highly recommended to meet the objective of the Green Deal agenda. Considering persistence properties, bioremediation remains a valid large-scale solution for the effective removal of PFAS which accumulated in environmental matrices over decades.

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List of abbreviations

AA-EQS	Annual Average Environmental Quality Standard
AFFF	Aqueous Film-Forming Foams
APFO	Ammonium pentadecafluorooctanoate
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	BioAccumulation Factor
BMDL	Benchmark Dose Level
CAR	Constitutive Androstane Receptor
CLP	Classification, Labelling and Packaging
Cl-PFESA	Chlorinated PolyFluorinated Ether Sulfonate
DWD	Drinking Water Directive
ECF	Electrochemical Fluorination
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EOF/AOF	Extractable or Adsorbable Organic Fluorine
EQS	Environmental Quality Standards
EQSD	Environmental Quality Standards Directive
ERα	Estrogen Receptor subunit Alpha
EU	European Union
FCCR	Food contaminants Commission Regulation
FCM	Food Contact Materials
GC/MS/MS	Gas Chromatography-tandem Mass Spectrometry
GHS	Globally Harmonised System
GWD	GroundWater Directive
HBGV	Health Based Guidance Value
HFPO	HexaFluoroPropylene Oxide
hL-FABP	Human Liver Fatty Acid Binding Protein
HPLC-MS/MS	High-Performance Liquid Chromatography-tandem Mass Spectrometry
IED	Industrial Emissions Directive
ITRC	Interstate Technology & Regulatory Council
LC/MS/MS	Liquid Chromatography-tandem Mass Spectrometry
LXR	Liver X Receptor
MAC-EQS	Maximum Allowable Concentration – Environmental Quality Standard
MEC	Measured Environmental Concentrations

OBS	Sodium p-perfluorous nonenoxybenzene sulfonate
OECD	Organisation for Economic Co-operation and Development
PBPK	Physiologically-based pharmacokinetic
PBT	Persistent, Bioaccumulative and Toxic
PFAA	PerFluoroAlkyl Acids
PFAS	Per- and PolyFluoroAlkyl Substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonic acid
PFCA	PerFluoroalkyl Carboxylic Acids
PFDA	Perfluorodecanoic acid
PFDS	Perfluorodecane sulfonic acid
PFDoA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHpS	Perfluoroheptane sulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFNS	Perfluorononane sulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PFPA	PerFluoroalkyl Phosphonic Acids
PFPIA	PerFluoroalkyl Phosphinic Acids
PFPeA	Perfluoropentanoic acid
PFSA	PerFluoroalkane Sulfonic Acids
PFUnDA	Perfluoroundecanoic acid
POPs	Persistent Organic Pollutants
PPAR	Peroxisome Proliferator-Activated Receptor
PXR	Pregnane X Receptor
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RPF	Relative Potency Factor
SNUR	Significant New Use Rule
SPE	Solid-Phase Extraction
SSD	Sewage Sludge Directive
SVHC	Substances of Very High Concern
TOF	Total OrganoFluorine

TOP	Total Oxidizable Precursor assay
TRβ	Thyroid Receptor Beta
TTR	Transthyretin Receptor
TWI	Tolerable Weekly Intake
USEPA	United States Environmental Protection Agency
WTP	Water Treatment Plant
vPvB	very Persistent and very Bioaccumulative
WFD	Water Framework Directive
WWTP	WasteWater Treatment Plant

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