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Article

Interlaboratory Comparison of Extractable Organofluorine Measurements in Groundwater and Eel (*Anguilla rostrata*): Recommendations for Methods Standardization

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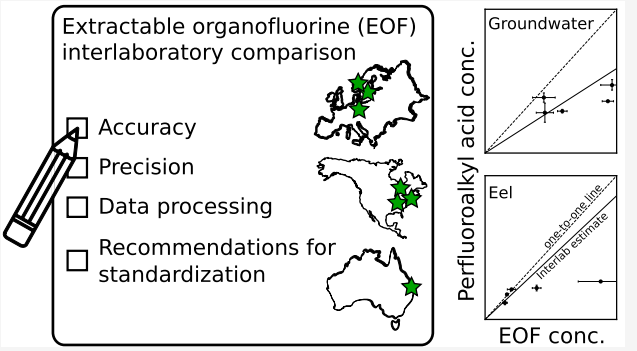
ABSTRACT: Research on per- and polyfluoroalkyl substances (PFAS) frequently incorporates organofluorine measurements, particularly because they could support a class-based approach to regulation. However, standardized methods for organofluorine analysis in a broad suite of matrices are currently unavailable, including a method for extractable organofluorine (EOF) measured using combustion ion chromatography (CIC). Here, we report the results of an international interlaboratory comparison. Seven laboratories representing academia, government, and the private sector measured paired EOF and PFAS concentrations in groundwater and eel (*Anguilla rostrata*) from a site contaminated by aqueous film-forming foam. Among all laboratories, targeted PFAS could not explain all EOF in groundwater but accounted for most EOF in eel. EOF results from all laboratories for at least one replicate extract fell within one standard deviation of the interlaboratory mean for groundwater and five out of seven laboratories for eel. PFAS spike mixture recoveries for EOF measurements in groundwater and eel were close to the criterion (±30%) for standardized targeted PFAS methods. Instrumental operation of the CIC such as replicate sample injections was a major source of measurement uncertainty. Blank contamination and incomplete inorganic fluorine removal may introduce additional uncertainties. To elucidate the presence of unknown organofluorine using paired EOF and PFAS measurements, we recommend that analysts carefully consider confounding methodological uncertainties such as differences in precision between measurements, data processing steps such as blank subtraction and replicate analyses, and the relative recoveries of PFAS and other fluorine compounds.



KEYWORDS: *Extractable organofluorine, combustion ion chromatography, per- and polyfluoroalkyl substances, analytical methods, interlaboratory comparison, aquatic contamination*

INTRODUCTION

Natural organofluorine is rare in Earth's biosphere.[1−4] The ubiquitous presence of organofluorine including aliphatic and aromatic nonpolymers and polymers in the modern biosphere thus reflects anthropogenic activity.[4] A subset of organofluorine consists of per- and polyfluoroalkyl substances (PFAS), a class of chemicals used widely in modern commerce with thousands of structures.[5] Less than 100 PFAS have commercially available analytical standards, making it difficult to construct mass budgets of the entire class of PFAS.[6] Further, the chemical-by-chemical approach to regulating PFAS is challenging and time consuming, leading experts to call for a class-based approach to regulation based on extreme persistence and potential harm.[7−10] Organofluorine measurements could support such a class-based approach to management, but a standardized method for measuring and interpreting data in a broad suite of matrices of regulatory concern is currently unavailable.

Extractable organofluorine (EOF) analysis using combustion ion chromatography (CIC) is a common organofluorine measurement technique that provides lower bound estimates of total concentrations of PFAS and other organofluorine

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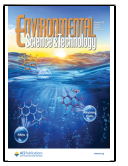

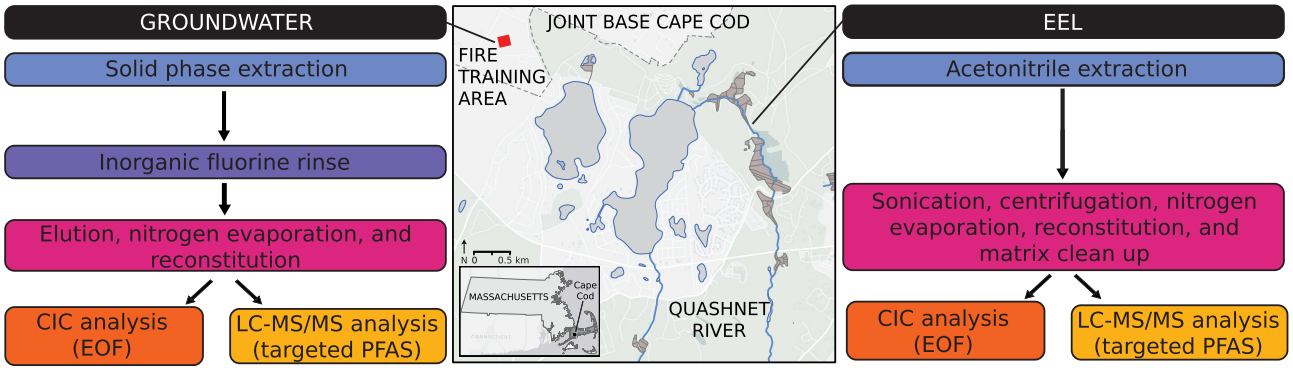

Figure 1. Sample map and overview of extractable organofluorine (EOF) workflow. The map was provided to the authors by Denis LeBlanc (USGS) for the purposes of this work.

compounds (e.g., pharmaceuticals)[11] in a sample. Compared to other organofluorine measurements, EOF has relatively low detection limits and can be applied to most sample matrices of interest (Table S1).[12] The steps for EOF analysis by CIC include extracting fluorine from a sample, separating the organic and inorganic fractions, oxidizing the fluorinated carbons (which releases fluoride) by hydropyrolytic combustion, and measurement using a conductivity sensor.[13]

Standardization of organofluorine analytical methods, reporting criteria, and data interpretation would help to ensure rigor and confidence in results and facilitate comparisons of data generated by multiple laboratories. Previous work has assessed the reproducibility and accuracy of EOF in groundwater and wastewater sludge among three laboratories.[14] However, there is limited information on (1) the sources and magnitudes of uncertainty in EOF measurements across laboratories, (2) effects of data processing steps such as blank subtraction, and (3) quality assurance and control criteria (QA/QC) including organofluorine recovery and inorganic fluorine removal.

Comparing EOF to targeted PFAS measurements using liquid chromatography tandem mass spectrometry (LC-MS/MS) is commonly referred to as the organofluorine mass balance. Since internal standards are not added prior to EOF extraction, comparisons are typically performed using the same extract split for CIC and LC-MS/MS analysis in which targeted PFAS concentrations are not recovery corrected. EOF concentrations tend to be greater than the sum of the fluorine concentrations from targeted PFAS across environmental, biotic, and manufactured media.[15−24] However, the difference between EOF and targeted PFAS concentrations is dependent on the number of targeted analytes quantified using LC-MS/MS methods (typically <50 out of thousands)[25−27] and whether other organofluorine subclasses are present.[11]

Here, we present EOF measurements in water and biotic tissue from an international interlaboratory comparison of seven independent laboratories representing academic, government, and commercial organizations. Specifically, we compared EOF and targeted PFAS measurements in groundwater and eel (*Anguilla rostrata*) from a well-characterized site where organofluorine primarily consists of PFAS derived from aqueous film-forming foam (AFFF).[22] The seven participating laboratories included Bureau Veritas (Canada commercial), Eurofins Environment Testing (Australia commercial), the Federal Institute for Materials Research and Testing (Germany government), Harvard University (USA academic), North Carolina State University (USA academic), Norwegian Institute for Water Research (NIVA: Norway nonprofit), and Örebro University (Sweden academic) (Table S2). We assessed the reproducibility, precision, and accuracy of EOF measurements along with data processing steps. Based on the results of this comparison, we provide recommendations for further standardizing and interpreting EOF measurements.

MATERIALS AND METHODS

Interlaboratory Coordination. The seven laboratories participating in this study were identified as groups that had previously published data measured using a CIC or based on information from instrument manufacturers on their clients in 2021. All laboratories were asked to measure groundwater and eel and associated QA/QC samples on the CIC and LC-MS/MS (if available). Laboratories were sent the methods used at Harvard University but were encouraged to follow their own standard operating procedures because one goal of this study was to assess the reproducibility of EOF results across different laboratories and methods. The Supporting Information (SI) contains example correspondence and materials sent to participating laboratories.

Experimental Design. Groundwater and eel samples were collected in June 2021 downstream from a former military fire training area in an AFFF-contaminated watershed on Cape Cod, Massachusetts, United States (Figure 1). Groundwater (10 L) was collected following established United States Geological Survey (USGS) protocols in collapsible low-density polyethylene containers.[28] A field blank was collected by pumping deionized water (10 L) through sampling equipment prior to groundwater collection. Water was subsampled (800 mL groundwater and 200 mL field blank) gravimetrically into new high-density polyethylene (HDPE) bottles and stored at 4 °C. Eel (*Anguilla rostrata*) samples were collected and euthanized by the Massachusetts Division of Fisheries and Wildlife using electrofishing techniques. Eel was chosen as the biotic sample of interest due to its unique life cycle as a migratory species, high fat content, and the fact that it is abundant in this region and elsewhere in eastern North America. Whole-body eel samples were composited (n = 20) and homogenized using a Black & Decker one-touch chopper and a hand-held OMNI International TH homogenizer. Homogenized tissues were divided into 25 g subsamples in 50 mL polypropylene tubes. Groundwater, the field blank, and homogenized eel were shipped on dry ice to participant laboratories in August 2021 along with inorganic fluoride and

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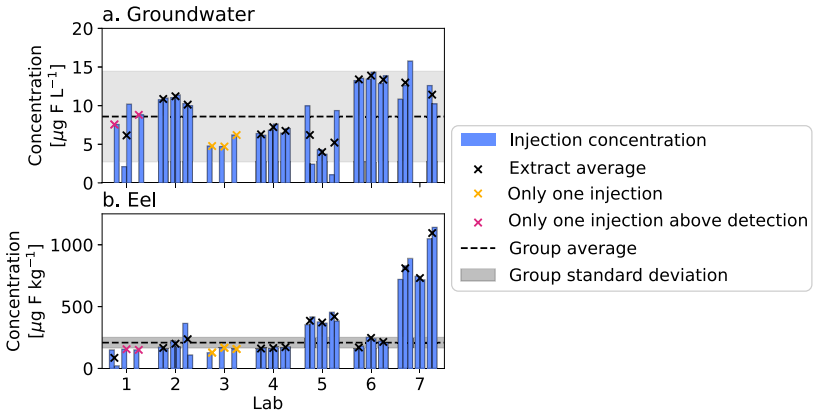

Figure 2. Extractable organofluorine (EOF) concentrations measured in groundwater (a) and eel (b; wet weight) by seven international laboratories. Each bar shows the concentration of each instrumental injection for triplicate extractions (only two groundwater extractions shown for Lab 7 since concentrations for one extract were below the detection limit after extraction blank subtraction). The marker represents the average concentration of duplicate instrumental injections (except for the first set of bars for Lab 7 because triplicate instrumental injections were performed) with different colored markers indicating only one injection performed or only one above the detection limit. The dotted line represents the interlaboratory average, and the shaded region represents the interlaboratory standard deviation (includes all laboratories for groundwater but excludes Lab 7 for eel since results were more than 3× results of other laboratories). Raw data are provided in Tables S4 and S6.

standard PFAS mixture (Wellington PFAC-24PAR, Guelph, Ontario, Canada) spikes (Table S3). Samples were analyzed by laboratories within six months of the initial sample collection.

Groundwater Extraction. All laboratories extracted groundwater using solid phase extraction with weak anion exchange cartridges. Specific protocols for each lab are reported in the SI. Triplicate extractions of 50 mL subsamples were performed on (1) groundwater, (2) groundwater + inorganic fluorine spike as sodium fluoride (Lab 2 = 1000 μg F L^{-1}, all other laboratories = 2000 μg F L^{-1}), (3) groundwater + organofluorine spike as a PFAS mixture (Lab 2 = 15 μg F L^{-1}, all other laboratories = 24 μg F L^{-1}), (4) the field blank, and (5) the extraction blank. Generally, extractions were performed by preconditioning the cartridges by using ammonium hydroxide in methanol, followed by deionized water equilibration. Following sample loading, cartridges were rinsed with an aqueous ammonium hydroxide solution to remove inorganic fluorine and then eluted with ammonium hydroxide in methanol. Samples were evaporated using nitrogen, and the extract was reconstituted in methanol (Figure 1). Extracts were split between the CIC for EOF analysis and LC-MS/MS for PFAS analysis. Internal standards were added to the split extract analyzed on the LC-MS/MS prior to instrumental injection (except Lab 2 which added internal standards prior to extraction, see SI). Reported EOF (Table S4) and PFAS (Table S5) data represent aqueous concentrations.

Eel Extraction. All laboratories used solid−liquid extraction using acetonitrile (ACN) to extract the PFAS in eel tissues. Specific protocols for each lab are reported in the SI. Extractions of 1 g of wet weight subsamples were performed in triplicate and included eel, eel + inorganic fluorine spike as sodium fluoride (Lab 2 = 1500 μg F kg^{-1}, all other laboratories = 1000 μg F kg^{-1}), eel + organofluorine spike as a PFAS mixture (all laboratories = 1200 μg F kg^{-1}), and extraction blank (1 mL deionized water). The extraction protocol was adapted from methods used in previous work.[29−31] Generally, extractions were performed by adding 4 mL of ACN into centrifuge tubes containing the sample, vortexing, sonicating for 30 min in a water bath, centrifuging, and decanting the supernatant to a new centrifuge tube. The process was repeated for a second time, and the combined 8 mL ACN extract was frozen at −20 °C for at least 4 h to allow for lipid precipitation. Extracts were centrifuged, decanted, evaporated by using nitrogen, and reconstituted in methanol. The reconstituted extract was subjected to ENVI-carb cleanup (dispersive + glacial acetic acid or cartridge) and split for CIC for EOF analysis and LC-MS/MS for PFAS analysis. At this point internal standards were added (except for Lab 2 where internal standards were added prior to extraction, see SI). Reported EOF (Table S6) and PFAS (Table S7) data represent the wet weight concentrations.

Instrumental Analysis and Quantification. Sample extracts were analyzed for EOF by CIC by all seven laboratories. Specific protocols including details of CIC instrumentation and operation as well as quantification of EOF concentrations are reported in SI. EOF results are presented in Tables S4 and S6.

For direct comparison to EOF results, five laboratories analyzed 28−45 PFAS in split extracts using LC-MS/MS following established methods.[14,22,25,32] The same five laboratories additionally reported recovery-corrected PFAS results from separate triplicate extractions where internal standards were added prior to extraction to compare with the EOF split extracts (Tables S5 and S7). All laboratories performed analysis in negative mode electrospray ionization, multiple reaction monitoring mode using Agilent 6495, Agilent 6460, or Waters Xevo TQ-S triple quadrupole LC-MS/MS instruments.

Quality Control. Quality control samples for both EOF and targeted PFAS analyses included in-house extraction blanks and field blanks consisting of deionized water. We also evaluated removal of inorganic fluorine using a sodium fluoride spike in samples (eq S1) and organofluorine recovery using a mixture of PFAS spikes in samples (eq S2). Standard analytical methods for targeted PFAS usually add internal standards prior to extraction.[25,26] In contrast, EOF methods typically add internal standards after the extracts are split for CIC and LC-MS/MS analyses to prevent bias from internal standard organofluorine concentration on CIC results. The difference in PFAS concentrations between the two procedures is determined by the PFAS extraction efficiency (eq S3). To assess PFAS extraction efficiency, targeted PFAS were

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quantified in separate triplicate extractions of groundwater and eel with internal standards added prior to the extraction and compared to the targeted PFAS concentrations in the split EOF extracts.

Throughout the paper, we compare recovery performance to the ±30% benchmark of standard targeted PFAS methods.[25,26] The ±30% window was selected in order to evaluate the performance of EOF to PFAS data but is not a formal recommendation for acceptable QA/QC criteria.

Data Handling and Statistical Analyses. All results were anonymized by assigning each lab a random number between one and seven, which are used consistently throughout the paper. This paper discusses results above the detection limit after instrument blank subtraction as the average ± standard deviation, unless otherwise noted. Extraction blank and/or field blank subtraction was performed only if two or more replicates were above the detection limit. Uncertainties in the measured data were propagated throughout the analyses using eq S4 and eq S5. The contribution of PFAS to EOF in the organofluorine mass balance was calculated using eq S6. Interlaboratory averages were calculated from the data using Markov-chain Monte Carlo (MCMC) analysis (see SI for further details; eq S7). MCMC analysis was performed in Python 3.10.9 using *emcee* version 3.1.1 to estimate the interlaboratory expected mean and standard deviation.[33] Other statistical analyses were performed in Python using *scipy* version 1.7.3.[34]

RESULTS AND DISCUSSION

Interlaboratory Reproducibility and Precision. Participants were asked to perform triplicate extractions and duplicate injections of each extract on the CIC to help distinguish between uncertainties arising from either extraction or instrumentation procedures. Duplicate injections were chosen instead of triplicate injections to limit runtime of the worklist (each sample injection ranged from 20 to 50 min depending on the laboratory). Figure 2 shows results for the concentrations of the EOF measured in (a) groundwater and (b) eel (wet weight). Bars represent the concentration of each injection, and markers represent the average extract concentrations. The average groundwater concentration across the seven laboratories was 8.6 ± 5.8 μg F L^{-1} (minimum = 4.00 μg F L^{-1}; maximum = 13.9 μg F L^{-1}). The average concentration for at least one extract from every lab fell within one standard deviation of the interlaboratory average. In one extract from lab 7, the concentration after blank subtraction was not measured above the detection limit in either injection replicate.

The interlaboratory average concentration for eel was 266 ± 70 μg F kg^{-1} (minimum = 119 μg F kg^{-1}; maximum = 869 μg F kg^{-1}). Only two laboratories reported concentrations that agreed within one standard deviation of the interlaboratory average concentration. Lab 7 reported concentrations more than 3× above the interlaboratory average and 2× higher than those in any other individual lab. We therefore recalculated the interlaboratory average, excluding Lab 7 data. The new interlaboratory average concentration was 208 ± 42 μg kg^{-1} (a 22% decline). The concentration for at least one extract from four of seven laboratories agreed within one standard deviation of the lower interlaboratory average.

Interlaboratory precision was determined by the coefficient of variance (COV: standard deviation/mean) of the interlaboratory average concentrations. The interlaboratory COVs were 67% for groundwater and 20% for eel (increasing to 26% when results from Lab 7 were included). The groundwater COV is higher than the ±30% precision benchmark used by most targeted PFAS methods.[25,26] Lower precision of EOF measurements in groundwater across laboratories mainly resulted from measurement variability within individual laboratories across triplicate extractions and duplicate injections (evidenced by the large differences in duplicate/triplicate injection of the same extract in Laboratories 1, 5, and 7 in Figure 2a).

Intralaboratory Precision. Sources of uncertainty can arise due to extraction (assessed by triplicate extractions) and instrumentation (assessed by duplicate injections of each extract). The COVs across the triplicate extractions within each lab (2%−35%) were less than the interlaboratory COVs across all laboratories for both groundwater and eel (Table S8). Duplicate injections showed a large variability among some laboratories (Figure 2). For groundwater, three of six laboratories reported percent differences ≤11% while the others reported differences >100% for at least one extract (duplicate percent differences were not calculated for some extracts from Laboratories 1, 3, and 7 as they either only injected each extract once or only had one determination above the detection limit). For eel, four of six laboratories reported percent differences ≤17% while the others reported differences >100% for at least one extract (Table S8). For two extracts from Lab 1, only one of the replicate determinations was above the detection limit, and Lab 3 only injected each extract once (Figure 2). These results emphasize that variable instrumentation procedures during analytical runs for EOF are a major source of uncertainty in measurements reported by some laboratories.

Accuracy in Organofluorine Spike Recovery. No standard reference materials were available for assessing the accuracy of EOF measurements; therefore, we used a mixture of 24 PFAS in Wellington's PFAC-24PAR product (Table S3). We selected this mixture because the samples in this study were primarily contaminated by AFFF, and the technical mixture contains many of the common PFAS found at AFFF-contaminated sites. Participant laboratories were asked to determine both the mixture concentration by direct injection (nominally = 12,059 μg F L^{-1}) as well as the recovery in triplicate-fortified matrix spikes of groundwater and eel. Laboratories 1 and 6 did not report the mixture concentration and were therefore excluded from subsequent analysis (recoveries calculated from the nominal concentration are shown for all laboratories in Figure 3 as comparison). Reported mixture concentrations were within ±20% of the nominal concentration for all laboratories that reported data (Figure S1). The diluted spike concentrations in the sample matrix were greater than the interlaboratory average EOF concentration by a factor of ∼2.5× in groundwater and ∼6× in eel (Figure 2). Therefore, the reported data are likely to represent PFAS recoveries at the concentration ranges measured in the samples.

Figure 3 shows the percent recovery of the PFAS mixture spike in groundwater (a) and eel (b). Organofluorine recovery was evaluated based on ±30% threshold that standard PFAS methods have established for acceptable analyte recoveries in water.[25,26] In groundwater, all laboratories reported average recoveries within ±30% of the measured spike concentration. In eel, three of five laboratories reported average recoveries with ±30% of the measured spike, and two laboratories reported average recoveries within ±40% of the measured

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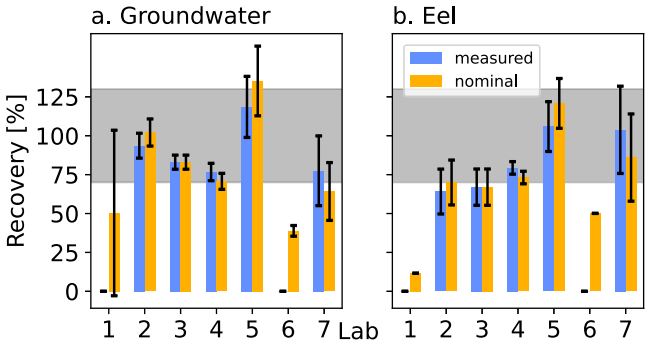

Figure 3. Percent recovery of Wellington's PFAC-24PAR PFAS mixture in (a) groundwater and (b) eel. Bars represent the average percent recovery, and error bars represent the standard deviation of triplicates (n = 3). Blue bars on the left represent the recovery calculated using the concentration of the spike measured on the CIC. Orange bars on the right represent the recovery calculated using the nominal concentration of the spike. The shaded region represents a ±30% recovery window that is generally considered acceptable for targeted PFAS analysis. Raw data are provided in Table S9.

spike. Lower recovery of PFAS in eel could be due to matrix effects which are commonly observed in biological tissues due to presence of large amounts of endogenous compounds.[35,36] For samples with <70% organofluorine recovery, the reported EOF concentration may be an underestimate of the actual amount of organofluorine present in the sample.

Effects of Data Processing Steps. Of the seven participant laboratories, six laboratories established a calibration curve using the entire CIC instrument (combustion unit and IC) while one established a calibration curve using only the IC. Four laboratories quantified EOF using a PFAS calibrant (three using perfluorooctanoate or PFOA, one using a PFAS mixture) and the other three using fluoride calibrant. Prior work showed the response of fluoride is 30% lower than PFAS on the CIC.[37] However, we found no significant difference in groundwater concentrations between calibrants (one-sided t test; p-value = 0.12; t-statistic = −1.2). Laboratories that performed quantification with a fluoride calibration curve reported significantly lower concentrations in eel compared to those that used PFAS (one-sided t test; p-value = 0.006; t-statistic = −2.7), but this result may be biased by the high concentrations reported by Lab 7 (Figure 2b). Therefore, we do not have enough data to assess whether the different calibration protocols had a significant effect on the EOF quantification.

Most CIC instruments have a detectable background fluorine peak even after replacement of most fluoropolymer components of the instrument and years of operation (some fluoropolymer components have no available replacement alternatives). This means that blank subtraction is an important step in EOF quantification. In this study, only one lab (Lab 2) had no detectable fluorine peak in their instrumental blanks (combustion of an empty boat). Typically, the sample peak area is corrected by subtracting the instrumental blank run immediately before the first sample injection and after the second sample injection or from the average of all instrumental blanks run intermittently throughout the worklist. Subtraction of instrumental blanks run before and after duplicate sample injections accounts for background contamination from the instrument and potential carryover due to incomplete combustion. Subtraction of the average across instrumental blanks only accounts for background instrumental contamination. Higher levels of fluorine indicated by greater peak area were commonly observed in the first instrumental blank run immediately after a sample injection, particularly in injections with concentrations >500 μg F L^{-1}. This is likely an indication of carryover due to incomplete combustion. We tested the impact of the instrumental blank subtraction by comparing EOF concentrations with and without the correction. Uncorrected concentrations were higher than corrected concentrations by up to 280% in groundwater and 80% in eel. The greater impact of the instrumental blank correction on groundwater concentrations compared to eel was likely due to smaller peak areas (lower concentrations) in the groundwater samples. While the greatest source of intralaboratory variability appears to arise from instrumental operation, variable instrumental blank correction procedures may explain the greater observed interlaboratory variability for groundwater compared to eel.

Additional fluorine contamination could be introduced during the extraction process from solvents and consumables and cross-contamination during sample preparation. In groundwater, two laboratories had EOF levels above detection limits in two or more extraction/field blanks. For laboratories with extraction/field blank contamination in groundwater, uncorrected concentrations were greater than the blank subtracted concentrations by 10% to 180% (Table S4). In eel, four laboratories observed EOF above detection limits in the extraction blanks (no field blanks were collected for eel). For laboratories with extraction blank contamination in eel, uncorrected concentrations were greater than the blank subtracted concentrations by 0.3% to 100% (Table S6). Therefore, subtracting any extraction/field blank contamination would help avoid overestimating the EOF concentrations in samples.

Participant laboratories calculated detection limits by following their own procedures. Three separate methods for determining detection limits were used including (1) the instrument limit of detection (iLOD), (2) the method reporting limit (MRL), and (3) the method detection limit (MDL). iLODs were calculated as the standard deviation of instrumental blanks and do not consider potential fluorine contamination introduced during sample preparation. Reported iLODs were 1 μg F L^{-1} for groundwater and 1 μg F kg^{-1} for eel (Table S4 and Table S6). MRLs were calculated as the lowest calibration point divided by the extraction factor and ranged between 0.26 and 1 μg F L^{-1} for groundwater and 13 and 50 μg F kg^{-1} for eel. MDLs were calculated as the average plus 3× the standard deviation of the extraction blanks and ranged between 0.68 and 2.18 μg F L^{-1} for groundwater and 19 and 84 μg F kg^{-1} for eel. The variation in detection limits calculated using different methods highlights the need to assess the most suitable calculation and for analysts to accurately identify the chosen limit applied to their reported data.

Effects of Fluoride Removal. Inorganic fluorine concentrations exceed organofluorine concentrations in most environmental matrices by several orders of magnitude.[4] Since organic and inorganic forms of fluorine are indistinguishable by CIC, the extraction procedure must effectively remove most inorganic fluorine to avoid overestimating EOF concentrations (Figure S2). We tested the removal of 2000 μg F L^{-1} inorganic fluorine introduced as a fluoride spike in groundwater (chosen to emulate concentrations relevant to drinking water; ~200×

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EOF concentrations) and 1000 μg F kg^{-1} inorganic fluorine as a fluoride spike in eel (~5× EOF concentrations). In groundwater, removal of inorganic fluorine was performed with 10 mL of 0.01%−0.03% v/v NH_4OH in deionized water rinse after the sample was loaded onto an SPE cartridge. Fluoride is insoluble in acetonitrile and is likely preserved in the tissue during EOF extraction. Overestimation of EOF can occur if any residual inorganic fluorine after removal exceeds the measured EOF concentrations by 30% (the standard accuracy/precision threshold for PFAS methods).[25,26] We estimated that separation methods had to remove ≥99.85% of the inorganic fluorine spike in groundwater and ≥94% of the inorganic fluorine spike in eel to achieve residual inorganic fluorine concentrations of less than 30% of the interlaboratory average EOF concentration (Figure S2).

Figure 4 shows the percent removal of the inorganic fluorine spike in (a) groundwater and (b) eel. In groundwater, six of seven laboratories achieved an average inorganic fluorine removal of ≥99.85%. The residual fluoride remaining in one lab (Lab 5) after extraction resulted in measured concentrations in the spiked samples that were up to 250% greater than those of the unspiked samples. In eel, four of seven laboratories achieved an average inorganic fluorine removal >94%. For Labs 2, 5, and 7, the residual inorganic fluorine increased measured concentrations by up to 80% above the unspiked samples. Despite the lower removal efficiency of inorganic fluorine in eel compared to groundwater, the residual inorganic fluorine affected reported EOF concentrations in groundwater more than eel because the inorganic fluorine spike was ~200× greater than the EOF concentration in groundwater but only ~5× greater than the EOF concentration in eel (Figure 4).

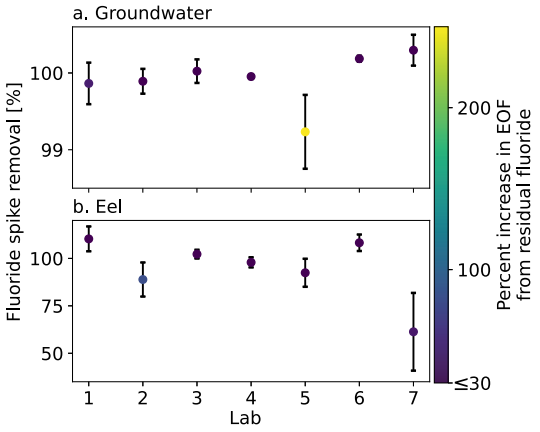

Figure 4. Inorganic fluoride spike removal in groundwater and eel. Points represent the average percent removal of the fluoride spike, and error bars represent the standard deviation (n = 3). The points are shaded based on the average increase in fluorine concentration expected compared to the unspiked sample due to the residual fluoride leftover. Raw data are listed in Table S10.

The impact of incomplete inorganic fluorine removal by some laboratories was demonstrated using high concentration sodium fluoride spikes. The spike was over 20 times the amount of inorganic fluorine concentration present naturally in groundwater based on measurements (<100 μg F L^{-1}). Based on this natural fluoride concentration, a >97% removal would be necessary to ensure residual fluoride did not impact EOF concentrations (which was achieved by all laboratories: Figure 4a). Therefore, the incomplete removal of naturally abundant fluoride likely had a negligible effect on the concentrations of EOF reported in groundwater (Figure 2a). For eel, fluoride was not measured in the original samples since only aqueous samples can be measured using ion chromatography. However, limited data on fluoride levels in eel suggest that it is less than 1350 μg F kg^{-1} (similar to our spike concentration), indicating that any levels of fluoride still present after extraction in eel likely also had a negligible effect on the concentrations of EOF reported.[38]

EOF Mass Balance. Concentrations of EOF and the sum of perfluoroalkyl acids (PFAA) measured in groundwater and eel are compared in Figure 5 for the five participant laboratories that reported both PFAS and EOF concentrations. Quantified polyfluoroalkyl precursors and ether-based PFAS made up no more than an additional 3% of EOF in either matrix (Tables S11 and S12). In groundwater, one lab reported PFAA and EOF concentrations that agreed with each other within one standard deviation, while the other four laboratories reported lower PFAA concentrations compared to EOF (Figure 5a). Linear regression including all data indicates the interlaboratory average estimate for the PFAA fraction of EOF was 58% in groundwater (standard deviation range: 45%−

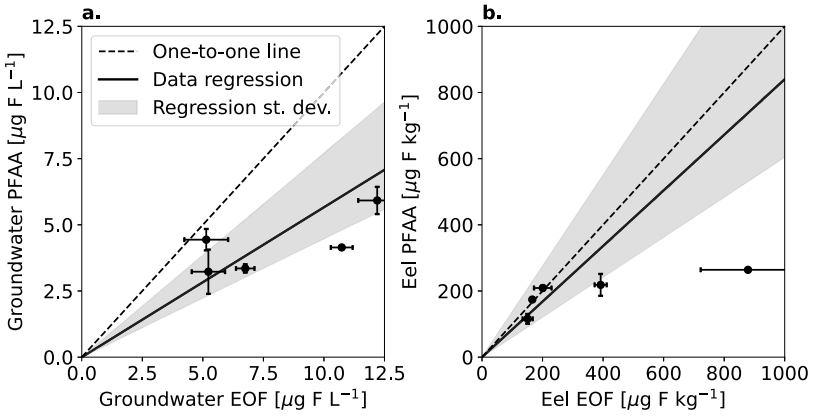

Figure 5. Comparison of the sum of PFAA and EOF in groundwater (a) and eel (b). Averages (circles) and standard deviations (error bars) from triplicate extractions (n = 3) are shown for each lab. The dotted black line represents the one-to-one line. The black data regression line represents the one-to-one line multiplied by the interlaboratory average estimate of the quantified EOF fraction. The standard deviation of the interlaboratory average estimate is shown in the shaded gray region. Raw data are provided in Tables S11 and S12.

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Table 1. Recommendations for Best Practices for Extractable Organofluorine (EOF) Analyses

	Recommendations	Future needs
Extraction and analysis	• If feasible, determine levels of targeted PFAS in sample prior to EOF extraction	• Standardized methods for measuring inorganic fluorine in nonaqueous matrices
	• Determine if sample contains high levels of inorganic fluorine that could potentially interfere with EOF results	• Standardized protocols outlining extraction and instrumentation procedures
	• Conduct replicate extractions on some samples within an extraction batch	
	• Analyze replicate injections of same extract	
	• Analyze duplicate instrument blanks between replicate sample injections	
QA/QC	• Assess organofluorine recovery with PFAS mixture representative of sample composition	• Standard reference materials for EOF
	• Assess inorganic fluorine removal efficiency with an inorganic fluorine spike at/above native levels in sample	• Approaches for quantifying the extraction efficiency of unknown EOF fraction
	• Measure extraction blanks to assess contamination and detection limits	• Standardized QA/QC criteria
	• Compare PFAS with internal standard added before and after extraction to quantify extraction efficiency	
Quantification and data processing	• Subtract instrument blanks from sample injections if background fluorine present	• Identification of appropriate calibrant and calibration protocol Identification and quantification of unknown EOF fraction
	• Subtract extraction/field blanks from samples if EOF detected in blanks	• Standardized quantification procedures including blank subtraction
	• Use appropriate detection limit metrics (iLOD vs MRL vs MDL)	

77%). The EOF fraction accounted for by PFAA in groundwater agrees with the historical average measured from the same groundwater sampling port (USGS well MA-SDW 425-0063) between 2002 and 2021 reported in previous work.[39] That work demonstrated PFAA accounted for 54 ± 16% of EOF and that remaining unquantified fraction were PFAA precursors measured by the total oxidizable precursor assay.

In eel, three laboratories reported PFAA and EOF concentrations that agree with each other within one standard deviation while two laboratories reported lower concentrations of PFAA compared to EOF. The interlaboratory average estimate for the PFAA fraction of EOF was 83% in eel (standard deviation range: 61%−140%). The interlaboratory uncertainty bounds for eel are indistinguishable from 100% (the dotted black line in Figure 5). Based on these results, PFAA likely accounts for the majority/all EOF in eel, and the presence of any potential unknown EOF cannot be confirmed. In summary, the mass balance approach yielded robust evidence across participant laboratories for non-PFAA organofluorine in groundwater but not in eel. In all cases, supplementary tools such as the total oxidizable precursor assay or nontargeted analysis are required to confirm or rule-out the presence of additional organofluorine.[12]

A given extraction method may be selective toward certain PFAS and other organofluorine compounds. Preferential losses of quantified PFAS relative to other organofluorine compounds during extraction can impact the fraction of quantified EOF when these two measurements. This impact is pronounced in scenarios where quantified PFAS are similar in magnitude to the unknown EOF fraction. We demonstrate this with example scenarios shown in Figure S3. For example, when the true fraction of quantified and unknown EOF is the same, small relative differences in extraction efficiencies alter the observed fraction. When the true fraction is primarily dominated by either quantified PFAS or unknown EOF, only very poor extraction efficiencies affect the fraction of EOF accounted for by PFAS. While there is presently no way to assess losses of the unknown fraction, targeted PFAS losses can be measured by comparing concentrations between methods that add internal standards before (standard PFAS analysis) and after (EOF analysis) the extraction step. For participating laboratories, the loss of PFAS due to extraction was generally ≤30% for all but one lab in both groundwater and eel matrices (Table S13). If greater losses are observed, it may be important to reconsider the extraction method used in comparing PFAS and EOF, unless the unknown EOF fraction is very large.

Recommendations and Next Steps. Based on interlaboratory results for the reproducibility, precision, and accuracy of EOF data, sample analysis/data processing steps, and organofluorine mass budget, we developed a table of best practices for sample extraction, instrumental analysis, QA/QC, and data processing (Table 1). Adherence to these recommendations would bolster confidence in future EOF measurements and can be used as a basis for evaluating the robustness of previously published EOF measurements in the literature. For example, careful consideration of how detection limits were calculated and whether the effects from blank contamination were accounted for in those calculations should be assessed before comparing results across data sets. To assist in EOF data assessment, we have provided a checklist that reviewers and evaluators can use in the SI (see the section EOF Data Evaluation Checklist).

Recommendations.

- Prior to EOF extraction, determine targeted PFAS concentrations and levels of inorganic fluorine expected in samples if feasible based on lab capability and sample amount available. This is useful for determining how much sample needs to be extracted to achieve detectable EOF levels, whether high concentrations of inorganic fluorine could impact those levels if not fully removed, and what spike concentrations to use for recovery assessments that are representative of the samples. Although current methods to assess fluoride in nonaqueous matrices are limited, analysts could consider assessing the concentration of residual fluoride that may be extracted during the method by evaporating the organic phase to dryness, reconstituting in water, and

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analyzing the aqueous extract using ion chromatography. If nonaqueous samples contain high concentrations of inorganic fluorine, additional cleanup steps may be necessary to ensure residual inorganic fluorine does not impact the reported EOF concentration. It is also beneficial to assess if high concentrations of other species (e.g., chloride, potassium, calcium, sodium) are present as these ions can cause devitrification of quartz combustion tubes and/or affect the combustion process. In instances where these other analyses are not available and/or are cost prohibitive (such as in high-throughput commercial laboratories or for large infrastructure projects), or when the sample composition cannot be characterized, strict adherence to QA/QC procedures can mitigate some of these confounding factors.

- The following QA/QC checks are recommended: (1) an organofluorine spike to assess EOF recovery (preferably with compounds that are present in the sample), (2) an inorganic fluorine spike at or above native inorganic fluorine levels to assess inorganic fluorine removal efficiency, (3) extraction blanks to assess contamination introduced by sample preparation and to calculate method detection limits, and (4) a comparison of PFAS quantified in the EOF extract and in a separate extract where internal standards are added prior to extraction to assess recovery and accurate quantitative comparison if conducting organofluorine mass balance calculations.
- For analysis and quantification, extracts should be injected from capped sample vials instead of manual injection on open ceramic boats to prevent evaporation. We recommend analyzing duplicate injections of a sample extract and duplicate injections of instrument blanks between samples for blank subtraction if background fluorine is observed during instrumental analysis to avoid overestimating sample EOF concentrations. Subtracting the instrument blank run before the first sample injection and the instrument blank run after the second sample injection is ideal for accounting for potential carryover due to incomplete combustion (i.e., instrument blank → sample injection #1 → sample injection #2 → instrument blank). Further method development to reduce carryover would also be beneficial. We recommend performing additional extraction blank subtraction if EOF is detected in the extraction blanks. We recommend using appropriate detection limit metrics that consider noise (background fluorine) from both the instrumental analysis and extraction procedure and clearly defining the chosen detection limit (iLOD vs MRL vs MDL).

Study Implications and Next Steps. Established protocols (e.g., extraction methods) that are compatible, efficient, and yield good performance across various sample matrices and instrumental analyses (i.e., EOF via CIC, targeted PFAS via LC-MS/MS, nontarget PFAS via high-resolution mass spectrometry) are needed for accurately assessing the total quantity of PFAS in samples. We recommend that future studies focus on further identifying and quantifying the organofluorine compounds that make up this fraction. For example, recent work has included quantification of fluorinated pharmaceuticals alongside EOF and PFAS measurements in wastewater biosolids.[11]

Results of this study highlight a need for best practices in handling and reducing background fluorine levels introduced during sample extraction for EOF measurements and instrumental analysis as well as methods for assessing and fully removing inorganic fluorine. Inorganic fluorine removal is especially critical for samples that have much higher fluoride concentrations compared to EOF such as in drinking/wastewater and seawater. Additional methods for measuring inorganic fluorine in nonaqueous samples (i.e., soil, blood, tissues) are currently limited and would be beneficial. Applications of CIC that perform direct combustion of the sample should not be interpreted as a measurement of total organofluorine (i.e., TOF) unless the absence of inorganic fluorine can be confirmed.

The development of a standardized EOF method would be extremely useful for the research community and would ensure the rigor and comparability of EOF data across laboratories. The United States Environmental Protection Agency issued a draft screening method 1621 for adsorbable organofluorine (AOF) in 2022[40] and the International Organization for Standardization is currently developing an AOF method,[41] but there is no equivalent method for EOF. We have provided a set of recommendations based on this interlaboratory comparison that could be incorporated into a standardized EOF method.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c04560.

Additional details for the Introduction, Methods, and Results including Tables S1−S13 and Figures S1−S3 and a EOF Data Evaluation Checklist for evaluators (PDF)

PFAS and EOF concentrations and QC corresponding to Tables S4−S7 and S11−S1 (XLSX)

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Notes

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