

Total Oxidizable Precursor (TOP) Assay—Best Practices, Capabilities and Limitations for PFAS Site Investigation and Remediation

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Cite This: <https://doi.org/10.1021/acs.estlett.3c00061>



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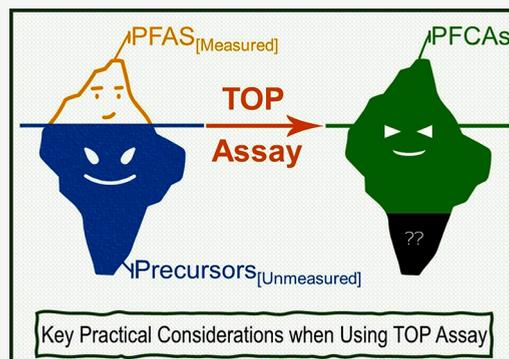
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ABSTRACT: The comprehensive characterization of per- and polyfluoroalkyl substances (PFASs) is necessary for the effective assessment and management of risk at contaminated sites. While current analytical methods are capable of quantitatively measuring a number of specific PFASs, they do not provide a complete picture of the thousands of PFASs that are utilized in commercial products and potentially released into the environment. These unmeasured PFASs include many PFAS precursors, which may be converted into related PFAS chemicals through oxidation. The total oxidizable precursor (TOP) assay offers a means of bridging this gap by oxidizing unknown PFAS precursors and intermediates and converting them into stable PFASs with established analytical standards. The application of the TOP assay to samples from PFAS-contaminated sites has generated several new insights, but it has also presented various technical challenges for laboratories. Despite the increased number of literature studies that include the TOP assay, there is a critical and growing gap in the application of this method beyond researchers in academia. This article outlines the benefits and challenges of using the TOP assay with aqueous samples for site assessments and suggests ways to address some of its limitations.

KEYWORDS: TOP Assay, Best Practice, PFAS Precursors, PFAAs, PFCAs, Conceptual Site Model



1. INTRODUCTION

1.1. The Growing Need of Investigating PFAS Precursors. Per- and polyfluoroalkyl substances (PFASs) are a diverse family of chemicals used in many commercial and industrial products since the 1950s. They are broadly defined by a fluorinated carbon backbone and a hydrophilic functional group (i.e., headgroup). PFASs represent a wide array of compounds with varied carbon chain lengths, chemical features such as ether linkages, repeating polymeric units, and/or other functional groups as presented in Figure 1.¹ The combination of a polar headgroup and a hydrophobic tail in PFAS molecules imparts unique chemical properties that affect their chemical behaviors including surfactant properties, hydrophobicity and lipophobicity, surface tension, chemical and thermal stability, and sorption.² PFASs have been used in many products, including firefighting foams, metal plating fluids, textiles, plastics, papers, electronics, photography and lithography products, cleaning products, coatings, pesticides, medical products, personal care products, explosives, and fuel and hydraulic fluid additives.^{3,4} PFAS-containing products typically include mixtures of PFAS and non-PFAS chemicals including processing residuals, glycols, lipids, solvents, and hydrocarbon surfactants.^{5,6} Recent estimates suggest that thousands of PFASs are already in the market and have entered the environment through direct use and/or discharge

through ventilation systems, wastewater, and landfills.^{3,4} Consequently, PFASs are found in all environmental compartments including water, soil, sediment, air, particulates, and biota.² The thermal and chemical stability of the carbon–fluorine bond makes some PFASs resistant to transformation and degradation and contributes to their persistence in the environment.

Perfluoroalkyl acids (PFAAs) are a class of highly stable compounds which include perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). PFAAs are unlikely to break down in the environment due to the stability of the carbon–fluorine bond and the oxidized state of the polar headgroups. However, other PFAS classes may undergo transformation depending on environmental conditions.⁷ Some PFAS precursors can be converted into intermediate or terminal PFAS degradation products through oxidative transformation processes.^{8–10} It is worth noting that not all PFAS precursors will fully oxidize to PFAAs under typical

Received: February 6, 2023

Revised: March 7, 2023

Accepted: March 7, 2023

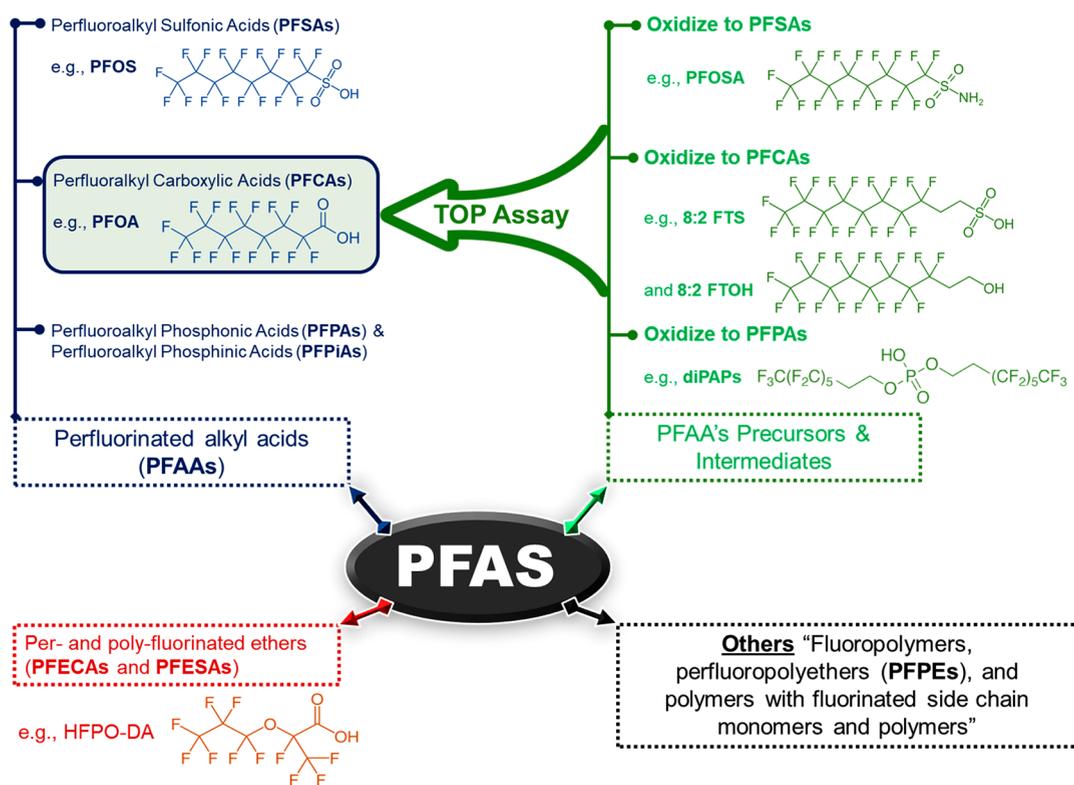


Figure 1. Groups of PFAS chemicals based primarily on common headgroups and other structural features. (For more extensive lists of groups see Buck, 2011; Backe, 2013; Wang, 2017.)

environmental conditions. In this article, the term “PFAS precursors” refers to PFASs with the potential for oxidative transformation, resulting in terminal products such as PFAAs. During the transformation of PFAS precursors into PFAAs, intermediate chemical compounds may be formed. These chemicals are referred to as “PFAS intermediates” and are also considered among potential precursors (e.g., 5:3 fluorotelomer carboxylic acid (FTCA) is relatively stable PFAS intermediates that might form from fluorotelomer alcohol (FTOH)).¹¹ Typically, “PFAA precursors” are identified based on documented evidence of oxidation. Some PFAS precursors, such as fluorotelomers and perfluorinated sulfonamides, are manufactured as replacements for PFAAs and used in the PFAS manufacturing processes and can be present also as intermediates. However, laboratories can only reliably measure a limited number of PFAS precursors due in part to lack of commercial availability of analytical standards (both native and isotopically labeled) of known identity and purity that are traceable to a national metrology standard such as ISO Guide 34.

The current understanding of precursors of PFASs, including molecular structures, physical and chemical properties, bioaccessibility, degradation rates, and environmental prevalence, among the scientific community is limited. Gaps exist in publicly available information pertaining to proprietary composition and production methods, abiotic and biotic degradation pathways, and the presence and quantities of PFAS precursors in the environment. The biotic and abiotic transformation processes of these precursors are not well understood, hindering our ability to fully comprehend the impact of PFAAs on human and ecological health.¹² Furthermore, the stability and persistence of PFAS precursors in various ecological settings have yet to be fully characterized,

partly due to limitations in analytical capabilities. In order to better understand and predict the behavior of these precursors in the environment, the scientific community would greatly benefit from access to a wider range of reliable tools and information.

1.2. Current Analytical Options for PFAS Analysis. To accurately evaluate migration and exposure pathways at PFAS-contaminated sites, a conceptual site model (CSM) is required.¹³ However, current PFAS CSMs are based on data for PFAAs and a limited number of precursors, potentially missing numerous other PFAS compounds. This data gap could result in an underestimation of PFAS migration, exposure, risk, and treatment needs. For example, certain oxidation technologies such as air sparging or advanced oxidation processes (AOPs) may convert precursors and intermediate PFAS on-site to PFAAs, thereby increasing targeted PFAA concentrations post-remediation.^{14–16} In addition, unmeasured PFASs may affect transport of total PFASs due to the surfactant nature of many of these molecules. The need for a thorough understanding of PFAS contamination to develop effective management strategies and robust remediation techniques is paramount. The rapid development of new PFAS compounds has exceeded the advancement of reference materials and standardized analytical methods. To address this gap, efforts should be made to develop novel methods for identifying and quantifying a broader spectrum of PFAS precursors and their oxidative transformation products or proxies that can be used to determine “total PFAS” content.

1.2.1. Targeted PFAS Analysis. ASTM International and federal government agencies, such as the US EPA, have developed targeted PFAS analytical methods and voluntary consensus standards to quantify ~40 PFASs using liquid chromatography-tandem mass spectrometry instrumenta-

tion.^{17–19} Target analytes in these methods include a range of PFCAs and PFASs as well as a smaller number of PFAS precursors and intermediate transformation products (e.g., partially oxidized PFAS precursors). These methods are called targeted PFAS analytical methods because they are used to determine the identities and concentrations of specific PFASs in environmental samples by comparison to commercially available certified reference materials. A common criticism of targeted analysis is the limited scope of compound detection. While the currently available targeted analytical methods provide information about many PFASs, the measured PFASs may be a fraction of the PFASs present in products or environmental sites due to the presence of PFAS precursors or intermediate degradation products that are not target analytes in the methods.^{5,20} Draft US EPA Method 1633²⁷ for non-drinking water sample matrices includes just 13 PFASs identified as precursors or intermediates, whereas ASTM D8421-21 includes 14 of them.²¹ Laboratories may modify these methods to test for more PFAS target analytes, but further expansion is limited by the commercial availability of appropriately certified reference materials and compatibility with existing analytical technologies.

1.2.2. Beyond Targeted Analysis. Various methods for identifying PFASs in different matrices, both directly and indirectly, have been rigorously reviewed previously.^{22–24} Indirect methods estimate the presence of PFASs and other fluorinated compounds in environmental compartments by measuring fluorine abundance,^{25,26} such as through the use of adsorbable organic fluorine (AOF), extractable organic fluorine (EOF), and total organic fluorine (TOF).^{27–29} AOF is a subset of EOF, which in turn is a subset of TOF. AOF is used to measure highly fluorinated compounds that can be adsorbed onto activated carbon, while EOF is used to measure all fluorinated compounds that can be extracted from a sample using an appropriate solvent or extraction method. TOF measures the total amount of organic fluorine in a sample. The US EPA has released a draft method for measuring organofluorine content in wastewater samples (draft EPA Method 1621),³⁰ which is sensitive to a range of fluorinated compounds, including PFAS and other fluorinated organic chemicals such as pesticides and pharmaceuticals that contain one or more carbon–fluorine bonds. However, these methods does not identify specific structures and may produce experimental artifacts during sample processing, potentially leading to the underestimation or overestimation of PFAS concentrations. These methods can be used as a screening method to evaluate if a water sample is impacted by high organic fluorinated compounds before considering further investigation of PFAS precursors. In contrast, nontarget analysis (NTA) detects PFAS compounds through the use of higher resolution mass spectrometry techniques such as quadrupole-time-of-flight tandem MS, allowing for the screening of thousands of PFASs using related analytical standards.³¹ However, NTA data are challenging to quantify without standards, requiring targeted methods for quantification purposes.³² Although NTA was used recently as a semi-quantification method, the results showed high variability and overestimated concentrations compared to TOP assay results in many cases.^{33,34} The bias in semi-quantified concentrations is in part due to limited analytical standards and in part due to the ionization or fragmentation differences when using surrogate reference standards. NTA is also time intensive,

requires extensive interpretive expertise, and is not ideally suited to routine analysis.

2. TOTAL OXIDIZABLE PRECURSOR (TOP) ASSAY FOR ENVIRONMENTAL APPLICATIONS

The TOP assay is a method used to convert oxidizable PFAS precursors into PFAAs, which are measured using a targeted PFAS analytical method. This oxidative sample pretreatment process was initially published in a journal article to better characterize PFASs in urban runoff, and the goal of this assay is to accurately quantify the PFAA precursors present in a sample (Figure 1).³⁵ One of the key benefits of the TOP assay is its compatibility with the same analytical instrumentation utilized in targeted analysis, which renders it more accessible to laboratories, as they do not need to invest in additional instrumentation. This feature has facilitated the application of the TOP assay to diverse matrices, including surface waters, soils, sediments, animal tissue, eggs, pesticides, and textiles.^{36–40} The TOP assay is gaining traction beyond research for its potential utility in site investigations and regulatory assessments. However, no clear guidance is offered for applications of the TOP assay in these different scenarios. Thus, this critical review minimizes this gap for researchers and practitioners by evaluating the benefits and challenges of the TOP assay for liquid aqueous samples. Particularly, the provided recommendations for addressing common obstacles encountered during the analysis will improve the development of CSMs.

2.1. Basics of the TOP Assay. To analyze a sample for the presence of PFAS precursors, duplicate samples are collected and tested, one of which undergoes pretreatment with the TOP assay while the other remains untreated. In the TOP assay procedures, aqueous samples undergo a heat- and alkaline-activated persulfate oxidation to promote radical formation (e.g., sulfate radicals after activation undergo further reactions to generate additional radical species such as hydroxyl radicals). This oxidation process converts PFAS precursors present in the samples to intermediate(s) or terminal oxidation product(s) with a carbonyl group, specifically a carboxylic acid group (–COOH). The duplicate samples are then analyzed with a targeted PFAS method, and concentrations of PFASs in the sample treated with the TOP assay are compared to concentrations in the untreated sample to evaluate the presence of precursors.^{35,41} The concentration of PFAS precursors present in the sample can be estimated by subtracting the concentrations in the pre-TOP assay sample from the concentration in the post-TOP assay sample. An increase in the summed PFAA concentrations in the post-TOP assay sample indicates the presence of PFAS precursors.

The procedures of the TOP assay used in solid matrices also varies with either (1) the PFAS being extracted first from the solid sample before oxidizing the extract or (2) the so-called direct TOP assay (dTOP) where the whole sample is oxidized first and then PFAS is extracted.⁴² Although the TOP assay for solid samples is out of the scope of this article, it is worth highlighting that the major differences in the PFAS TOP assay method for aqueous and solid samples are related to sample preparation, matrix interference, detection limit, and calibration.⁴³ For instance, solid samples require an additional step of extraction with solvents such as methanol or acetonitrile before the liquid–liquid extraction.⁴⁴ Solid samples often contain more matrix interference than aqueous samples, which can lead

to higher detection limits for PFASs in solid samples than for aqueous samples.⁴⁵

No published, standardized reference method or consensus guidelines exist for the use of the TOP assay, although it has been used in varied field assessments. Australia is one of the few countries that integrates the TOP assay into their PFAS management. The Queensland Department of Environment and Heritage Protection uses the TOP assay in regulating fluorine contents of aqueous film forming foams (AFFFs).⁴⁶ The composition of fluorine-containing AFFFs has evolved over time, and a potentially large number of PFAS precursors may be present at AFFF-impacted sites, including PFAS precursors containing cationic, anionic, and/or zwitterionic functional groups,⁴⁷ that would contribute to the presence of more PFAAs through biotic and abiotic transformation over time.^{48,49} While many modern AFFF formulations contain limited PFAA content, some of them contain broad mixtures of proprietary PFAS precursors including fluorotelomer sulfonamido betaines and perfluoroalkyl sulfonamide amino carboxylates.⁵⁰ Thus, sites contaminated with AFFF could benefit from TOP assay data to reconstruct the composition in AFFF formulations⁵¹ and AFFF-contaminated sites.⁵² Some precursors are not in the original AFFF formulations but are transformation intermediates. For example, the perfluoroalkane sulfonamides and perfluoroalkane sulfonamido substances are transformation intermediates from other precursors and can be transformed into PFSAAs and PFCAs,³⁶ and fluorotelomer sulfonates can be transformed into PFCAs.⁴⁵ Recent applications of the TOP assay include environmental forensics applications, since precursor identification may be useful for source identification.^{42,53} More research is needed to support this emerging application.

2.2. Technical Challenges with the TOP Assay. There are several technical challenges that may be encountered when using the TOP assay to analyze environmental samples. For example, precursor oxidation is not specific, producing a range of intermediate PFAS transformation products and PFAAs. The distribution of these transformation products can vary based on the properties of the sample and the oxidation conditions.⁵⁴ It is currently challenging to determine the specific precursor or intermediate for post-TOP analytes in environmental samples. For instance, recent studies showed that results from the TOP assay might need to be combined with other statistical methods, such as Bayesian inference,⁵¹ to reconstruct the concentrations of oxidizable precursors and their perfluorinated carbon chain length and manufacturing origin.⁵² In addition, the results from aqueous samples can be highly variable due to factors such as the presence of natural organic matter, co-contaminants, and other variables. These limitations should be taken into consideration, as oxidation of precursors may not be complete when using the TOP assay with aqueous samples for environmental analysis. The following examples explain some possible limitations.

2.2.1. Example 1. During the oxidation process, it is possible to produce unmeasurable/nontargeted intermediates or ultrashort chain PFASs, such as trifluoroacetic acid (TFA) and perfluoropropionic acid (PFPrA).^{36,47} As shown in Figure 2, PFPrA can make up a significant portion of the oxidation products' yield for certain precursors, up to 35%. However, apart from the D8421-22 method, currently available analytical methods have not been validated for measurement of PFPrA. If ultrashort chain PFASs like PFPrA are not taken into account, the TOP assay may underestimate the total concentration of

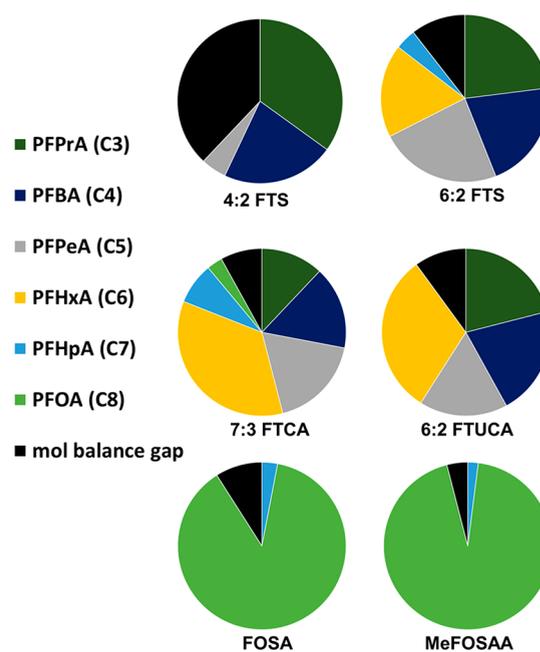


Figure 2. Molar yield and mole balance gap of selected PFAS precursor after TOP assay (This is a reproduction of data collected from Martin et al.³⁶). **Note:** PFPrA is not commonly measured.

PFAS precursors in a sample. It is therefore important that the analytical methods used in the TOP assay include the measurement of ultrashort chain PFASs when possible.

2.2.2. Example 2. Some PFASs contain ether functional groups, which may be resistant to oxidation or may produce oxidation products that are not typically measured by targeted PFAS methods.⁵⁵ For example, a replacement for perfluorooctanoic acid (PFOA) called 4,8-dioxo-3H-perfluorononanoic acid, or ADONA, has two ether linkages and a fluorine-to-hydrogen substitution on C3, next to one ether bond. In the TOP assay, ADONA was completely converted to an intermediate, perfluoro-3-methoxypropanoic acid, or PFMO-PrA, with a yield of $98 \pm 20\%$.⁵⁵ This intermediate is not commonly measured by the targeted analytical methods. Other PFAS precursors with ether functional groups may not oxidize readily using the TOP assay (e.g., F-53B, a chlorofluoroalkyl ether carboxylic acid) or may generate transformation products that are not readily identified with liquid chromatography/mass spectrometry (e.g., Nafion byproduct). Therefore, TOP assay values may underestimate the actual total concentration of PFAS precursors in a sample when PFAS ethers and other novel PFASs are present.

2.2.3. Example 3. In the analysis of the TOP assay, the presence of certain target precursors before and after the assay can indicate that the oxidation of these substances was incomplete.⁵⁴ Incomplete oxidation may be due to the presence of co-contaminants in the sample matrix that consume some of the oxidant, leading to an insufficient amount of chemical oxidant available to fully oxidize the sample. For example, the detection of fluorotelomer sulfonates (FTSs) in post-TOP assay samples may be indicative of this phenomenon. While the detection of target precursors in both pre- and post-TOP assays could suggest incomplete oxidation of the chemicals, it could also indicate incomplete oxidation of other precursors. In this circumstance, the TOP assay results may underestimate the concentration of precursors present.

Table 1. Suggestions to Consider When Using the TOP Assay

	Current practice	Better practice
Lab Selection	<ul style="list-style-type: none"> ○ No available US EPA certified or consensus method. ○ The TOP assay procedures vary from lab to lab. 	<ul style="list-style-type: none"> ✓ Ask laboratory to provide TOP assay SOP, data quality information, and a demonstration of capability. ✓ The TOP assay SOP should be reviewed before committing to the TOP assay analysis.
TOP Assay Sample Collection	<ul style="list-style-type: none"> ○ Follow PFAS sampling SOP. ○ No differences when collecting TOP assay samples 	<ul style="list-style-type: none"> ✓ Precursor concentrations generally decrease downgradient from the source areas, collecting PFAS samples from low to highly impacted locations. ✓ Make note on chain of custody for the samples which are collected from the highly impacted areas. ✓ Laboratory screening analysis may need to be performed to determine proper sample dilution. ✓ Collect sample replicates for analysis and QA needs ✓ Minimize air exposure during sample collection to minimize oxidation of sample or loss of semi-volatile precursors.
Target PFAS Analysis	<ul style="list-style-type: none"> ○ LC/MS/MS methods vary – often single lab validated 	<ul style="list-style-type: none"> ✓ The list of targeted PFAS to be measured should include ultrashort-chain PFAAs such as PFPrA (see ASTM D8421) ✓ Analyze samples using a multi-laboratory validated test method, wherever possible ✓ Laboratory should supply SOP, data quality information, and a demonstration of capability in relevant matrices
Replication	<ul style="list-style-type: none"> ○ Following the laboratory guidance for TOP assay 	<ul style="list-style-type: none"> ✓ Triplicate samples of each with and without TOP oxidation and recommended to provide estimates of measurement uncertainty for results.
QA/QC samples	<ul style="list-style-type: none"> ○ None recommended 	<ul style="list-style-type: none"> ✓ Spike 20% of samples with precursors and intermediates that can be reliably measured with the targeted analytical method and analyze replicate spiked samples with and without TOP assay oxidation steps to evaluate extent of oxidation. ✓ Sample should be rerun/reoxidized when precursors are detected in post-TOP assay samples. ✓ Include method blanks, blank spikes, method reporting limit spikes, and surrogates
Additional Analytical Methods	<ul style="list-style-type: none"> ○ None recommended 	<ul style="list-style-type: none"> ✓ Collect data on salinity, organic carbon content, and pH for each sample
Data Reporting	<ul style="list-style-type: none"> ○ Laboratory reports target PFAS detections in concentrations 	<ul style="list-style-type: none"> ✓ Calculate mole yield and degree of oxidation in matrix spike samples, review replication data (see Section 4.3)

2.2.4. *Example 4.* Significant variability has been observed in interlaboratory studies of the TOP assay.⁵⁴ This variability may be due to, in part, the fact that there is no standardized method that participating laboratories can follow. After TOP treatment, differences in the concentrations of total PFASs were observed both within and between laboratories. For instance, one TOP assay study shows data variations for perfluorodecanoic acid (PFDA) and perfluorohexanesulfonic acid (PFHxS) from 72% to 218% and 85% to 128%, respectively, of the spiked values. The study report did not recommend any measures to reduce this variability.

2.2.5. *Example 5.* The TOP assay has been employed in several studies to evaluate the efficacy of biological and physical-chemical treatment technologies in managing PFAS risks. It has been employed to confirm the generation of intermediates during the electrochemical destruction of PFASs.⁵⁶ Although intermediates were identified using targeted analysis, some intermediates were not converted

into PFCAs after the TOP assay, confirming that precursor transformation is complex and suggesting that the TOP assay and targeted analytical methods do not capture all the intermediates.

3. BEST PRACTICE TO SAMPLE AND PERFORM THE TOP ASSAY

The likelihood of detecting PFAS precursors at a particular location or facility can vary based on several factors. For example, PFAS precursors are more likely to be found in areas associated with (1) releases of PFAS-containing AFFF, (2) manufacturing facilities that produce or use PFAS precursors, (3) leachate from municipal solid waste landfills, and (4) biosolids or effluent from municipal wastewater treatment facilities that receive a wide range of source waters.^{57,58} Drinking water utilities, on the other hand, generally have a low to moderate probability of detecting PFAS precursors in their source water, depending on the proximity to any of the

forementioned point sources. If no point source has been identified at a contaminated site or facility, and no PFAS precursors are detected with a targeted analytical method, the probability of precursors being found using the TOP assay is lower.

There are several considerations that should be taken into account when conducting the TOP assay, as listed in Table 1. It is important to carefully consider who will be responsible for conducting the assay on samples and who will be responsible for analyzing the sample extracts for PFAS analysis. While there are several commercial laboratories that offer the TOP assay, it is advisable to review and compare their quality assurance protocols and practices before selecting a vendor. Alternatively, researchers with access to the necessary laboratory equipment and instrumentation may choose to conduct the TOP assay themselves. In this case, it is strongly recommended that appropriate sample collection, PFAS analysis, and quality assurance practices are followed.

3.1. Sample Collection and Storage. It is important to note that many PFASs are known to have surfactant properties with high mobility and may disperse easily. To ensure the accuracy of the results, samples for PFAS analysis should be collected following a standard operating procedure (SOP) to document collection methods. It is advisable to minimize the use of materials that may contain PFASs during the sampling process and to collect field blanks to assess any cumulative background contamination that may be contributed by sampling equipment and practices, collection containers, and/or the testing laboratory. In addition, samples for related PFAS analyses (e.g., targeted analysis, AOF) should be collected at the same time as samples for the TOP assay for comparison purposes. Precursors are likely to oxidize in aerobic environments. Previous site data may be helpful in identifying aerobic zones and appropriately planning for sampling. In general, samples should be taken in close proximity to the suspected sources to characterize precursor presence. Samples may be frozen to minimize potential breakdown of precursors during storage.

3.2. Extraction and Targeted Analysis. There are several methods, such as EPA method 537.1, SW846 3512/8327, and draft EPA Method 1633, that utilize LC-MS/MS for targeted analysis of PFAS compounds. However, these methods have limitations in terms of the number of targeted analytes that can be analyzed, and none currently include ultrashort chain PFASs such as PFPrA as target analytes.^{47,59,60} To improve the accuracy of the TOP data, it may be beneficial to expand traditional targeted methods to quantify a wider range of PFASs, including PFPrA. When selecting a laboratory to conduct TOP analysis, the specific analytical method, extraction protocol, and target analytes should be considered. Therefore, if conducting the TOP assay in-house, it is recommended to consider the various TOP method adaptations that have been described in the literature.^{7,35,42,43,47}

3.3. Oxidation Efficiency Evaluation with Surrogates. A variety of quality assurance and quality control (QA/QC) samples may be included in project planning documents, such as field duplicates/replicates, field blanks, equipment rinse blanks, internal calibrant spikes, method blanks, blank spikes, matrix spikes, and surrogate spikes.^{45,54} Isotopically labeled standards used for the quantification of PFASs during targeted analysis should be added to samples after oxidation to prevent degradation of internal standards. However, matrix spikes and

surrogate spikes should be spiked into samples prior to the TOP assay to assess oxidation efficiencies. Matrix spikes of a known precursor such as 6:2 FTS can help to determine the efficiency of the oxidation process.⁵⁴ The degree of oxidation can be assessed by comparing the PFAS precursor concentration in a matrix spike subjected to the TOP assay relative to the concentration in an untreated matrix spike, with >95% loss of the added precursor(s) indicating acceptable levels of oxidation.⁵⁴ However, it is important to note that complete oxidation may not always be achieved in environmental samples due to the presence of co-contaminants, high concentrations of precursors, or variations in laboratory procedures.⁶¹ As discussed previously, incomplete oxidation may indicate that the TOP assay underestimates precursor quantities. Other precursors, such as isotopically labeled ¹³C perfluorooctanesulfonamide (FOSA) or *n*:2 FTS compounds other than 6:2 FTS, may also be useful for monitoring or evaluating the extent of precursor oxidation.³⁶

3.4. Minimizing Variability. The success of the TOP assay is dependent upon adding enough oxidant.⁶² While the use of the TOP assay for precursor detection is relatively new, AOPs for contaminant degradation have been widely employed in the treatment of wastewater and groundwater. Confirming complete oxidation can be technically challenging, but several laboratory proxies can aid in this process. For example, monitoring pH and residual oxidants can provide evidence of complete oxidation.^{63,64} Persulfate oxidation via hydroxyl radical formation is nonspecific, and background constituents affect its performance. It is also important to note that certain factors can inhibit the formation of radicals and change formation pathways of different radical species. Known radical scavengers include halide ions, carbonates,⁶⁵ humic acid,⁶⁶ and other co-contaminants.⁶⁷ Thus, the efficiency of oxidation in environmental samples may vary due to the chemical properties of the sample. To gain a deeper understanding of these discrepancies, it would be useful to collect physicochemical data about the samples before analysis, including information on salinity, organic carbon content, and pH. These measurements can provide indirect evidence to help explain data variability and confirm the complexity of the oxidation process.

3.5. Mole Balance and Data Reporting for the TOP Assay. Ideally, the TOP assay procedure should convert each PFAS precursor into PFAAs that can be measured using targeted analytical methods. For most precursor PFASs, the TOP assay converts them to PFCAs.³⁵ It is important, however, to note that some recent studies have reported the production of other PFAAs, including some PFSAs, in soil samples post TOP oxidation.⁶⁸ Since post-TOP analytical methods include both PFCAs and PFSAs, it is recommended that all PFAS in the analytical method be included when evaluating TOP results.^{69,70} Until further research can explain the instances of PFSA production from TOP, it is recommended that data is focused on PFCAs only. The conversion process can be tracked using moles, to avoid the complications of changing molecular masses due to chemical transformations. Mass-based concentrations are influenced by both the amount present and the molecular weight of the chemical. Mole balances or mole yields have been used to compare spiked samples (e.g., 4:2 FTS) with and without TOP treatment. If some PFASs (e.g., low molecular weight PFASs) are not included in the targeted PFAS analytical method, there may be significant gaps in mole yield.⁷¹ These gaps may

indicate that the total PFAS estimate after TOP treatment is biased. In general, because the TOP assay estimates previously unmeasured precursor amounts, mole yields should be greater after TOP treatment.

One way to determine mole yields is to use quality assurance/quality control samples spiked with known quantities of PFAS precursors and intermediates. By measuring these samples before and after TOP treatment, it is possible to calculate the mole yields of the spikes. This information can be useful in evaluating the uncertainty of the TOP assay at a specific site. A poor degree of oxidation (less than 95%) suggests that complete oxidation may not have occurred and that the total PFAS concentrations in the original sample (e.g., soil, groundwater) may be higher than the measured amounts. Other considerations in poor mole yields include the formation of PFAS outside of targeted analysis, such as short chain compounds or volatile PFASs, such as FTOHs.⁷²

3.6. Considerations for Monitoring PFAS Treatment Performance. It is important to consider the monitoring of PFAS precursors in treatment systems to treat PFASs and other contaminants. Section 3 of this article provides some suggestions to determine if a facility is likely to be impacted by PFAS precursors from potential sources. The tools available for characterizing these precursors are outlined in Section 1. When a facility is likely to be affected by PFAS precursors, the impact of these precursors on the CSM and mass balance should be considered throughout the treatment processes. Bench-scale testing may be conducted to understand the fate, transport, and treatment of precursors. The analysis of precursors may begin with targeted PFAS analysis to determine the presence and concentrations of specific precursors. The TOP assay can help understand the movement and transformation of precursors (such as transforming precursors into PFAAs) throughout the treatment process. For example, in a previous pilot study of groundwater treatment, the TOP assay was used to investigate the need for more frequent granular activated carbon (GAC) changeouts when precursors were present in the influent. The full-scale GAC treatment system, which did not consider the presence of PFAS precursors, experienced significantly increased operating costs due to the unanticipated need for more frequent changeouts. The pilot study, which simulated the conditions of the full-scale system, revealed the presence of PFAS precursors in the influent and allowed for the monitoring of GAC sorption of these precursors and their breakthrough from the system using the TOP assay.^{73,74}

4. THE WAY FORWARD

The potential negative health impacts of PFASs have led to increased regulations and restrictions on their use. There has been also an increased use of PFAS precursors, which are chemicals that can break down into PFAAs under certain conditions. Thus, understanding PFAS precursors is important to describing and appropriately managing PFAS risks. The TOP assay emerged as a tool to estimate the quantities of PFAS precursors present in the environment. Important considerations when using the TOP assay include sample collection, targeted analysis, and the use of matrix spikes to evaluate the efficiency of sample treatment. It is important to carefully review the quality assurance protocols and practices of commercial laboratories before selecting a vendor for the assay or to follow appropriate practices if conducting the assay in-house. It is also essential to consider the potential matrix effects that can impact the accuracy of the assay, as the

presence of other background constituents in the sample can interfere with the measurement of PFAS precursors. The use of matrix-spiked standards and internal standards can also help to address these matrix effects. Finally, the TOP assay may be used for estimating the presence of PFAS precursors at the environmental sites when careful consideration of sample collection, analysis, and quality assurance practices is performed for accurate and reliable results.

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Notes

The authors declare no competing financial interest.

†Retired.

ACKNOWLEDGMENTS

The authors thank Dr. Troy Strock from EPA's Office of Land and Emergency Management (OLEM) and Dr. Jean Van Buren from EPA's Office of Research and Development (ORD) for reviewing the document and providing valuable comments.

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