Analytical and Bioanalytical Chemistry (2024) 416:1777–1785
https://doi.org/10.1007/s00216-024-05125-y

FEATURE ARTICLE

# PFAS ghosts: how to identify, evaluate, and exorcise new and existing analytical interference

**Jacqueline Bangma[1] · Kitrina M. Barry[2] · Christine M. Fisher[3] · Susan Genualdi[3] · Theresa C. Guillette[4] · Carin A. Huset[2] · James McCord[1] · Brian Ng[3] · Benjamin J. Place[5] · Jessica L. Reiner[5] · Anna Robuck[1] · Alix E. Rodowa[5]**

Received: 2 October 2023 / Revised: 13 December 2023 / Accepted: 18 December 2023 / Published online: 27 January 2024


## Abstract

With increasing public awareness of PFAS, and their presence in biological and environmental media across the globe, comes a matching increase in the number of PFAS monitoring studies. As more matrices and sample cohorts are examined, there are more opportunities for matrix interferents to appear as PFAS where there are none (i.e., "seeing ghosts"), impacting subsequent reports. Addressing these ghosts is vital for the research community, as proper analytical measurements are necessary for decision-makers to understand the presence, levels, and potential risks associated with PFAS and protect human and environmental health. To date, PFAS interference has been identified in several matrices (e.g., food, shellfish, blood, tissue); however, additional unidentified interferents are likely to be observed as PFAS research continues to expand. Therefore, the aim of this commentary is several fold: (1) to create and support a publicly available dataset of all currently known PFAS analytical interferents, (2) to allow for the expansion of that dataset as more sources of interference are identified, and (3) to advise the wider scientific community on how to both identify and eliminate current or new analytical interference in PFAS analyses.

**Keywords** PFAS · Interference · Analytical methods · Method development

## Introduction

Analytical measurements by liquid chromatography-mass spectrometry (LC–MS) are predicated both on high measurement sensitivity and the method specificity. Nevertheless, in low-resolution mass spectrometry, the specificity of individual reaction monitoring transitions (e.g., multiple reaction monitoring (MRM)/selected reaction monitoring (SRM)) can be difficult to ensure. For the analysis of per- and polyfluorinated alkyl substances (PFAS) in particular, the necessity of low detection limits and the ubiquity of environmental PFAS contamination makes measurements susceptible to bias from systematic contamination and interfering compounds from complex matrices. Careful method validation and awareness of analytical limits is necessary to prevent one from "seeing ghosts" of PFAS interferences where they are not present.

During PFAS analyses, systemic issues related to PFAS *contamination* that may arise from the unintentional introduction of PFAS to samples during sample collection, preparation, or analysis can be resolved through experimental and laboratory design [1–3]. This type of contamination, while an important concern in PFAS analysis, is outside the scope of this paper. Instead, this paper will focus on analytical *interferences*, which are non-PFAS based substances, interferents, that produce a signal indistinguishable from the target analyte within the context of the analytical method (e.g., identical MRM transition and (similar) retention time) [4]. Interferents can impact any measurement, and the techniques here are broadly applicable to other chemical classes, but this manuscript focuses on PFAS due to the relatively high probability and impact of interference. Compounds interfering with PFAS MRM transitions have been observed as closely or co-eluting chromatographic peaks and as persistent background [5, 6]. These elevated or incorrectly selected peaks can result in

✉ Jacqueline Bangma
Bangma.jacqueline@epa.gov

1 Center for Environmental Measurement and Modeling, US Environmental Protection Agency, Durham, USA

2 Minnesota Department of Health, St. Paul, MN, USA

3 Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, USA

4 Arcadis G&M of North Carolina, Inc, Raleigh, USA

5 National Institute of Standards and Technology, Gaithersburg, USA

erroneously elevated quantitative values for targeted PFAS measurements and can significantly alter the scientific conclusions of a study and lead astray resulting decisions. For example, false positive detections of PFAS occurrence may lead to inaccurate assumptions about which PFAS are present in the environment or bioaccumulating in a study population. They may also obscure true PFAS sources. For example, researchers reported apparent false detection of 6:2 fluorotelomer sulfonic acid (6:2 FTS) due to interference from an emerging PFAS [5, 6], which would have suggested significantly different origins (e.g., aqueous film-forming foams (AFFFs) vs. fluoropolymer manufacturing) for the PFAS contamination and markedly different follow-up source identification and control steps.

Additionally, analytical interference that yields quantitative biases of present PFAS can have significant impacts on our understanding of PFAS behaviors. A study by Bangma et al. 2020 identified a perfluorobutanoic acid (PFBA) interference in placenta, with radically different conclusions if the interferent had not been noticed [7, 8]. The apparent high prevalence and abundance of PFBA in human placenta described by the authors would have radically departed from our understanding of short- vs. long-chain PFAS accumulation and toxicokinetic behavior. Instead, correction of the analytical measurements by removing inclusion of the interferent yielded expected distributions of PFAS, and no radical adjustment of current dogma was necessary.

To serve the community performing PFAS analysis, this communication describes important observations or "red flags" that may indicate that an interferent is leading to a false positive or elevated PFAS detection. To demonstrate this, we present a few recent examples of PFAS interferents and their characterization. Additionally, we propose actions to confirm and resolve suspected PFAS interference. Finally, this work introduces a centralized resource for cataloging known interferences for specific PFAS compounds, the PFAS Interferents List (PIL), and includes information based on LC–MS/MS data and sample matrices where these interferents have been observed. The interferents list has been created as a consultation resource when suspected interferences occur, and as a living document for continual collation of observed interferents as they are discovered. The PIL can be accessed via the National Institute of Standards and Technology via the static DOI: 10.18434/mds2-3040 or at https://data.nist.gov/od/id/mds2-3040.

## Origins and documenting known analytical interferents for PFAS

The literature record on PFAS interference began in 2007 when Benskin et al. reported that isomers of taurodeoxycholic acid (TDCA) in human serum samples could interfere with the 499 m/z → 80 m/z MS/MS transition for perfluorooctane sulfonic acid (PFOS) [9]. Since 2007, the literature has expanded significantly, with the recognition of endogenous steroid sulfates interfering with the 399 m/z → 80 m/z and 399 m/z → 99 m/z transitions for perfluorohexane sulfonic acid (PFHxS) [10], and reports of lipid interferents for measurements of perfluorinated carboxylic acids in placenta [8], chocolate [11], oysters [12], and condensate water [12], among others (DOI 10.18434/mds2-3040).

The PIL currently contains the reference literature for these interferents reported in various sample matrices, but while the examples given are varied, they should not be interpreted as an exhaustive listing of possible matrix interferences. For example, a lipid interferent for perfluorobutanoic acid (PFBA) observed in the 2021 study of Bangma et al. was hypothesized to be a tissue-specific interferent and unlikely to be observed in environmental matrices (e.g., surface water). However, shortly thereafter, a PFAS study on indoor air observed several isomers of that interferent in condensate collected from an air conditioning unit [12].

Likewise, cholic acids, such as TDCA mentioned previously, are commonly observed in matrices associated with the bile duct, including serum and egg/egg-yolk, and these species were also found in other egg-containing products (e.g., ranch dressing, ravioli, baby food, soup) analyzed as part of a total diet study [11]. These compounds have also been observed in commonly consumed seafood products (e.g., cod, tuna) [13]. To avoid credulous interpretation of PFAS results, it is important to assume analysis of complex matrices will be impacted by unidentified or understudied interferents; therefore, strategies must be developed to differentiate incurred PFAS from matrix interferences.

## Recognizing possible analytical interference

Identification of an analytical interferent requires recognition that results are suspicious, outliers, or otherwise abnormal. Typically, interferents present themselves in different ways, but suspicion is most likely to arise during the data review stage, rather than during data collection. Observing an interferent is easiest in the raw data; however, given the prevalence of automated workflows and data processing software, interference may go unnoticed until multiple processing steps have passed (e.g., peak integration, quantification, data interpretation) or even multiple personnel have interacted with the results. While recognizing "suspicious" data is subjective and heavily dependent on analyst experience, there are numerous situations where PFAS measurements may require follow-up investigation. These include, specific concentrations being

unexpectedly and/or uniquely elevated in a sample set or individual PFAS being present in unexpected samples, and/or uncorrelated to similar species. The raw data of seemingly unusual or suspect samples can be interrogated, where close examination can reveal indicators such as elevated background signal or variations in QC metrics like retention times and/or qualitative transitions. A detailed list of potential red flags that could indicate an analytical interferent with literature examples is provided in Table 1. In addition, Fig. 1 shows visual representations of several of the red flags.

All of the examples provided share one aspect, the lack of correlation of a single PFAS measurement with other members of the class and/or with the analyst expectations for the system under study. The likelihood of analytical interference must be weighed against the likelihood of specific, novel behavior for a single PFAS when such PFAS are normally correlated. When the possibility of interference is indicated, there are several considerations for resolving the interferent from the analysis.

## Approaches for investigating and removing analytical interference

Despite the specificity of MS/MS transitions, there is always some degree of risk of analytical interference that contaminates the transition. During method validation and implementation, there are several key methods for resolving this interference.

(a) Critical analysis of QA/QC validation

Careful monitoring of quantitative transitions in MRM can reveal deviations consistent with interference and allow for flagging of the affected data. For example, maintaining strict performance metrics for baseline abundance and/or retention time deviations can flag samples, or entire matrices, for issues, even if peaks are still integrated by processing software. A recent recommendation by the European Union specified a relative retention time deviation between analyte and matched internal standard should not exceed 1% for PFAS analysis in food and feed [14]. Unusual distributions of apparent isomers or the inclusion of misshapen peaks (i.e., shouldering) can be the result of close eluting interferents and can be compared to spike-in controls to determine whether it is the result of chromatographic deviations, or additional species. These types of QA/QC checks can extend to modification of the analytical methods in response to frequent interferents.

(b) Alternate transitions can be used for confirmation of PFAS or known interferents

For some compounds, the examination of qualitative secondary transitions can confirm the presence of the analyte of interest and/or known interferents. Secondary transitions can also, if necessary, be used as quantitative transitions, trading sensitivity for higher specificity. The classic example of TDCA interference in the 499 m/z → 80 m/z transition can be resolved because a prevalent 499 m/z → 99 m/z transition is specific to PFOS over the interferent [9]. Unfortunately, secondary qualitative transitions for the target analyte are not always available, for example PFBA and PFPeA lack secondary transitions, in which case, the secondary transition must be specific to the suspected interferent(s). For example, 499 m/z → 124 m/z has been used to confirm the presence of cholic acid, while 263 m/z → 175 m/z has been used to confirm certain lipid interferents for PFPeA [12] and 263 m/z → 114 m/z has been used to confirm an interferent commonly found in chocolate [11, 12]. Authors recommend PFAS results should be labeled uncertain if only one transition is available for confirmation and note that confirmatory transitions or supporting analytical techniques are required for unequivocal identification of compounds in certain regulatory schemes [15].

(c) Adjusting method parameters to reduce or remove interferents

Eliminating interference can also be accomplished by adjusting the method gradient, altering the column stationary phase (e.g., using a fluoro-column), or using a longer column to see if secondary peaks can be resolved. In the total diet study mentioned above, PFBA and PFPeA detections were confirmed to contain interferents after the use of a longer analytical column revealed unacceptably large relative retention time shifts. Researchers may also consider modifications to their sample preparation to remove specific interferents. For example, simple "dilute-and-shoot" extraction methods may be more susceptible to interference compared to extensive cleanup methods using weak-anion-exchange and/or carbon cleanup. Adding graphitized carbon clean-up to remove TDCAs is one example where this approach has worked well [16]. Note that method modifications should still be validated to be consistent with accreditation for specific numbered methods or internal methods. When an interferent is characterized, it may be possible to purchase a chemical standard (and/or isotopically labeled analog) to validate that a preparation or analysis method is suitable for resolving the interferent from the analyte of interest. Validation of the exact identity and structure of interferent will likely require secondary analytical techniques.

(d) Leveraging other analytical techniques to confirm PFAS/interferents

**Table 1** Common red flags, description, and examples for the identification of possible analytical interference

| Red flag | Description | Example(s) | Figure 1 visual representation | Approaches (see Sect. "Approaches for investigating and removing analytical interference") |
|---|---|---|---|---|
| Detected at a high frequency | When observing a dataset, one PFAS is observed at a higher frequency than expected throughout all samples. May be observed in several of the related matrices in a multi-matrix study | As in Bangma et al., 2021, PFBA was observed in 75% of collected placenta tissue [7]. Of note, this red flag may appear to be a background contaminant in a matrix blank (if included in analysis), but is not present in solvent blanks, quality control, or other quality assurance samples | A | 2, 3, 4 |
| Specific PFAS detected in an unexpected matrix | An observation for an unlikely, individual PFAS is seen in a known matrix type. Of note, this observation is based on knowledge about the sample source, suspected contamination source, or historical knowledge of the matrix type | As documented in Bangma et al., 2022, PFPeA was observed at a high concentration in oysters as a single analyte, not as part of a homologous series [12] | B | 2, 3, 4 |
| Concentration is way too high | A concentration will be measured significantly higher for a single analyte (e.g., an order of magnitude or greater) relative to any other PFAS included in an analyte list or the concentration is outside of the expected range for a single analyte in the matrix type. Of note, this type of observation is based on experience or knowledge of the general concentration range of a given matrix | A better known interferent, TDCA was observed in eggs [8], and was quantified as a combined concentration of TDCA plus PFOS. Left unidentified, TDCA would have inflated PFOS quantitation by an order of magnitude over true concentration | C | 1, 2, 3, 4 |
| Retention time is shifted | Observed in a dataset or a single sample, the retention time for an individual PFAS is shifted outside of the expected threshold of acceptable drift. This red flag is more apparent in the presence of a matched internal standard which has the expected retention time | This red flag seems to be the most common, but often overlooked since individual retention times are not commonly reported per analyte per sample. Examples include, but are not limited to, PFPeA interferents in oyster tissues [12] and a PFPeA interferent in food items containing chocolate [11] | D | 1, 2, 3, 4 |
| Loss of low-end calibration point(s) | Loss of a low-end calibration point or points and little or no detection for an individual PFAS especially when the analyte is expected. This is typically indicative of an elevated baseline | Anecdotally, a loss of PFPeA was observed in low end calibrants for the 263- > 219 transition (DOI:10.18434/mds2-3040). This was compounded by the fact that PFPeA only has one reliable transition as noted in Red Flag 5 | E | 2, 4 |

**Table 1** (continued)

| Red flag | Description | Example(s) | Figure 1 visual representation | Approaches (see Sect. "Approaches for investigating and removing analytical interference") |
|---|---|---|---|---|
| Can't use confirmation transitions | An observed positive detection for smaller compounds with only one, non-diagnostic (e.g., loss of $CO_2$), transition (e.g., PFBA and PFPeA), especially without any long chain homologues | Any of the PFBA and PFPeA examples mentioned in this table and/or the text. Note: this may be indicated in the data by any of the visual representations in Fig. 1. Confirmation by an alternate method is recommended for any positive detections of PFAS compounds with a single transition | A–E | 2, 3, 4 |

To supplement low-resolution MS/MS analysis such as triple-quadrupole measurements, high-resolution mass spectrometry (HRMS) can enable higher specificity detection of both target precursors and suspected interferents. Exact mass (mass accuracy error < 10 ppm) is normally sufficient to resolve PFAS from interferents; however, high-resolution MS/MS may still be necessary for unequivocal confirmation; for example, an exact mass interference between 6:2 FTS and the novel PFAS HYDRO-EVE has been reported [17]. Charbonnet and colleagues have published a comprehensive resource on communicating confidence during novel PFAS identification using HRMS [18]. Another option is to use ion-mobility mass spectrometry as an additional dimension of separation, which can readily separate multiple classes of chemical species. This has been demonstrated to identify PFAS from complex mixtures with biological molecules and xenobiotic compounds [19]. True confirmation of an interferent may require acquisition of an authentic chemical standard, but any of the techniques discussed may be enough to confirm the presence of an unidentified interferent.

## Expanding the list of observed PFAS interferents

Accurate detection/quantitation of PFAS is important and given the challenges associated with the prevalence of interferents across diverse matrices and classes of PFAS, there is significant value in cataloging these interferences as they are discovered. To address this need, we have created "The PFAS interferents List" (PIL). This list is formatted as a simple excel table and includes the following data columns:

- PFAS:
  - Compound name and identifier (DTXSID)
  - Precursor mass and transition with observed interferent
- Interferent:
  - Chemical name or any identifying information if the compound is unknown
  - Precursor *m/z*
  - Standard availability
  - Diagnostic MS/MS transition (secondary)
  - Citation or reference to work where the interferent has been observed/documented

The PIL resource can be accessed at https://datapub.nist.gov/od/id/mds2-3040 and is available with a static

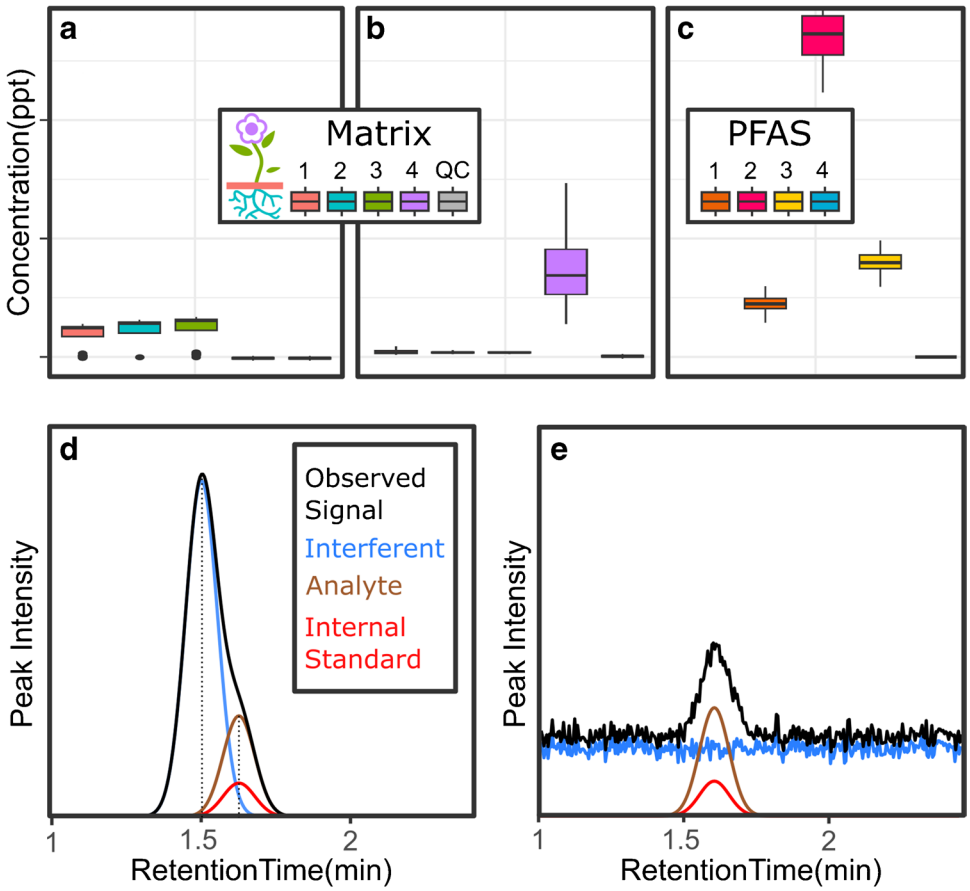


**Fig. 1** "Red flags" indicating potential PFAS interferents in a multi-matrix dataset (**a**, **b**), a sample set in a single matrix (**c**), and in a single-sample (**d**, **e**). **a** Elevated detection of an easily interfered species in an unexpected subset of a multi-matrix sampling (pink, blue, green). **b** Unique detection of a single PFAS in a single matrix (purple). **c** Unusually high reporting of a single PFAS (dark pink). **d** Minor retention time shifting and shouldering of observed PFAS signal caused by a coeluting interferent, but which could be resolved from the internal standard. **e** Elevated, persistent background which reduces the signal-to-noise for the analyte of interest and may result in the loss of quantitative accuracy and limit of detection

DOI:10.18434/mds2-3040. We recognize that it is common for researchers to come across relevant interferences in their work, quickly deal with them, and move on, often without publishing this valuable information. This dataset is intended to be a "living" resource to capture both published and un-published interferents once they have been found. As such, the dataset will be maintained and updated by NIST researchers as additional interferents are identified. We encourage researchers in the PFAS analysis community to submit observed interferents as they find them in different matrices so they can be added to this dataset ad hoc. To submit new interferents, please use the template included in the PIL website (https://datapub.nist.gov/od/id/mds2-3040) and send it to pfas@nist.gov. Ideally, interferents will be associated with DOIs for a citable resource to direct other researchers, but anecdotal "author communications" are also accepted. With more contributions, this resource will become more powerful for the community. Currently, support for verification of new interferents added to the PIL are only being accepted for PFAS, please visit https://data.nist.gov/od/id/mds2-3040 for any updates.

## Conclusions

Overall, analytical interferences with PFAS measurements may introduce uncertainty and reduce confidence in results. Informed decision making relies on accurate and precise data. If that data lacks confidence, then potential actions can be delayed and require further investigations—which may increase overall costs of site investigations, risk assessments, and regulatory decisions and thus postpone needed remedial actions. It is our hope that the solutions provided in this commentary for identifying PFAS interferents can improve the quality of data available to the community, and that it acts as a centralized repository for previously identified problems to accelerate acquisition of new PFAS measurements.

**Funding** C. Huset was supported by NIEHS Grant number 5U2CES026533-05.

## Declarations

**Conflict of interest** The authors declare no competing interests.

**Disclaimers** This manuscript has been reviewed in accordance with the policy of the Center for Environmental Measurement and Modeling, US Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the view and policies of the Agency.

Certain commercial equipment or instruments are identified in the paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by NIST, US EPA, or the US FDA, nor does it imply that the equipment or instruments are the best available for the purpose.

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**Jacqueline Bangma** PhD (she/her/hers) is a chemist at the US Environmental Protection Agency. Her research focuses on non-target analysis (NTA) applications for identifying novel PFAS in surface water and industrial mixtures. She is interested in utilizing environmentally sourced mixtures of novel PFAS to understand mammalian bioaccumulation and mixture toxicity.

**Kitrina Barry** M.S. (she/her/hers) is a research scientist with the Minnesota Department of Health in the Chemical Threats and Biomonitoring Unit. She serves as a clinical quality specialist and specializes in method development using liquid chromatography with tandem mass spectrometry. Her research interests include bioanalytical chemistry focusing on emerging contaminants in environmental and human biological matrices.

**Christine Fisher** PhD (she/her/hers) is a chemist at the US Food and Drug Administration (FDA) in the Center for Food Safety and Applied Nutrition (CFSAN). Since 2017, her research interests have focused on developing tools, methods, and quality controls for non-targeted analysis and suspect screening using liquid chromatography/high resolution-mass spectrometry for food safety applications.

**Susan Genualdi** PhD (she/her/hers) is a research chemist at the US Food and Drug Administration (FDA) in the Center for Food Safety and Applied Nutrition (CFSAN). Since 2011, her research has focused on developing methods for direct and indirect food additives using gas chromatography/mass spectrometry, liquid chromatography/mass spectrometry, and ion chromatography.

**Theresa Guillette** PhD (she/her/hers) is a technical subject matter expert with Arcadis' North American PFAS group. Her primary research areas are analytical method development for per- and polyfluoroalkyl substances (PFAS), forensics analysis with high resolution mass spectrometry, and ecological toxicity in crocodilian species. She received her doctoral degree from the Medical University of South Carolina with a dissertation focused on developing lipidomic methods to assess oxidative stress in Nile crocodiles. She has extensive expertise in assessment and monitoring of PFAS in solid, aqueous, and biological samples, with experience discovering new chemical byproducts in effluent and animal models. She is interested in researching new high-resolution mass spectrometry methods to detect and visualize PFAS on solid surfaces and soil cores.

**Carin Huset** PhD (she/her/hers) is a research scientist in the Chemical Threats and Biomonitoring Unit at the Minnesota Department of Health and is a co-chair of the National Biomonitoring Network Steering Committee. She works on the development and implementation of new analytical methods, and also coordinates with partners to plan, design, and implement biomonitoring projects.

**James McCord** PhD (he/him/his) received his PhD in analytical chemistry from North Carolina State University and is currently a research chemist at the US EPA in the Center for Environmental Measurements and Modeling. He collaborates with state and regional laboratories across the country to investigate novel and emerging chemical pollutants and human exposures. Currently, his primary area of focus is methods development and chemical characterization of per- and polyfluorinated alkyl substances (PFAS) in multimedia, with a focus on water matrices. He also explores applications of high-resolution mass spectrometry and non-targeted analysis for environmental exposure screening and metabolomics.

**Brian Ng** PhD (he/him/his) is a staff fellow chemist at the US Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN). He received his PhD in chemistry from Florida International University where he worked on the development of non-targeted analysis (NTA) methods using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). He is currently working on non-targeted analysis of per- and polyfluoroalkyl substances (PFAS) and the determination of interferences that can hinder their accurate quantitation.

**Benjamin Place** PhD (he/him/his) is a research chemist at the National Institute of Standards and Technology, working in the Chemical Sciences Division to develop methods, reference materials, and data analysis tools for food and environmental contaminants. His current work focuses on the development of methods, materials, and data tools for identifying per- and polyfluoroalkyl substances (PFAS) in a wide range of matrices using nontargeted analytical techniques. In addition, he is the technical program lead for multiple current and upcoming reference materials for the targeted, quantitative analysis of PFAS in environmental, commercial, and food matrices.

**Jessica Reiner** PhD (she/her/hers) is a research chemist at the National Institute of Standards and Technology and a member of the Biochemical and Exposure Science Group located at the Hollings Marine Laboratory. She has expertise in improving analytical measurements for chemicals of emerging concern, mainly per- and polyfluoroalkyl substances, through the certification of reference materials, instrument development, methods development, and quality assurance activities.

**Anna Robuck** PhD (she/her/hers) is an environmental chemist and principal investigator with the US Environmental Protection Agency in the Office of Research and Development. Her research characterizes legacy and novel contaminants in environmental matrices, with particular focus on discovery of novel PFAS in tissues, sediments, and water using high-resolution mass spectrometry.

**Alix Rodowa** PhD (she/her/hers) is a research chemist for the National Institute of Standards and Technology. Her research interests include emerging environmental contaminants of concern. She works primarily on the quantitative analysis of per- and polyfluoroalkyl substances (PFAS) in environmental and food matrices including groundwater, drinking water, aqueous film-forming foams, textiles, and food. Her research goals include improved analytical instrumentation methods and quantitation, contributions to reference materials, and improving data quality.