Quantitative Method Validation for the Emerging Hexafluoropropylene Oxide Acids (HFPOA) and Related Perfluoroether Carboxylic Acids (PFECAs)

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ABSTRACT

Legacy perfluorinated compounds exhibit significant environmental persistence and bioaccumulation potential, which has spawned an ongoing effort to introduce replacement compounds with reduced toxicological risk profiles. Many of these emerging chemical species lack validated quantitative methods, and frequently appropriate analytical standards for accurate monitoring and identification. To fill this knowledge gap, a general method for the quantitative determination of perfluoroether carboxylic acids (PFECAs) by LC-MS/MS was single-lab validated on spike-recovery samples in surface, drinking, and wastewater for a variety of perfluorinated ether standards. Relative error measurements for spike-recovery samples in each

matrix ranged from 0.36% to 25.9%, with an average error of 10% overall. Coefficient of variation (CV) for each compound ranged from 10 to 28% with an average of 17%. The quantitative methodology was applied during a continuous monitoring effort of the Cape Fear River during remediation of the PFECA hexafluoropropyloxide dimer-acid (HFPO-DA), brand name "GenX." Concentration estimates for emerging PFECA compounds lacking analytical standards was also carried out using surrogate calibration curves and mass labeled HFPO-DA as an internal standard. Quantitative estimates of the emerging compounds were possible using matched standards, but may possess up to an order of magnitude, or more, in uncertainty due to the difficulty of matching with an appropriate standard. Nevertheless, the estimation biases are primarily systematic rather than stochastic, enabling the collection of time-course data; both HFPO-DA and the emerging compounds were reduced in surface water and drinking water concentration of several orders of magnitude after removal of the source waste stream.

INTRODUCTION

Poly- and perfluoroalkyl substances (PFASs) are recalcitrant environmental pollutants which have become a focus of significant public health interest in recent years.[1] In particular, the widely used compounds perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) were found to have a negative toxicological profile and bioaccumulation potential,[2-3] resulting in a drinking water health advisory,[3-4] international restrictions,[5] and pressure for banning under the Stockholm Convention on Persistent Organic Pollutants.[6] The primary manufacturers of these molecules phased out US production of PFOS/PFOA and related chemical species in the 2000s.[7-8] However fluorinated surfactants such as PFOA and PFOS, as well as the fluorinated polymer products whose production they support, exhibit unique chemical properties that are of substantial value in

the textiles and consumer products industries.[9] As a consequence, numerous alternative chemistries have emerged to replace the discontinued species.[10-11]

A broad class of replacement chemicals are fluorinated ether compounds,[11] which consist of a series of short chain perfluorinated carbons linked by ether oxygens and are capped with an acidic head group (Table 1). Many species demonstrating this general fluorinated ether structure have been detected in locations where fluorochemical manufacturing impacts the water supply,[12-14] but toxicological data of these emerging compounds are limited. One particular chemical, 2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy) propanoic acid, also known as hexafluoro propylene oxide dimer acid (HFPO-DA), or the trade name of its ammonium salt, GenX,[15] was designed as a fluorochemical processing surfactant that meets the requirements of the EPA PFASs management program.[16] GenX, and other HFPO related products are present in significant concentrations in the discharge from certain fluorochemical manufacturing sites.[12, 14, 17] The limited toxicological studies of the GenX compound supplied by DuPont[18-21] indicate that *acute* toxicity and metabolism are of limited concern for the replacement chemical, but long-term exposure effects are similar to PFOA and cause for some concern.[20-21] The detection of the compound in the Cape Fear River in North Carolina sparked an intensive state level investigation and health goal[22] amid concerns over its long-term effects.

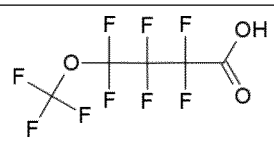
GenX is but one of an amalgamation of related fluorochemical compounds emerging as pollutants of interest in surface, ground and drinking water, and, while existing non-targeted methodologies have allowed for the detection of novel structures,[12-14] quantitative methods will be necessary for long term environmental monitoring and health effects studies regarding new fluorochemicals like HFPO-DA /GenX. Existing methodologies, such as EPA method 537, were developed for the

analysis of legacy compounds, straight chain perfluorinated carboxylic acids and sulfonates, in drinking water, but there is substantial need for general methods sufficient to analyze known and emerging compounds in environmental matrices. Further, as emerging contaminants frequently lack authenticated standards, methods for tracking and estimating fluorochemical concentrations are needed for monitoring environmental levels of unknown contaminants. Here we demonstrate a quantitative sample preparation and LC-MS/MS method suitable for emerging perfluoroether compounds, validate it in multiple water source matrices, and apply it to the ongoing monitoring in an impacted watershed. Further, we apply surrogate species calibration curves to the concentration estimation and time-course monitoring of perfluoro-ether compounds with no existing standards.

MATERIALS AND METHODS

Preparation of PFECA Standards

Reference standards of perfluoroether carboxylic acids were obtained from Synquest Laboratories (Table 1) along with $^{13}C_3$ labeled 2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy) propanoic acid (HFPO-DA/GenX) for use as an internal standard (Wellington Labs). A stock solution of each standard was prepared in 95:5 methanol:2.5M NaOH to prevent the formation of the methyl ester. From these stocks, a mixed PFECA standard was prepared containing equal mass of each native species and diluted to the desired final concentrations in methanol.

Compound Name	CAS	Formula	Structure
Perfluoro(4-oxapentanoic acid) (“Surrogate 1”)	863090-89-5	$C_5HF_9O_3$	
Perfluoro(3,6-dioxaheptanoic acid) (“Surrogate 2”)	151772-58-6	$C_5HF_9O_4$	

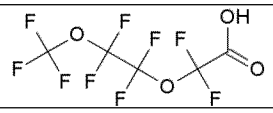
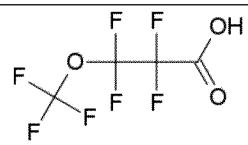
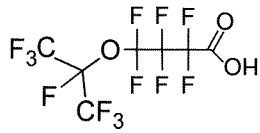
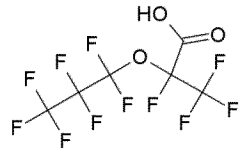
			
Perfluoro(3-oxabutanoic acid) (“Surrogate 3”)	377-73-1	$C_4HF_7O_3$	
Perfluro(5-oxa-6-dimethylhenxanoic) acid (“Surrogate 4”)	801212-59-9	$C_7HF_{13}O_3$	
Undecafluoro-2-methyl-3-oxahexanoic acid (“HFPO-DA”)	13252-13-6	$C_6HF_{11}O_3$	

Table 1. Reference perfluoro-ether carboxylic acids included in the PFECA standard mixture.

Preparation of PFECA Spike Recovery Samples

Standard PFECA solution mixtures were prepared using water sourced from three locations as the background matrices. Background matrix water was sourced as followed: surface water collected from Discovery Lake, Research Triangle Park, NC (35.8813 N, -78.8755 W), waste water collected from Jacksonville Wastewater Treatment facility effluent, Jacksonville, NC, and drinking water collected from a faucet tap in the Environmental Protection Agency office in Research Triangle Park, NC. Each source sample was divided into one liter pre-cleaned high-density polyethyelene (HDPE) Nalgene bottles and spiked with a standard mixture of PFECAs (Table 1) at either 0, 50, or 200 ng/L. Five of these matrix spiked samples were prepared for each combination of background matrix (Surface Water, Waste Water, Drinking Water) and PFECA level (Blank, Low Spike, High Spike) resulting in 45 samples. The prepared spiked samples were stabilized by pH adjustment to pH = 2 with the addition of nitric acid. A calibration curve of the PFECA mixture at 0, 10, 25, 50, 100, 150, 200, and 250 ng/L was also prepared in deionized water (DI) and processed in tandem.

Environmental Sampling

Fluorochemical contaminated water samples were acquired from North Carolina Department of Environmental Quality (NCDEQ) at a location near the industrial waste outfall for a local fluorochemical manufacturer, as well as from downstream drinking water treatment plants. Location 1 was the source water drawn from the Cape Fear River for a downstream water treatment facility and Location 2 was finished drinking water delivered to Wilmington, NC. Each sample was collected in one liter HDPE and stabilized by pH adjustment with nitric acid as previously described.[23] Samples were analyzed within five days of the sampling date.

Solid Phase Extraction of PFECAs from Aqueous Matrix

The previously prepared, stabilized samples were decanted into a 1000 mL HDPE graduated cylinder and their total volume recorded; water in excess of 1000 mL was discarded. The sample bottle was then rinsed with 10 mL of methanol to remove any compounds that partially adhere during sample processing and storage; the retained water was returned to the bottle alongside the methanol. An internal standard of $^{13}C_3$ labeled HFPO-DA was added to each water sample at a concentration of 100 ng/L of sample, and shaken vigorously before being vacuum filtered through a Whatman GF/A glass filter.

Oasis weak anion exchange (WAX) Plus (225 mg) solid phase extraction (SPE) cartridges (Waters Corporation) were conditioned according to the manufacturer instructions, as follows: the SPE cartridges were equilibrated under 10 mL/min methanol flow for 2.5 minutes, followed by 2.5 min of deionized water (DI) at 10 mL/min; flow was maintained through a SPC10-P Sep-Pak concentrator (Waters Corporation). Samples were concentrated at a flow rate of 10 mL/min for 50 minutes (500 mL total volume) and SPE cartridges were removed for later elution. The

Sep-Pak system was flushed with 25 mL of methanol (2.5 min at 10 mL/min) between sample cartridges to prevent sample carryover.

After sample concentration, the SPE cartridges were washed with 4 mL of 25 mM pH 4.0 acetate/acetic acid buffer, followed by a wash of 4 mL of neutral methanol. Compounds of interest were then eluted with 4 mL of freshly prepared 0.1% ammonium hydroxide in methanol and evaporated to ~ 1 mL under a dry nitrogen stream at 40 ºC. The evaporated samples were diluted 4-fold in 2.5 mM ammonium acetate to match the starting conditions for liquid chromatography.

LC-MS/MS Data Acquisition and Quantification

LC-MS/MS quantification took place on a Waters Acquity ultra performance liquid chromatograph which had been modified to replace OEM PTFE filters and solvent plumbing with stainless steel or PEEK tubing and incorporate a PFC isolation column (Waters P/N 176001744). The chromatographic separation was carried out using a Waters ACQUITY UPLC BEH C18 column (2.1 mm x 50mm, 1.7 µm), a flow rate of 500 µL/min, 50 µL injection volume, and a binary mobile phase gradient with mobile phases A (95:5, 2.5mM ammonium acetate**:** Methanol) and B (95:5, Methanol**:** 2.5 mM ammonium acetate). The gradient program was a follows: 10-85% B (5min), 85-100% (0.1 min), 100% hold (1.9 min), 100 – 10% (0.1 min), 10% hold (1.9 min). Detection and quantitation was performed with an interfaced Waters Quattro Premier XE triple quadrupole mass spectrometer with individual MRM parameters for each PFECA species analyzed (Table 2). Stable isotope labeled (SIL) labeled HFPO-DA as used as an internal standard for all measured compounds.

Species	Molecular Formula	Precursor Mass (Da)	Product Mass (Da)	Cone Voltage (V)	Collision Energy (eV)

Surrogate 1	$C_5HF_9O_3$	279.0	84.9	14	22
			134.8	22	24
Surrogate 2	$C_5HF_9O_4$	295.0	84.9	10	26
			200.9	10	10
Surrogate 3	$C_4HF_7O_3$	229.0	84.9	10	10
			184.9	22	18
Surrogate 4	$C_7HF_{13}O_3$	379.0	184.8	16	12
			134.9	16	34
HFPO-DA	$C_6HF_{11}O_3$	329.2	168.9	10	12
			286.9	10	6
HFPO-DA IS	$[^{13}C]_3C_3HF_{11}O_3$	332.2	168.9	10	12
			286.9	10	6
PFMOAA	$C_3HF_9O_5$	178.9	84.9	10	12
PFO2HxA	$C_4HF_7O_4$	245.0	84.9	22	18
			178.8	22	4
PFO3OA	$C_5HF_9O_5$	311.1	84.9	22	20
			150.8	22	4

Table 2. MRM table for surrogate and emerging PFECAs analyzed in this study

MRM transitions were previously optimized for the four surrogate perfluoroether compounds and HFPO-DA using available standards. Additional transitions for three emerging PFECAs previously described (Supplemental Figure 1-3),[14] but currently lacking analytical standards, were selected using direct infusion of extracts prepared from the Cape Fear River. For method validation, the MRM method contained only the surrogate and HFPO-DA transitions, while environmental samples included the three MRM transitions for the emerging PFECAs.

The PFECA mixture was used to prepare an eight-point calibration curve for each constituent PFECA standard for quantitation. Ion abundances were normalized to the HFPO-DA internal standard intensity and calibration curves were fitted as a concentration weighted (1/x) quadratic curve. For spike recovery experiments, quantitation was derived from the matching standard curve. For quantitative measurements of HFPO-DA and semi-quantitative estimates for the

emerging PFECAs in environmental samples, systematic application of each surrogate standard curve was used to calculate estimated concentrations.

RESULTS AND DISCUSSION

Spike-Recovery Validation

Representative figures of merit were calculated for the quantitative spike recovery assessment based on the five sample preparations for each combination of background matrix and concentration (Figure 1). For each replicate, the relative percentage error (RE) ranges from 0.36% to 25.9%, with an average of ~10% when aggregated across compounds and background matrices (Table 3). Sample precision was calculated with average coefficient of variation of 17% across matrices and spike levels (Table 3). A method limit of detection (LoD) for PFECAs was estimated at 16 ng/L using a 3X signal-to-noise cutoff. The method noise was estimated as the standard deviation of repeated injections of the 10 ng/L calibration curve point. This reported LoD value is not a physical limitation, and can be reduced through modifications to the SPE concentration step, sample blowdown, or injection volume to increase the on-column analyte load and improve sensitivity.

Figure 1. Measured PFECA concentrations for a synthetic PFECA mixture in varying matrices at 200 ng/L (HS), 50 ng/L (LS), and 0 ng/L (BL) spike levels, with 95% confidence intervals.

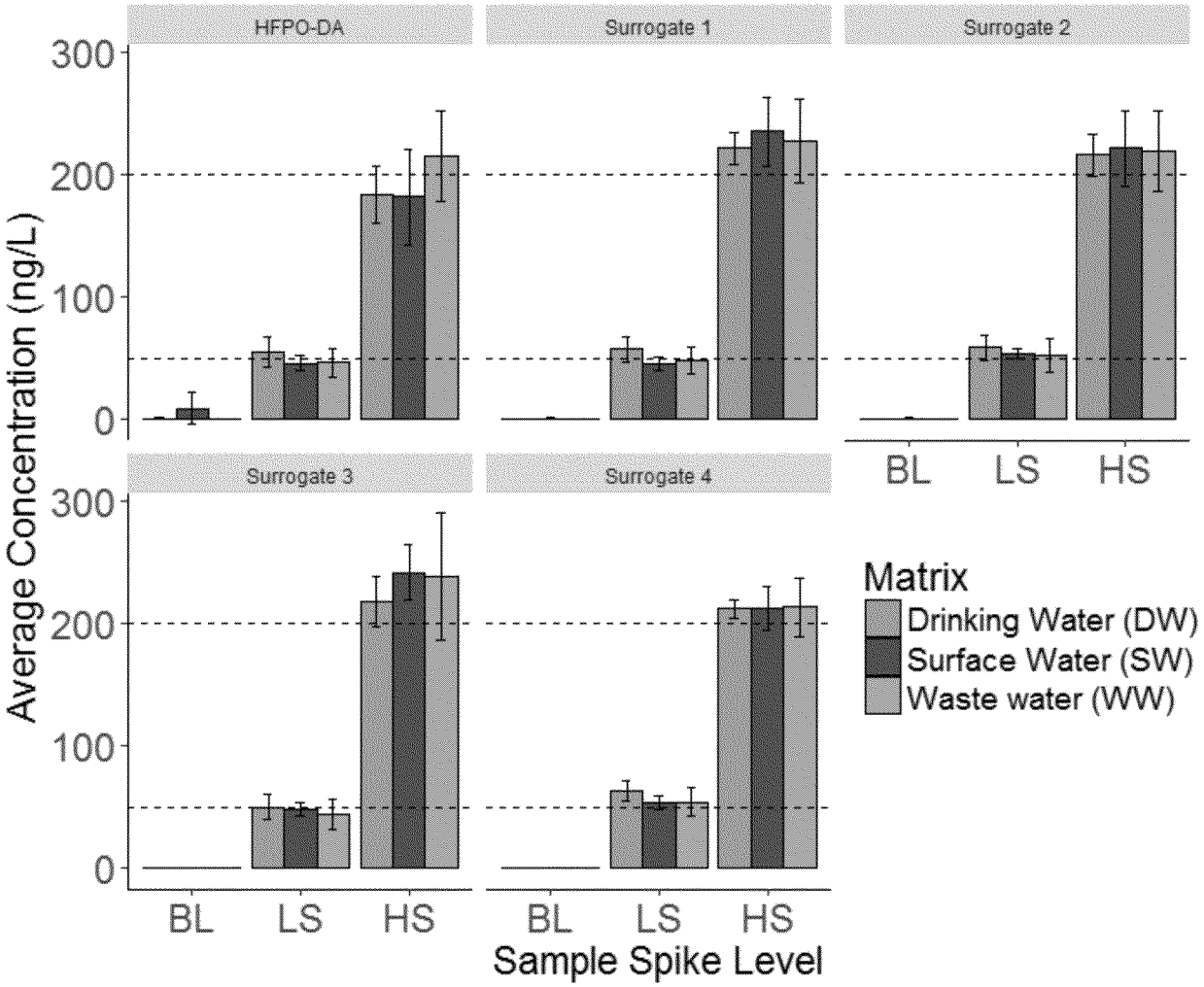


Table 3. Calculated relative percentage error (RE) and coefficient of variation (CV) for measurements (n=5) in waste water (WW), surface water (SW) and drinking water (DW) matrices for a series of PFECA compounds.

Compound	Matrix	RE (%)	CV (%)
HFPO-DA	DW	9.2	20%
	SW	8.6	20%
	WW	7.4	25%
Surrogate 1	DW	12.9	14%
	SW	13.4	14%
	WW	9.1	22%
Surrogate 2	DW	13.0	14%
	SW	9.2	12%
	WW	7.5	23%
Surrogate 3	DW	4.7	17%
	SW	12.2	12%
	WW	15.6	28%

Surrogate 4	DW	15.9	10%
	SW	7.0	10%
	WW	7.5	19%

For the HFPO-DA compound, the measurement retains accuracy regardless of matrix or sample concentration within the range of the calibration curve; all measurements correspond to their theoretical values within the 95% confidence interval and demonstrate acceptable RE (<10%) and CV (<30%) values. However, the other PFECA compounds demonstrate a small, systemic overestimation of their concentration in some background matrices. This effect is attributable to a non-matched internal standard, and can be corrected in the future as appropriate stable isotope labeled species become available. The reproducibility of the assay is still high; CVs of the surrogate compounds are equivalent or better than HFPO-DA with one exception, and the RE remains under 20% in all cases, with results from each matrix being statistically indistinguishable from each other. Thus, the methodology can be generally applied even in absence of matched internal standards. Of note, a single replicate of waste water – high spike was excluded from the collection sample set. The excluded values were a 3.5-sd outlier for all surrogate species due to significant signal suppression (>5-fold) of HFPO-DA in that preparation; caution remains necessary when using non-matched internal standards, especially in heterogeneous matrices such as waste water.

Application in Continuous Monitoring of HFPO-DA

Initial environmental samples obtained in June 2017 from fluorochemical manufacturing outfall, surface, and drinking water far exceeded the designated calibration range and required significant dilution to enter the calibration curve range for accurate quantitation. Following this sampling, the

manufacturer ceased waste stream emissions from one of their manufacturing lines and the measured concentrations of all monitored species dramatically declined in both the direct outfall and downstream locations (Figure 2, 3). Of note, local contamination from pumps and piping at the manufacturing site are hypothesized to be the cause of the persistently high concentrations in the waste outfall through the third sampling week in spite of the immediate decline observed at downstream locations. Continuous monitoring of both outfall and impacted drinking water sites demonstrated minor daily variation but consistent reduction below North Carolina's targeted long term health goal of 140 ng/L.[22]

Figure 2. Measured HFPO-DA concentrations in manufacturing outfall over time. The vertical line indicates diversion of PFECA generating waste stream, the horizontal line indicates targeted HFPO-DA long term health goal (140 ng/L), inset shows shaded region.

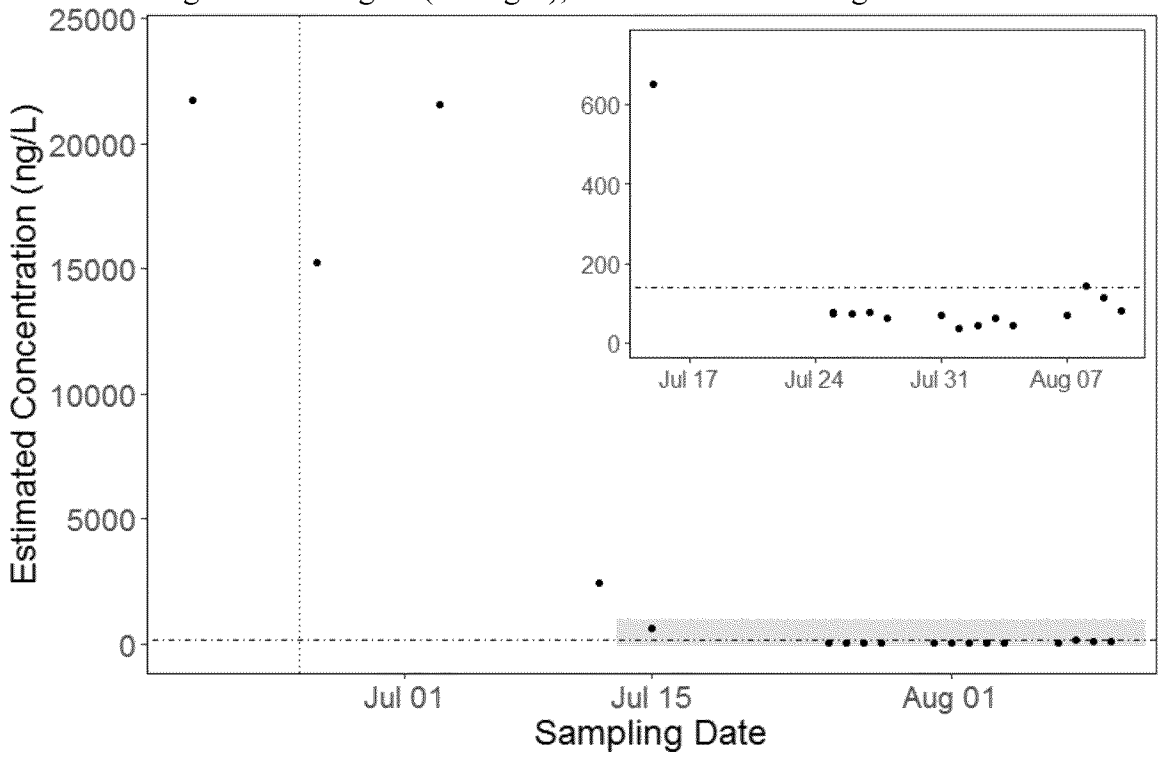
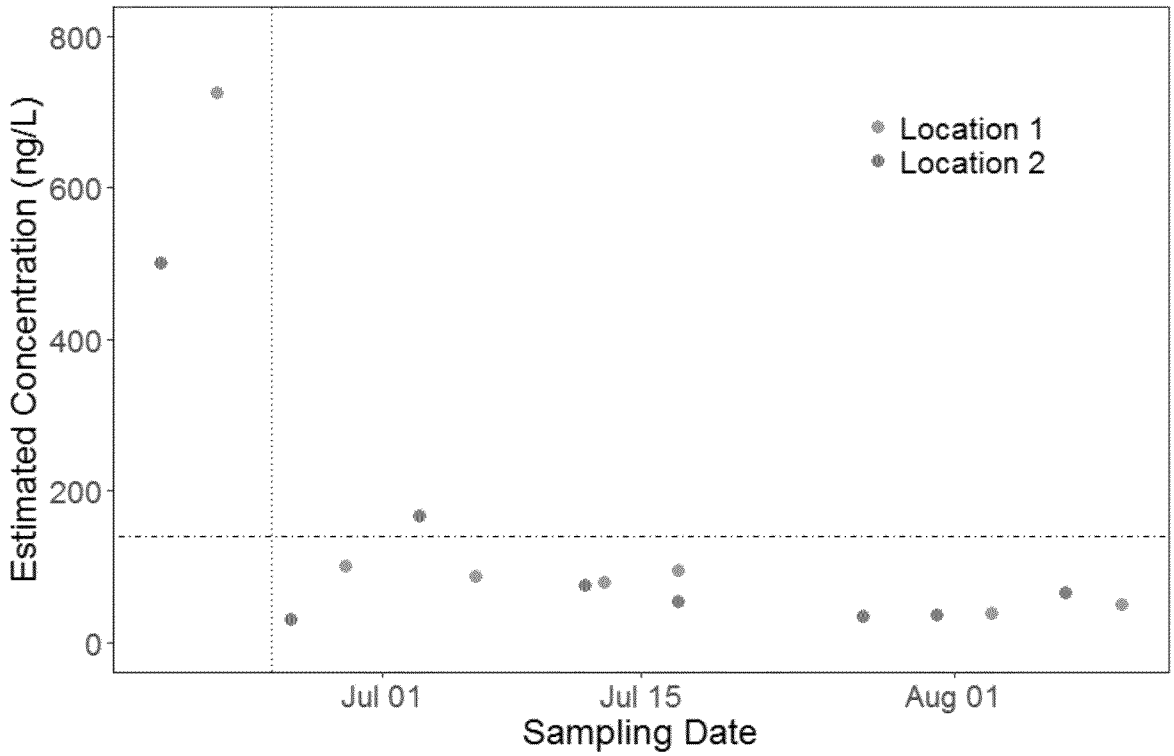


Figure 3. Measured HFPO-DA concentrations at drinking water treatment plants located in the Cape Fear River basin. Vertical line indicates diversion of PFECA generating waste stream, horizontal line indicates targeted long term health goal (140 ng/L)



Blank, low spike, and high spiked standard mixtures were carried to the sampling site as field blanks each week and returned for QC analysis. Throughout the sampling course, QC samples measured were quantified with an average RE of 22%. Each week, a randomly selected 10% subset of the environmental samples were replicated and inter-replicate CVs were <10%, demonstrating long term method stability and precision.

Semi-Quantitative Estimates of Emerging PFECAs

For compounds tentatively identified as PFMOAA, PFO2HxA, and PFO3OA,[14] the lack of a matched standard and reliance on a surrogate internal standard limits the potential accuracy of quantitative efforts. Nevertheless, semi-quantitative estimates can inform public health decisions

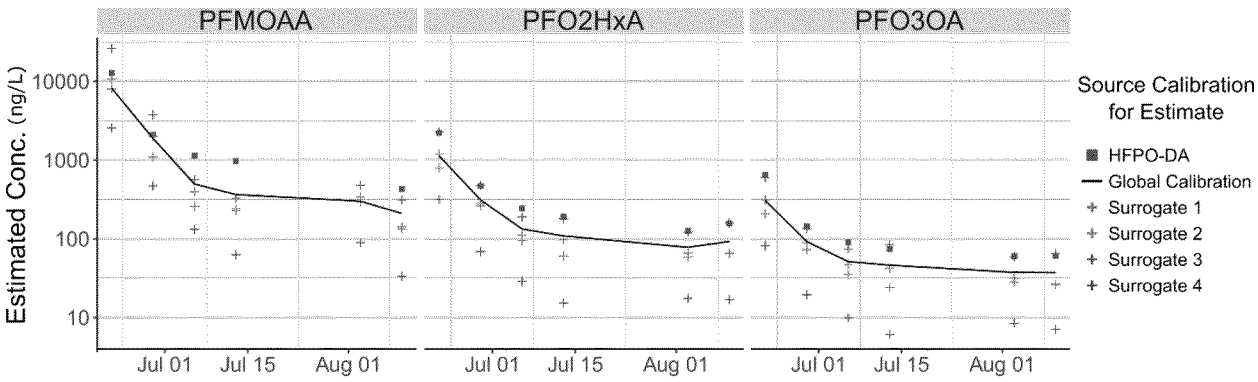
and provide focus for further development of analytical methods.[24] The PFECA mixture was used to prepare an eight-point calibration curve for each constituent PFECA standard. Ion abundances were normalized to the HFPO-DA internal standard intensity and calibration curves were fitted as a concentration weighted (1/x) quadratic curve. Estimated concentrations for the three emerging compounds, which lack standards, and HFPO-DA were obtained using each of the five calibration curves each sampling week, as well as a global estimate composed of the data points from all five surrogates.

To establish an estimate of the level of error when using surrogate compounds for concentration estimates, the HFPO-DA value from its matched calibration curve was compared to values obtained using the calibration curves from the four surrogate compounds. The surrogate estimated concentrations yielded average errors of 1.3%, 0.6%, 48%, and 0.3% for Surrogates 1-4 respectively. The three closely matching surrogates, 1, 2 and 4, elute within a 1-minute window, very near the HFPO-DA peak (Supplemental Figure 4). As consequence, they demonstrate remarkably little bias in estimated concentrations for HFPO-DA, unlike Surrogate 3, which elutes much earlier in the chromatographic run, and has significant peak distortions resulting from its more hydrophilic character than the other species. For the HFPO-DA, therefore, a best case scenario of a closely matched chemical structure for the calibration curve and internal standard yields accurate results within the error margins of the matched curve and standard, while a poorly matched species yields a several-fold, but still manageable, level of bias in estimation. Matching of surrogates can be performed empirically, by selecting similar chemical structures and confirming similar chromatographic and MS performance, but a general solution to determining “good” surrogate matches is beyond the scope of this paper.

The emerging species exhibit significantly more variability in estimated concentrations compared to the surrogate standards (Figure 4). Structurally, the emerging compounds appear similar to the surrogate PFECA compounds, however, their elution time indicates substantially higher hydrophilicity, with PFMOAA eluting nearly in the void volume, and PFO2HxA falling somewhere between the PFMOAA and surrogate standards (Supplemental Figure 5).

The systematic dissimilarity between the lower molecular weight emerging compounds and the surrogate standards impacts the concentration estimates. The predicted concentrations are consistent in their ordering, with HFPO-DA estimate being the highest value and Surrogate 4 underestimating that value by ~10-fold.

Figure 4. Estimated concentrations of three emerging PFECA compounds at Location 2, using five surrogate calibration curves and a global calibration of all species.



The systematic bias of estimated concentration is consistent across sampling location and date, implying it is intrinsic to the process, likely the result of hydrophobic bias in the chromatography and/or electrospray that cannot be corrected for without more closely matched calibration curve and/or internal standard. A conservative application of the variation between the five closely related PFECA standards suggests the error of the global average is on the order of ten-fold and

can be considered only semi-quantitative at best. Regardless, using the global average calibration curve of the PFECA standards provides a rough estimate of concentration that can be useful for directing further work. The most recent PFMOAA estimates still bracket the health goal for HFPO-DA and it is worthy of further scrutiny, while the other compounds have been reduced below that target level, even when using the highest estimate of the concentration.

Nevertheless, all three of the non-standard PFECA compounds demonstrated substantial reduction over time as the result of halted upstream emission, similarly to the behavior of the HFPO-DA compound. This signifies that all the related PFECA compounds likely derive from the single fluoropolymer manufacturing process whose waste was diverted. Further, time trends indicate that the overall concentration has dropped ~2 orders of magnitude, similarl to the HFPO-DA, regardless of the calibration source used. This methodology is therefore suitable for tracking temporal changes in compounds lacking standards even though the specific estimation of concentrations remains highly uncertain.

CONCLUSION

The ongoing proliferation of new perfluorinated species to serve industrial chemical need in absence of the legacy compounds PFOA and PFOS is a burgeoning environmental and public health issue. Monitoring efforts for these new species require a robust, generalized method for quantitative determinations of exposure. The availability of a SIL internal standard for HFPO-DA/GenX allows for robust analysis of this species, and allows its use as a surrogate internal standard for other compounds barring the production of matching SIL perfluoroethers. The methodology presented here is robust to variations in the environmental matrix (tap, surface, and waste water) and offers an extended range of sensitivity through the use of multiple concentration

steps. Nevertheless, the calibration range utilized, with a method limit of detection ~10 ng/L, is sufficient for exposure monitoring, as the existing health advisory/goal levels for both the legacy and emerging perfluorinated compounds are around an order of magnitude higher. Lower health targets for legacy species exist in some states,[25] and increased concentration factors would be necessary to achieve lower method detection limits.

The same sample preparation technique can be applied to the analysis of only partially characterized species assuming chemically similar standards are available for purchase. Using one or more related species to construct calibration curves, even with surrogate internal standards, allows for an estimation of concentrations that can inform future research and policy efforts in environmental exposure. This can act as supplemental and supporting information to other mass spectrometry related monitoring efforts in non-targeted analysis. For species with some available structural information and a closely related standard, the error is on the same order as the method variation and suitable for quantitative work. The estimates from less closely matched calibrations curves vary by > 10-fold on the selected calibration species, but nevertheless can reveal accurate time-trend data for determining the impact of systemic changes and determining whether multiple species have correlated behaviors in response to an intervention.

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REFERENCES

1. Lindstrom, A. B.; Strynar, M. J.; Libelo, E. L., Polyfluorinated Compounds: Past, Present, and Future. *Environ. Sci. Technol.* **2011,** *45* (19), 7954.
2. Stahl, T.; Mattern, D.; Brunn, H., Toxicology of perfluorinated compounds. *Environmental Sciences Europe* **2011,** *23* (1), 38.
3. Lifetime Health Advisories and Health Effects Support Documents for Perfluorooctanoic Acid and Perfluorooctane Sulfonate. United States, E. P. A., Ed. Federal Register, 2016; pp 33250-33251.
4. Provisional Health Advisories for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS). United States Environmental Protection Agency: Washington DC, 2009.
5. Report of the Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants on the work of its fourth meeting **2009**.
6. Proposal for a COUNCIL DECISION on the submission, on behalf of the European Union, of a proposal for the listing of additional chemicals in Annex A to the Stockholm Convention on Persistent Organic Pollutants. Council of the European Union: 2016.
7. Fact Sheet: 2010/2015 PFOA Stewardship Program. United States Environmental Protection Agency: 2006.
8. EPA and 3M Announce phase out of PFOS. https://yosemite.epa.gov/opa/admpress.nsf/0/33aa946e6cb11f35852568e1005246b4.
9. Kissa, E., *Fluorinated surfactants and repellents*. CRC Press: 2001; Vol. 97.
10. Scheringer, M.; Trier, X.; Cousins, I. T.; de Voogt, P.; Fletcher, T.; Wang, Z.; Webster, T. F., Helsingør Statement on poly- and perfluorinated alkyl substances (PFASs). *Chemosphere* **2014,** *114*, 337-339.
11. Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K., Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and their potential precursors. *Environ. Int.* **2013,** *60*, 242.
12. Sun, M.; Arevalo, E.; Strynar, M.; Lindstrom, A.; Richardson, M.; Kearns, B.; Pickett, A.; Smith, C.; Knappe, D. R. U., Legacy and Emerging Perfluoroalkyl Substances Are Important Drinking Water Contaminants in the Cape Fear River Watershed of North Carolina. *Environmental Science & Technology Letters* **2016,** *3* (12), 415-419.
13. Newton, S.; McMahen, R.; Stoeckel, J. A.; Chislock, M.; Lindstrom, A.; Strynar, M., Novel Polyfluorinated Compounds Identified Using High Resolution Mass Spectrometry Downstream of Manufacturing Facilities near Decatur, Alabama. *Environmental Science & Technology* **2017,** *51* (3), 1544-1552.
14. Strynar, M.; Dagnino, S.; McMahen, R.; Liang, S.; Lindstrom, A.; Andersen, E.; McMillan, L.; Thurman, M.; Ferrer, I.; Ball, C., Identification of novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) in natural waters using accurate mass time-of-flight mass spectrometry (TOFMS). *Environ. Sci. Technol.* **2015,** *49* (19), 11622.
15. DuPont™ GenX Processing Aid for Making Fluoropolymer Resins. Chemours: 2010.
16. Risk Management for Per- and Polyfluoroalkyl Substances (PFASs) under TSCA. United States Environmental Protection Agency: Washington DC, 2015.

17. Pan, Y.; Zhang, H.; Cui, Q.; Sheng, N.; Yeung, L. W.; Guo, Y.; Sun, Y.; Dai, J., First Report on the Occurrence and Bioaccumulation of Hexafluoropropylene Oxide Trimer Acid: An Emerging Concern. *Environmental Science & Technology* **2017,** *51* (17), 9553-9560.
18. Gannon, S. A.; Fasano, W. J.; Mawn, M. P.; Nabb, D. L.; Buck, R. C.; Buxton, L. W.; Jepson, G. W.; Frame, S. R., Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. *Toxicology* **2016,** *340*, 1-9.
19. Caverly Rae, J. M.; Craig, L.; Slone, T. W.; Frame, S. R.; Buxton, L. W.; Kennedy, G. L., Evaluation of chronic toxicity and carcinogenicity of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in Sprague–Dawley rats. *Toxicology Reports* **2015,** *2*, 939-949.
20. Hoke, R. A.; Ferrell, B. D.; Sloman, T. L.; Buck, R. C.; Buxton, L. W., Aquatic hazard, bioaccumulation and screening risk assessment for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate. *Chemosphere* **2016,** *149*, 336-42.
21. Rushing, B. R.; Hu, Q.; Franklin, J. N.; McMahen, R. L.; Dagnino, S.; Higgins, C. P.; Strynar, M. J.; DeWitt, J. C., Evaluation of the Immunomodulatory Effects of 2,3,3,3-Tetrafluoro-2-(Heptafluoropropoxy)-Propanoate in C57BL/6 Mice. *Toxicological Sciences* **2017,** *156* (1), 179-189.
22. NC DHHS Preliminary Risk Assessment FAQ for GenX (Perfluoro2-propoxypropanoic acid). North Carolina Department of Health and Human Services: 2017.
23. Nakayama, S. F.; Strynar, M. J.; Reiner, J. L.; Delinsky, A. D.; Lindstrom, A. B., Determination of perfluorinated compounds in the Upper Mississippi River Basin. *Environ. Sci. Technol.* **2010,** *44* (11), 4103.
24. *Environmental decisions in the face of uncertainty*. National Research Council: 2013.
25. Maximum Contamination Level Recommendation for Perfluorooctanoic Acid in Drinking Water. New Jersey, Drinking Water Quality Institute: 2017.