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Widening the lens on PFASs: Direct human exposure to perfluoroalkyl acid precursors (pre-PFAAs)

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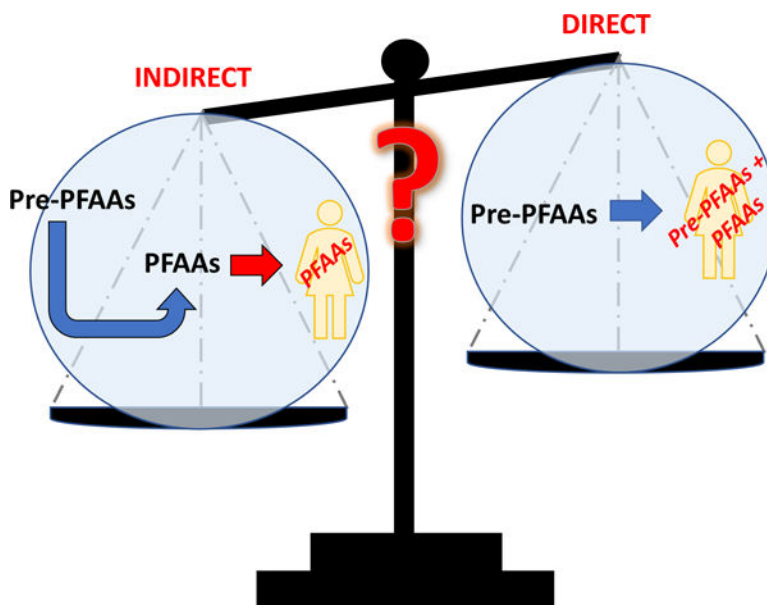
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Abstract

Determining health risks associated with per- and polyfluoroalkyl substances (PFASs) is a highly complex problem requiring massive efforts for scientists, risk assessors, and regulators. Among the most poorly understood pressing questions is the relative importance of pre-PFAAs, which are PFASs that degrade to highly persistent perfluoroalkyl acids. How many of the vast number of existing pre-PFAAs are relevant for direct human exposure, and what are the predominant exposure pathways? What evidence of direct exposure to pre-PFAAs is provided by human biomonitoring studies? How important are pre-PFAAs and their biotransformation products for human health risk assessment? This article outlines recent progress and recommendations towards widening the lens on human PFAS exposure to include the pre-PFAA sub-class.

Graphical Abstract

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Synopsis:

We highlight significant developments and knowledge gaps related to direct human exposure to fluorinated precursors, a poorly understood component of human PFAS exposure.

Keywords

PFASs; per- and polyfluoroalkyl substances; precursor; bioaccumulation; biotransformation; nontarget analysis; risk assessment; human exposure

WHAT MAKES A PFAS A “PRECURSOR”?

Widespread human exposure to perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and other persistent perfluoroalkyl acids (PFAAs) is a serious public health concern due to their adverse health effects. PFAAs are the most well-studied sub-class of per- and polyfluoroalkyl substances (PFASs). PFASs are often characterized as “forever chemicals” that do not break down with natural processes. This characterization is appropriate for PFAAs, which fit the REACH definition of “very persistent.”^{1,2} The two main types of PFAAs, perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAAs), are resistant to degradation under natural conditions on geological timescales. However, many diverse PFASs fall under the sub-class of “precursors” that can degrade to highly persistent PFAAs. The body of knowledge about precursors is relatively small compared to PFAAs, particularly with respect to human exposure, pharmacokinetics, and health effects.

Typically the “precursor” definition is applied to organofluorine substances that undergo transformation under oxidative conditions to produce PFAAs (*i.e.* compounds that can be measured via the total oxidizable precursor (TOP) assay). As more structures are incorporated into PFAS investigations, the precursors definition becomes more complicated. For example, some compounds classified as PFAAs degrade to other PFAAs

as terminal products (e.g. perfluoroalkyl phosphinic acids (PFPIAs) transforming to perfluorophosphonic acids (PFPAs)).³ Perfluoroalkyl ether sulfonates and carboxylates (PFESAs and PFECAs), sometimes classified as PFAAs,⁴ are considered highly persistent and do not degrade under strong oxidizing conditions.⁵ Chlorine-substituted PFESAs may also be resistant to oxidation, but transform to hydrogen-substituted polyfluoroalkyl ether sulfonates via reductive dehalogenation.^{6,7} Such novel structures do not fit neatly into the precursor sub-class, nor the PFAA sub-class. In some cases, precursors are defined with respect to a specific product of concern. For example “PreFOS” is a term for all PFASs that degrade to PFOS.⁸ This article addresses the importance of direct human exposure to the “pre-PFAA” sub-class, defined as all PFASs that could potentially degrade to highly persistent ubiquitous PFAAs (PFCAs and PFSAs; Figure 1). The pre-PFAA sub-class includes fluorotelomers, per- and polyfluoroalkyl sulfonyl compounds, and polyfluoroalkyl ethers.

While many estimates of the total number of PFASs exist, the Organization for Economic Cooperation and Development (OECD) classified 4,186 compounds as “potential precursors to PFAAs in environment/biota” out of 4,730 identified PFAS-related CAS numbers.⁴ Among the 1,629 nonpolymeric PFASs identified by Glüge et al.,⁹ only 227 are PFAAs, while 1,048 are “PFAA-based substances,” most of which are known or likely pre-PFAAs. Like PFAAs, pre-PFAAs are used in many different applications where stability and reduction of surface tension are desired, including cleaning solutions, brake fluid, personal care products, non-stick and stain-resistant coatings, and aqueous film-forming foams (AFFFs).⁹ However, for the majority of pre-PFAAs, little to no information on human exposure, toxic effects, or potential for biotransformation is publicly available.

DIRECT HUMAN EXPOSURE TO PRE-PFAAS

PFAAs (smallest circle in Figure 1) are globally ubiquitous due to remarkable persistence and long-range transport potential, leading to widespread human exposure through contaminated drinking water, food, dust, and other media. Pre-PFAAs can be environmentally transformed to PFAAs, thereby contributing to human PFAA exposure. We refer to this as “indirect pre-PFAA exposure” (see TOC art). There are also pre-PFAAs (second largest circle in Figure 1) to which humans are exposed directly. Additionally, many pre-PFAAs do not appear to be widespread in the environment and are only known from patents, consumer product analysis, or field studies of highly polluted sites (largest circle in Figure 1).

Previous studies have noted uncertainties in the degree to which PFAAs in human serum result from direct exposure to PFAAs, versus exposure to pre-PFAAs and subsequent biotransformation.¹⁰ However, few studies have deeply investigated direct exposure to pre-PFAAs, which may or may not lead to formation of PFAAs *in vivo*. This is partly due to the absence of reliable exposure monitoring methods. Regulatory bodies have rapidly developed methods to extract and measure a fairly large list of PFASs in matrices relevant for human exposure, but methods for quantitation of pre-PFAAs remain limited. Current US EPA methods for drinking water analysis include three fluorotelomer sulfonates (Method 533) or two sulfonamido acetic acids (Method 537.1). The ISO 21675:2019 method for PFASs

in water includes four perfluorooctane sulfonamido-based substances, two fluorotelomer sulfonates, one unsaturated fluorotelomer acid, and one polyfluorinated phosphoric acid diester. Standardized methods are under development to measure PFAS exposure via other pathways such as house dust and ambient air, which are significant reservoirs for many pre-PFAAs.^{11–13}

Direct pre-PFAA exposure is likely distinct in terms of predominant exposure pathways, pharmacokinetics, and toxicological effects when compared to exposure to the terminal PFAA product. There are multiple sources of human exposure to PFASs, including diet (both foodstuffs and food packaging/wrappers), drinking water, air (particularly indoor dust), and commercial products,¹⁹ but their relative importance for direct pre-PFAA exposure has not been investigated extensively. The relative importance of direct versus indirect exposure for a specific pre-PFAA will vary with primary exposure pathways depending on proximity of application or emission (where or how the compound was used) and transport potential (stability and mobility in the environment).

Pre-PFAAs in Consumer Products.

Many pre-PFAAs are used in household and personal care products, likely leading to direct exposures. The ubiquitous presence of pre-PFAAs in residential wastewater highlights widespread use of PFASs in everyday life.²⁰ Greater PFAA content in treated effluent compared to influent in wastewater treatment plants also implies significant contributions from pre-PFAAs transformed during treatment processes.²¹

In studies of household products, a significant fraction of total identified PFASs were pre-PFAAs. Kotthoff et al. found that fluorotelomer alcohols (FTOHs) were the dominant PFASs in cleaners and stain repellents by 4–5 orders of magnitude, and in outdoor textiles by one to two orders of magnitude.²² In a survey of cosmetic products in Sweden, Schultes et al. reported 200 to 500 µg/g polyfluoroalkyl phosphoric diesters (diPAPs).²³ In the same products, total PFAAs were 1.5 to 2 µg/g. Another recent survey of North American cosmetics found abundant polyfluorinated alkyl phosphate esters (PAPs), FTOHs, and fluorotelomer methacrylates (FTMAs) in the tested products.²⁴

Many studies of consumer products have suggested significant contributions from unknown PFASs through measurement of extractable organic fluorine (EOF) or total fluorine.^{24–26} However, these methods do not differentiate between pre-PFAAs and other fluorinated organics, so it is not known how many of these unknown compounds are pre-PFAAs. Furthermore, surrogate methods such as EOF and TOP assay often miss novel PFASs (e.g. volatile compounds) that are not amenable to sample extraction techniques. Very few studies of commercial products have used TOP assay,^{26,27} likely due to difficulties adapting this method for complex matrices. Robel et al. noted significant contributions from pre-PFAAs measured via TOP assay in papers and textiles, and observed that the majority of pre-PFAA content was unidentified.²⁶

Direct exposure of humans to uncharacterized and under-studied pre-PFAAs is a likely source of PFAS exposure (and potentially internal PFAA exposure after biotransformation). A survey of personal care product ingredient listings²⁸ indicated

frequent inclusion of organofluorine substances using INCI (International Nomenclature of Cosmetic Ingredients), which holds ambiguity in identifying exact chemical structures. Examples include perfluorononyl dimethicone, perfluorononyl ethyl carboxyldecyl PEG-10 dimethicone, and polyperfluoroethoxymethoxy difluoroethyl PEG phosphate, none of which have available commercial standards nor have been identified through targeted chemical analysis. However, their presence in product listings highlights widespread use of uncharacterized pre-PFAAs. Human exposure to novel pre-PFAAs lacking data on toxicity and potential to transform *in vivo* is a substantial knowledge gap and should be a major focus of current and future research.

Pre-PFAAs in Water and Food.

PFAAs, rather than pre-PFAAs, typically predominate in drinking water. In surveys of finished drinking water, targeted pre-PFAAs are often under detection limits.²⁹ Pre-PFAAs may be transformed to PFAAs during transport prior to reaching drinking water intakes, resulting primarily in indirect exposure. Pre-PFAAs can transform abiotically through hydrolysis and photolysis^{30–32} and may transform during drinking water treatment.^{33,34} Some fairly persistent ionizable pre-PFAAs such as fluorotelomer sulfonates (FTSs) and FASAs have been intermittently detected at low levels (typically < 5 ppt) in drinking water.^{35,36} Sites of AFFF use or PFAS manufacturing often contain significant amounts of pre-PFAAs^{15,37,38} and contribute PFAS contamination to drinking water sources.^{38,39} However, studies of PFAS transport from AFFF-contaminated source zones demonstrate that many novel pre-PFAAs (particularly zwitterions and cations) are not likely transported far from AFFF source zones prior to transformation.^{18,40} These pre-PFAAs may transform to mobile intermediate pre-PFAAs such as FASAs. FASAs were widespread in drinking water wells in a recent study of an AFFF-impacted community in Colorado Springs.³⁸

Diet (particularly seafood, meat, and dairy) is a major source of human exposure to PFAAs.⁴¹ Sources of pre-PFAAs to the food-chain are difficult to constrain due to potential contributions from pre-PFAAs in the food itself, as well as in packaging and wrapping. In a market basket study, pre-PFAAs (preFOS and diPAPs) were found predominantly in meat, fish, and eggs.⁴² Biotransformation of these pre-PFAAs was estimated to contribute little to total PFAA exposure resulting from consumption of these foods, but there was a great degree of uncertainty in estimated biotransformation factors. In a study of fishery workers from Tangxun Lake, consumption of contaminated fish was identified as the source of elevated perfluorooctane sulfonamide (FOSA) in human serum, and potentially contributed to internal PFOS exposure.⁴³ The data demonstrate that diet can be a source of pre-PFAA exposure, particularly in highly contaminated environments. For the general population, other exposure pathways such as indoor dust inhalation, inadvertent dust ingestion, and close contact with consumer products are likely more significant sources of direct pre-PFAA exposure.

Pre-PFAAs in Outdoor Air.

The significance of atmospheric pre-PFAAs transported from diffuse sources as a contributor to human exposure are difficult to constrain due to myriad potential sources. Many pre-PFAAs such as FTOHs are widespread in air and undergo long-range transport.

Perhaps the most persuasive evidence of indirect diffuse exposure to pre-PFAAs is the elevated concentrations of PFAAs concurrent with pre-PFAAs in wildlife in remote environments. Previous research has presented compelling evidence linking atmospheric oxidation of FTOHs followed by trophic magnification.⁴⁴ Humans are exposed to much higher levels of pre-PFAAs through indoor air, dust, and commercial products, and relative contributions of pre-PFAAs such as FTOHs in ambient outdoor air are likely relatively minor outside of highly contaminated areas. However, understanding relative environmental persistence of pre-PFAAs remains essential to determine baseline exposure levels that would persist if pre-PFAAs were phased out of in-use products.

PRE-PFAAS IN THE HUMAN BODY

Some of the strongest evidence of direct exposure to pre-PFAAs is their presence in human samples. However, most biomonitoring campaigns include few pre-PFAAs, due in part to difficulties inherent in analyzing these structurally diverse compounds using widely available methods. The number of PFASs measured by the U.S. National Health and Nutrition Examination Survey (NHANES) has increased since 2011 but to date only one precursor (methyl-perfluorooctane sulfonamido acetic acid, N-MeFOSAA) has been regularly included. MeFOSAA concentrations in blood serum from the general U.S. population were low compared to the predominant PFAAs, ranging from 0.121 to 0.140 ng/mL for samples collected in the 2017/2018 campaign.⁴⁵

In biomonitoring studies using expanded lists of target analytes, sulfonamide and sulfonamido acetic acid precursors to PFOS (including methyl and ethyl derivatives), diPAPs, and FTSs are the most widely reported pre-PFAAs in serum samples from the general population.^{46–48} Detection frequencies and concentrations of serum pre-PFAAs have typically been low relative to PFOA and PFOS. In matrices besides blood, there are only a few studies that have analyzed pre-PFAAs, and detections were infrequent.^{49–51} These low concentrations are likely not representative of total exposure due to pre-PFAA transformation to intermediate products that are missed by targeted analysis, or formation of terminal PFAAs. Some of the most compelling evidence of exposure to pre-PFAA in wildlife is the presence of pre-PFAA intermediate metabolites. Likewise, human biomonitoring should widen its scope of target analytes.

Analyzing Pre-PFAAs in Human Samples.

The diversity of pre-PFAA structures and paucity of information about their physicochemical properties confounds the development of truly comprehensive methods for PFAS analysis in blood serum. Various preparatory and analytical steps preclude the detection of neutral volatile precursors, cationic and zwitterionic precursors, and ultra-short chain PFASs. Widely used sample preparation and analysis methods for analyzing PFASs in serum are optimized for a narrow range of anionic PFASs with medium to long perfluorinated chain-lengths (C6 – C11). These methods are highly effective for measuring trace levels of common PFAAs but they are limited to detecting the subset of pre-PFAAs amenable to negative electrospray ionization and reversed-phase liquid chromatography. Recoveries for structurally diverse PFASs also vary dramatically based on the extraction

method used.⁵² Volatile pre-PFAAs account for a significant portion of precursor exposure, and are important intermediates in biotransformation reactions.^{53,54} Fully understanding contributions of these compounds requires GC-based HRMS analysis of biological samples, which has not been developed to the same extent as LC-based analyses for PFASs. Among the subset of pre-PFAAs potentially detectable using typical methods, only a much smaller subset can be monitored using traditional targeted mass spectrometry (LC-MS/MS) due to the lack of available analytical standards. Fluorotelomer and sulfonamide precursors can form metabolic conjugates and will be missed if conjugates are not cleaved.^{55,56} For example, Dagnino et al. identified an FTOH-sulfate conjugate in human serum as a probable biomarker for FTOH and PAP exposure.⁵⁷

Even the choice of matrix (traditionally serum or plasma) may inhibit understanding of pre-PFAA exposure and bioaccumulation. Serum and plasma are appropriate choices for PFAA biomonitoring based on the propensity for PFAAs to associate with serum proteins.^{58,59} However, pre-PFAAs including sulfonamides and PFPIAs are known to associate strongly with the red blood cell fraction. Depending on the sample matrices used for biomonitoring studies, pre-PFAAs might be overlooked or falsely assumed to be transformed.

Taken together, analytical limitations are serious roadblocks to developing a more comprehensive understanding of the sources and extent of human PFAS exposure. Progress in this area will greatly improve understanding of the importance of human exposure to pre-PFAAs and will contribute to closing the mass balance on organofluorine in human blood. Total organic fluorine measurements indicate the likely presence of unknown PFASs in human blood from the general population,^{60,61} but only a handful of novel PFASs have been identified in human serum.^{38,62–65} The presence of unidentified pre-PFAAs, including intermediate transformation products, has been raised as a potential contributor to the unknown PFAS fraction in serum from the general population.⁶⁶

TOXICOLOGICAL RELEVANCE OF PRE-PFAAS

A wide variety of toxic effects have been associated with PFAAs, including carcinogenicity, liver toxicity, metabolic disruption, and immunosuppression.⁶⁷ The adverse effects of pre-PFAAs are much less well studied. Direct exposure to pre-PFAAs may cause adverse effects in humans by (i) transformation to toxic PFAAs *in vivo*, (ii) toxic effects caused by pre-PFAAs themselves, or (iii) toxic effects induced by intermediate transformation products.

Pre-PFAAs as a Source of PFAAs in the Human Body.

The biotransformation of pre-PFAAs to form known toxic PFAAs has been studied *in vitro*^{56,68} and *in vivo*,^{69,70} and detailed reviews of biotransformation pathways have been published.^{53,71} An example of a generalized biotransformation pathway for fluorotelomers is shown in Figure 2.

Constraining contributions from pre-PFAAs to human PFAA body burdens is hampered by the lack of informative biomarkers of pre-PFAA exposure. A better understanding of the toxicokinetics of pre-PFAAs and identification of novel transformation products will aid in identifying which pre-PFAAs may accumulate or form semi-persistent intermediate

products *in vivo*. To date, evidence of the importance of pre-PFAAs in contributing to PFAA bioaccumulation is mostly inferred from biomonitoring studies. These studies can rarely differentiate between direct and indirect pre-PFAA exposure due possible *in vivo* transformation of pre-PFAAs. Patterns of branched and linear PFOS isomers have been proposed as an approach to track contributions of preFOS to total PFOS concentrations in biological samples because branched isomers tend to be metabolized preferentially,⁸ forming branched products. However, branched isomers may also be degraded preferentially in the environment via microbial degradation and differences in isomer degradation rates are highly complex.⁷²

Studies of highly exposed populations provide some of the clearest evidence of pre-PFAA exposure leading to bioaccumulation of pre-PFAAs as well as to PFAAs formed via metabolism. Nilsson et al. found highly elevated PFOA and other PFCAs in ski wax technicians, which was traced to occupational exposure to fluorotelomers.⁷³ Here, 8:2 FTOH concentrations in the breathing zone corresponded to 92,800 ng/m³, much higher than a typical indoor air concentration of 5 ng/m³. Further evidence for 8:2 FTOH exposure was the presence of intermediates in the biotransformation pathway, 8:2 and 7:3 fluorotelomer unsaturated carboxylates (FTUCAs). In air samples, PFOA was detected in low concentrations whereas 8:2 FTUCA and 7:3 FTUCA were below detection limits. Taken together, the data suggest the ski wax technicians were directly exposed to pre-PFAAs (8:2 FTOH) and following biotransformation, developed elevated PFOA concentrations in their bloodstream.

In the general population, unexpectedly long half-lives for PFCAs in human serum suggest significant contributions of pre-PFAAs to human PFCA body burdens.^{10,48} Additionally, several studies applying pharmacokinetic models to human exposure data underestimated PFHxS concentrations in serum.⁷⁴ Exposure to PFHxS precursors is a potential explanation for the discrepancy. However, there are many other sources of uncertainty in these studies, such as dust ingestion rates and overlooked sources of exposure. Many studies that seek to develop human pharmacokinetic models measure only PFAAs. In such studies, discrepancies between observed and predicted human serum levels may be due to contributions of unmonitored pre-PFAAs or other sources of uncertainty. The importance of direct pre-PFAA exposure to human body burdens and adverse health effects cannot be accurately constrained without comprehensive analytical methods to monitor direct exposure to known and novel pre-PFAAs.

Toxicological Effects of Pre-PFAAs.

While the toxicological evidence for pre-PFAAs and their intermediates is scant, what is emerging indicates that results from studies of PFAAs may be insufficient for understanding the toxicity of the diverse array of pre-PFAAs and their intermediates. Strong binding to nuclear receptors, including peroxisome proliferator activated nuclear receptors (PPARs), thyroid receptor, and several others, has been identified as one potential pathway by which PFAAs and FTOHs induce toxicity.^{75,76} These pathways have not been investigated in detail for other pre-PFAAs. Studies have suggested that FTUCAs and fluorotelomer unsaturated

aldehydes (FTUALs) produced via pathways shown in Figure 2 may induce toxicity via covalent protein binding leading to protein deactivation or disruption.⁷⁷

Some pre-PFAAs and their intermediates are relatively short-lived in biota and thus may be assumed to pose “minimal risks.” However, these pre-PFAAs and/or their metabolic intermediates may be more potent than the PFAA product, raising concerns regarding continuous exposure. Rand et al. observed greater cytotoxicity associated with intermediate fluorotelomer metabolites (saturated fluorotelomer aldehydes (FTALs), FTUALs, FTCAs, and FTUCAs shown in Figure 2) compared to the terminal PFCA products.⁷⁸ FTCAs and FTUCAs were also more toxic than PFCAs *in vivo*.⁷⁹ In studies of 6:2 FTOH,⁸⁰ kidney toxicity occurred at lower administered doses of 6:2 FTOH than one of its terminal degradation products, PFHxA. Another intermediate, 5:3 FTCA, appears to have slower clearance than PFHxA and may either be precipitating kidney toxicity or interacting with PFHxA to enhance kidney toxicity. This suggests that PFHxA is not a suitable indicator of toxicity for 6:2 FTOH or other intermediates.

In addition, toxic non-PFAS byproducts may be produced during biotransformation. Little attention has been paid to these compounds, including quaternary ammonium compounds¹⁶ and hydrofluoric acid (HF),⁸¹ HF generation may explain enhanced toxicity of FTCAs compared to FTUCAs observed in aquatic organisms.⁷⁹ Impacts of HF generation would only be observable in toxicity tests using metabolically active organisms or cell lines.⁷⁸ Short-lived intermediates and transformation byproducts require further study, especially with respect to pre-PFAAs that are metabolically labile and likely to continuously generate transformation products *in vivo*.

FUTURE PERSPECTIVES AND PATHS FORWARD

Much effort has gone into cataloging PFAS uses and production and creating comprehensive databases. Now, more work is needed to elucidate the structures of pre-PFAAs, particularly in commercial products, and to understand the health implications of direct human exposure. Constraining the contributions of pre-PFAAs to total PFAS exposure in humans will require innovative approaches that (1) incorporate commercially-relevant complex mixtures so that novel pre-PFAAs can be identified and studied, (2) use nontarget and surrogate analytical techniques to understand contributions of pre-PFAAs to total PFAS burdens, and (3) develop and use high-throughput bioanalytical tools to investigate how diverse pre-PFAAs interact with and impact biological systems.

Using Complex Mixtures to Understand pre-PFAA Bioaccumulation and Toxicity.

Understanding mechanisms of xenobiotic bioaccumulation, biotransformation, and toxicity typically requires the use of highly controlled laboratory experiments using high-purity standards. This has been a serious challenge for pre-PFAA research because humans are exposed to a wide range of pre-PFAAs, some with unknown structures or no available neat standards. To circumvent this issue, a handful of studies have used commercial products, such as AFFF formulations, to dose *in vivo*^{82–86} and *in vitro*^{87,88} models and measure known and novel transformation products. Others have used environmental samples containing complex PFAS mixtures.⁸⁹ These approaches offer a way forward to

uncover bioaccumulation mechanisms for novel pre-PFAAs, prioritize bioaccumulative pre-PFAAs for further study, and identify biomarkers of pre-PFAA exposure. However, results must be interpreted carefully due to mixture complexity. For example, unknown PFASs as well as non-PFASs present in commercial or environmental mixtures (for example, hydrocarbons present in AFFF)⁹⁰ could be responsible for some portion of toxicological effects observed in mixture studies. Additionally, care must be taken when extrapolating *in vitro* measurements to make inferences about *in vivo* toxicity.

Nontarget and surrogate analytical techniques.

While some information about the impact of pre-PFAAs can be inferred from analyzing the terminal PFAA products, a deeper understanding of pre-PFAA sources, pharmacokinetics, and toxic effects requires structural elucidation of novel pre-PFAAs and their biotransformation products. The use of high-resolution mass spectrometry is essential for this task, as many pre-PFAAs are not available as analytical standards.⁹¹ Harmonized systems for reporting novel PFASs are also critical, as discovery of new pre-PFAAs and other PFASs is currently hampered by inconsistent naming and reporting. Surrogate analytical techniques have been useful in inferring the presence of unknown precursors in environmental samples. The use of predictive models to estimate precursor composition based on observed TOP products⁹² and the combination of TOP and HRMS⁵ are promising paths forward to improve the specificity and comprehensiveness of surrogate techniques. Surrogate approaches⁹³ or sample preparation techniques⁹⁴ that target specific subsets of pre-PFAAs based on their chemical properties could also prove useful in probing the composition of the unknown pre-PFAA pool.

High-throughput bioanalytical tools.

To tackle the complexity of pre-PFAAs and their interactions with living organisms, the development of high-throughput bioanalytical tools is essential for rapid screening and prioritization for additional toxicity testing. A very promising approach for filling in knowledge gaps is the use of read across techniques to predict toxicokinetics and toxicity of data-poor pre-PFAAs using information from well-studied PFASs.⁶⁷ ToxCast assays have been used to rapidly assess the activity of more than 100 PFASs (including several pre-PFAAs) with respect to multiple nuclear receptors.⁹⁵ However, as of yet, these assays cannot fully capture the effects of transformation products. High-throughput zebrafish assays were recently used to assess the metabolism of large libraries of PFASs and identify their transformation products.⁹⁶ *In silico* approaches are useful for predicting the physico-chemical properties and binding interactions of diverse pre-PFAAs and other novel PFASs,⁹⁷ as well as for expanding protein targets.⁹⁸ However, validation of binding through *in vitro* and *in vivo* studies remains an important component of molecular simulations.

IMPLICATIONS FOR RISK ASSESSMENT

Recently there have been arguments made to regulate all PFASs, including pre-PFAAs, as a class.¹ Given that pre-PFAAs will ultimately break down to highly stable end products, current risk-based classification is often done by grouping pre-PFAAs with their PFAA products (the “arrowhead approach”).⁹⁹ This approach, along with the use of

surrogate methods such as TOP assay, are vital given the intractability of elucidating all pre-PFAA structures. However, there may be scenarios in which risk is underestimated by characterizing pre-PFAAs based solely on their PFAA products. Key questions remain regarding whether pre-PFAAs may be more bioaccumulative and/or toxic than their end products, and whether there is a high probability of direct exposure to these pre-PFAAs (highly likely for pre-PFAAs in consumer products). Due to the sheer number of pre-PFAAs, it is not practical to evaluate them all individually. Access to more information on production volume and usage could facilitate prioritization of representative compounds for testing. Data generated for representative compounds could then be used to inform read-across approaches and uncover structure-activity relationships. However, in some scenarios, such as human exposure in communities near PFAS-contaminated sites, there is a much higher likelihood of exposure to novel pre-PFAAs due to their close proximity. In these cases, using nontarget analysis to identify specific pre-PFAAs and surrogate approaches to understand the full extent of PFAS exposure is essential to fully evaluate risk.

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Biography

Dr. Carrie McDonough is an Assistant Professor in the Department of Civil Engineering at Stony Brook University. Her research focuses on understanding the impacts of organic contaminants on aquatic ecosystems and human health using high-resolution mass spectrometry and bioanalytical techniques. Current research objectives include describing uptake and bioaccumulation of complex PFAS mixtures and identifying novel products of PFAS biotransformation.



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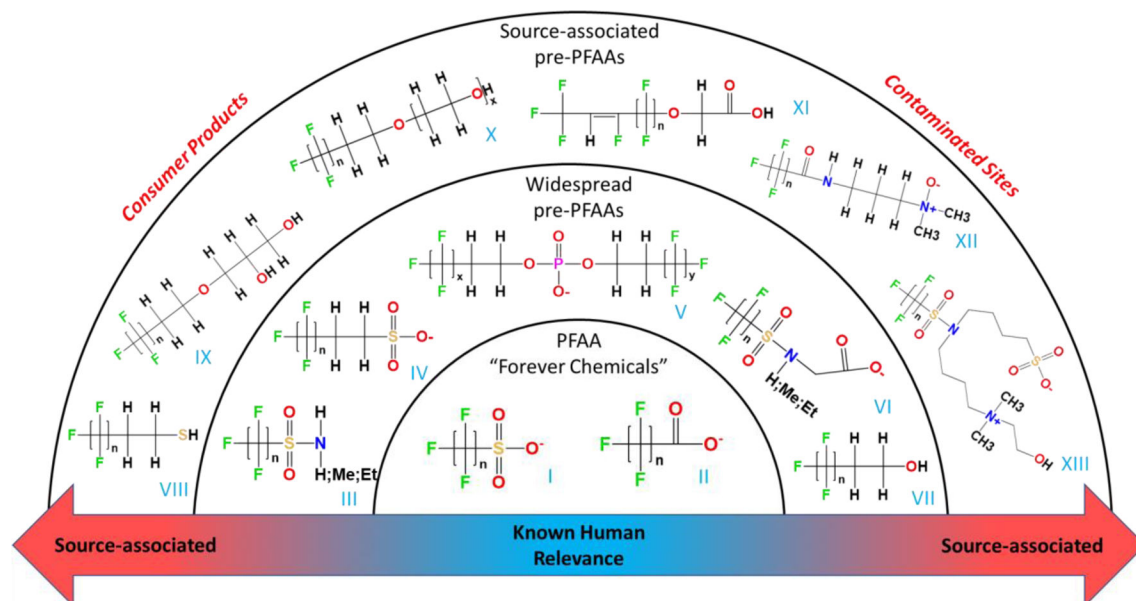
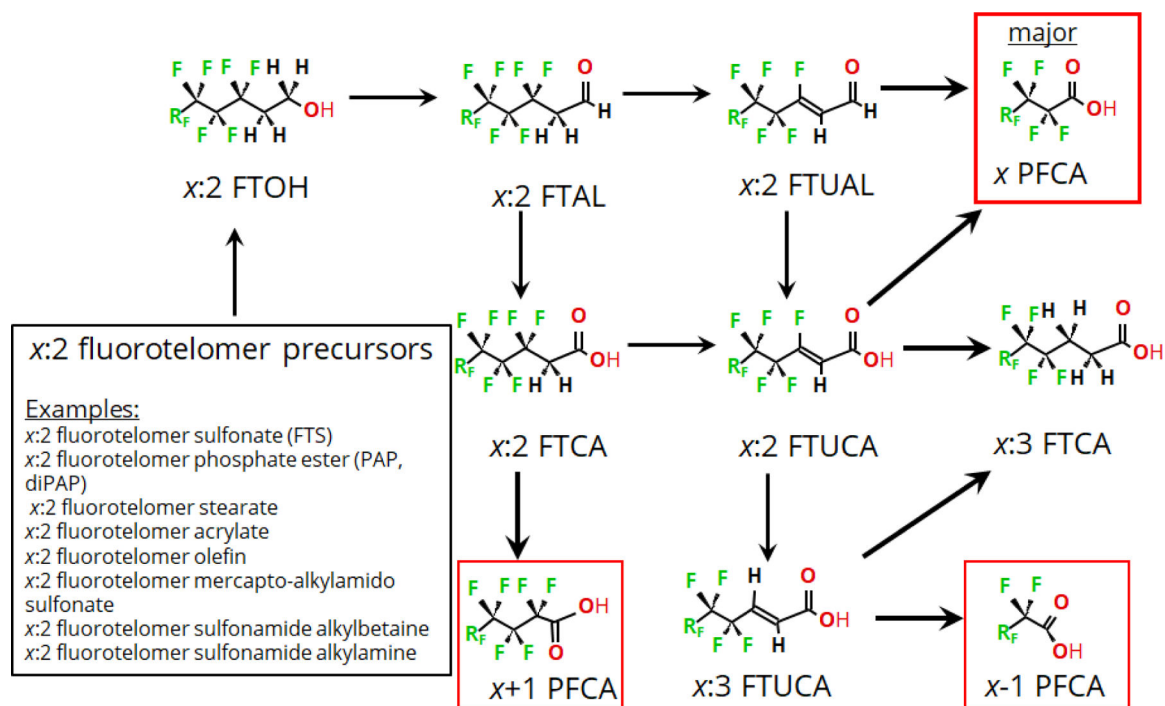


Figure 1.

Examples of PFAAs and pre-PFAAs, and their known relevance for direct human exposure. PFAAs are shown in the center [I: perfluoroalkyl sulfonates (PFSA); II: perfluoroalkyl carboxylates (PFCAs)]. Examples of widespread pre-PFAAs likely relevant for direct human exposure are in the second semi-circle [III: perfluoroalkyl sulfonamides (FASAs); IV: fluorotelomer sulfonates (FTSs); V: polyfluoroalkyl phosphoric acid diesters (diPAPs); VI: perfluoroalkyl sulfonamido acetic acids (FASAAs); VII: fluorotelomer alcohols (FTOHs)]. Examples of source-associated pre-PFAAs with poorly understood importance for direct human exposure are shown in the outer circle and include compounds with known or suspected use in consumer products [VIII, IX, X]^{9,14} and structures identified in highly-impacted areas (fluorochemical manufacturing site [XI]¹⁵ and AFFF source zones [XII; XIII].¹⁶⁻¹⁸)

**Figure 2.**

Generalized biotransformation pathway for a diverse suite of x:2 fluorotelomer precursors producing PFCAs with x carbons as major product and minor pathways yielding PFCAs with x:1 and x-1 carbons. Also shown is formation of x:3 fluorotelomer carboxylate (FTCA) which is known to be a stable final product. R_F = fluorinated aliphatic moiety.