Source apportionment of serum PFASs in two highly exposed communities

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HIGHLIGHTS

- Novel use of source apportionment methods in human biological samples
- Identification of human exposure from blood samples
- Identification of now controlled exposure sources
- Methods have limitations but show promise for expanded uses.

GRAPHICAL ABSTRACT
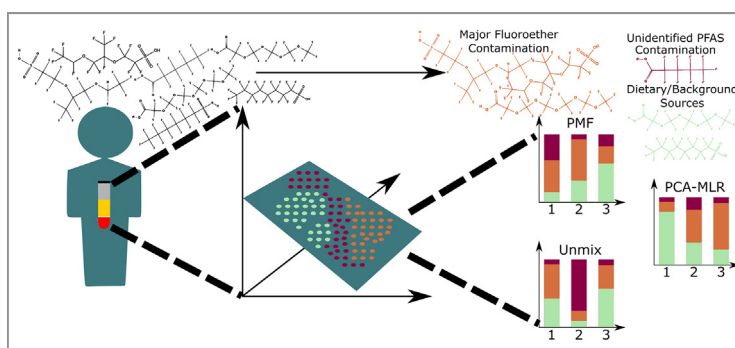
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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) are synthetic chemicals that are ubiquitous in environmental and biological systems, including human serum. PFASs are used in many products and industrial processes and are tied to numerous health effects. Due to multiple sources and exposure pathways, methods are needed to identify PFAS sources in communities to develop targeted interventions. We assessed effectiveness of three source apportionment methods (UNMIX, positive matrix factorization [PMF], and principal component analysis - multiple linear regression [PCA-MLR]) for identifying contributors to human serum PFAS concentrations in two highly exposed populations in Colorado and North Carolina where drinking water was contaminated via upstream sources, including a Space Force base and a fluorochemical manufacturing plant. UNMIX and PMF models extracted three to four potential PFAS exposure sources in the Colorado and North Carolina cohorts while PCA-MLR classified two in each cohort. No sources were characterized in NHANES (National Health and Nutrition Examination Study). Results suggest that these three methods can successfully identify sources in highly exposed populations. Future PFAS exposure research should focus on analyzing serum for an expanded PFAS panel, identifying cohorts with other distinct point source exposures, and combining biological and environmental data to better understand source apportionment results in the context of PFAS toxicokinetic behavior.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a class of thousands of chemicals that have many uses from stain and water-repellent products to

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aqueous film forming foams (AFFF) (Buck et al., 2011). PFASs have a range of mechanisms of action in the human body and varied behavior in the environment (Buck et al., 2011; Agency for Toxic Substances and Disease Registry (ATSDR), 2021). Though few PFASs have been studied extensively, exposure to these substances at occupational and environmental levels has been associated with an array of adverse health effects (Agency for Toxic Substances and Disease Registry (ATSDR), 2021; Sunderland et al., 2019). Since production began in the 1930s, PFASs have become globally pervasive due to their widespread use, persistence and mobility (Sunderland et al., 2019; Vestergren and Cousins, 2009; Armitage et al., 2009; Mueller and Yingling, 2017; Land et al., 2018). Because of this, and varied exposure pathways, it is imperative that investigators identify key sources of exposure to develop targeted interventions.

Receptor-based methods for source apportionment are often used for identifying contamination sources and source contributions in environmental media. Some of these models (i.e. chemical mass balance [CMB]) require known source profiles (Hopke, 2016; Miller et al., 1972), which are rarely available for PFAS-contaminated communities. Models have been developed to resolve sources in mixtures without a priori knowledge of sources (Hopke, 2016; Henry, 2002; Paatero and Tapper, 1994). Such models include United States Environmental Protection Agency (USEPA)-developed UNMIX, positive matrix factorization (PMF), and principal component analysis-multiple linear regression (PCA-MLR) (Hopke, 2016; Paatero and Tapper, 1994; Henry, 2003; Harrison et al., 1996; Larsen and Baker, 2003; Reff et al., 2007; Thurston and Spengler, 1985). These models have been successfully used for source apportionment of PFASs in environmental media (Li et al., 2020; Kuroda et al., 2014; Liu et al., 2019; Qi et al., 2017; Qi et al., 2016; Xu et al., 2018; Xu et al., 2013). While many source apportionment studies have used receptor models to determine source contributions from environmental media, the models have rarely been used for biological media (Rodenburg and Delistraty, 2019; Rodenburg et al., 2015; Jovanović et al., 2019). Many studies in exposed communities lack a priori knowledge of all exposure sources and/or do not have access to environmental samples from the time of the contamination. This means that biological measurements are often the best source for reconstructing prior exposure. Therefore, an understanding of model utility for biological matrices is important. This work uses multivariate receptor models– UNMIX, PMF and PCA-MLR–to evaluate putative sources of PFASs measured in serum samples from cohorts in Colorado (CO) and North Carolina (NC), United States (U.S.), along with a population representative of U.S. background exposures from 2015 to 2016. While some sources of contamination are known in these communities, many other PFAS concentrations in the blood samples are elevated relative to the expected background exposure, indicating multiple unknown sources of contamination. Measurements of the water sources for both the NC and CO cohorts also indicate exposure sources beyond the known sources of PFAS exposure (Kotlarz et al., 2020; McCord and Strynar, 2019; Hopkins et al., 2018; Sun et al., 2016; Strynar et al., 2015; Nakayama et al., 2007). These cohorts lacked sufficient environmental samples or information on important exposure metrics like diet and, therefore, using source apportionment methods on biological samples may help reconstruct prior exposure. The overall objective of this work was to assess the utility of multiple source apportionment methods while elucidating source contributions using serum samples from populations exposed to varying PFAS sources. We expected to identify at least one source that aligns with known sources of PFAS contamination in these populations and additional sources that represent currently unidentified sources of exposure. This allows us to estimate the exposure contributions, the total contribution to overall exposure, of the known sources of PFASs and an indication of the magnitude of the other unidentified sources.

2. Methods

2.1. Colorado and North Carolina communities and contamination sources

Data from two on-going research studies, occurring in PFAS-exposed communities in Colorado (CO) and North Carolina (NC), were used to assess the

utility of these models in determining exposure contributions of serum PFAS. The CO site is located in El Paso County, where approximately 80,000 people were exposed to high concentrations of perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS) and other PFASs originating from AFFF use at nearby Peterson Space Force Base (formerly Peterson Air Force Base) (Barton et al., 2020). The NC site is in New Hanover County, where approximately 200,000 people were exposed to PFAS-contaminated drinking water sourced from the Cape Fear River (Kotlarz et al., 2020). This PFAS contamination was made up in part by perfluoroalkyl acids (PFAAs; e.g., PFOS, PFOA, and PFHxS) from currently unidentified sources upstream of the drinking water intake and in part by PFASs known as novel fluoroethers which were released from a fluorochemical manufacturing facility (Kotlarz et al., 2020); fluoroethers are a newer class of PFASs that have the traditional perfluoroalkyl carbon chains characteristic of PFAAs, such as PFOA, but interrupted by ether oxygen(s). Fluoroethers, including hexafluoropropylene oxide dimer acid (HFPO-DA, a.k.a. GenX), and perfluoro-3,5,7,9-tetraoxadecanoic acid (PFO4DA), were discharged into the Cape Fear River by a fluorochemical facility 80 miles upriver from the public water utility intake (McCord and Strynar, 2019; Sun et al., 2016; Strynar et al., 2015; Zhang et al., 2019; Wagner and Buckland, 2017; McCord et al., 2018).

Select characteristics from each cohort are presented in Supplemental Table 1. In Supplemental Fig. 1 distributions of select serum PFAS concentrations from both studies are displayed along with U.S. national reference range concentrations measured in the 2015–2016 National Health and Nutrition Examination Survey (NHANES) (NHANES, n.d.). NHANES is an annual survey that collects information from a representative sample to assesses the health of the U.S population (NHANES, n.d.).

2.2. CO dataset

The CO study population, design, and procedures are described in detail elsewhere (Barton et al., 2020). Briefly, in spring 2018, 213 non-smoking adults who resided for at least two years in an area with AFFF-contaminated drinking water were recruited for the study. Blood samples were collected, and a questionnaire administered. Participants relied on a PFAS-impacted private well (N = 16 participants) or resided in one of three PFAS-impacted water districts (Fountain, Security, or Widefield) (N = 197 participants). PFAS water concentrations in the water districts displayed a north to south gradient (Security > Widefield > Fountain) moving away from the AFFF contamination source, as did certain PFAS (i.e., PFHxS) serum concentrations (Barton et al., 2020). While AFFF in drinking water is a known source of exposure in this cohort, the contribution of the AFFF has not been quantified and additional samples to characterize other local exposures (e.g., local diet, indoor and outdoor air) have not been collected. Blood samples were analyzed for 48 PFASs at the Colorado School of Mines. Details of the laboratory methods are described elsewhere (McDonough et al., 2021).

Eleven PFASs were detected in the serum of ≥50 % of CO study participants (Table 1). Four PFASs (PFOA, PFHxS, PFOS, and perfluorononanoic acid (PFNA)) were detected in ≥98 % of participants. Other PFASs detected in ≥50 % of participants included: Perfluoro-n-heptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluoropentanesulfonic acid (PFPeS), perfluoroheptanesulfonic acid (PFHpS), 2-(N-Methylperfluorooctanesulfonamido) acetic acid (MeFOSAA), and the tentatively identified unsaturated perfluorooctanesulfonic acid (U-PFOS), which contains two fewer fluorines and one double bond within the perfluorinated chain (McDonough et al., 2021).

2.3. NC dataset

The NC study population, design, and procedures are also described in detail elsewhere (Kotlarz et al., 2020). Briefly, 344 residents of New Hanover County, ages 6 years and older, living in a home served with Cape Fear Public Utility Authority (CFPUA) drinking water for at least 12 months prior to November 2017, were recruited for the study. Blood

Table 1
PFASs detected in >50 % of participants for each cohort used as a starting point for inclusion in the three models. Not all PFASs listed below were included in the final selected models.[a]

PFAS	Full name	DTXID[b]	CO cohort		NC cohort	
			% Detected	Detection limit[c] (ng/mL)	% Detected	Detection limit[c] (ng/mL)
Perfluoroalkyl carboxylic acids (PFCAs)						
PFHpA	Perfluoroheptanoic acid	1037303	56	0.01–0.10	63	0.1–0.3
PFOA	Perfluorooctanoic acid	8031865	100	0.01–0.10	100	0.1–0.5
PFNA	Perfluorononanoic acid	8031863	98	0.01–0.20	97	0.1–0.9
PFDA	Perfluorodecanoic acid	3031860	85	0.01–0.20	–	–
PFUdA	Perfluoroundecanoic acid	8047553	66	0.01–0.20	–	–
Perfluorosulfonic acids (PFSAs)						
PFPeS	Perfluoropentanesulfonic acid	8062600	81	0.01–0.20	–	–
PFHxS	Perfluorohexanesulfonic acid	7040150	100	0.11–1.0	98	0.1–1.8
PFHpS	Perfluoroheptanesulfonic acid	8059920	99	0.01–0.04	–	–
U-PFOS	Unsaturated perfluorooctanesulfonic acid	NA	89	0.01–0.20	–	–
PFOS	Perfluorooctanesulfonic acid	3031864	100	0.10–2.0	99	0.1–0.5
MeFOSAA	2-(N-Methylperfluorooctanesulfonamido)acetic acid	10624392	52	0.01–0.20	–	–
Novel fluoroethers						
Nafionbp2	Perfluoro-2-{[perfluoro-3-(perfluoroethoxy)-2-propanyl]oxy}ethanesulfonic acid	10892352	–	–	99	0.1–0.12
PFO4DA	Perfluoro-3,5,7,9-tetraoxadecanoic acid	90723993	–	–	98	0.1–0.11
PFO5DoA	Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	50723994	–	–	88	0.1

[a] UNMIX and positive matrix factorization (PMF) allow user to add and delete species to evaluate how these changes impact the fit of the resulting solution.
[b] DTXSID is a unique substance identifier used in the U.S. EPA CompTox Chemistry Dashboard (Williams et al., 2017).
[c] Multiple analytical runs were used to analyze sample sets, causing some run-to-run variation in detection limits. The range of detection limits for each compound is provided.

samples were collected, and a questionnaire administered. Samples were analyzed at U.S. Environmental Protection Agency (EPA) in Research Triangle Park, North Carolina. Eight PFASs were detected in ≥50 % of NC study participants (Table 1). Two fluoroethers (Nafion byproduct 2 and PFO4DA) and four PFAAs (PFOS, PFOA, PFHxS, and PFNA) were found in ≥97 % participants. Other PFASs detected in ≥50 % of participants included perfluoro-3,5,7,9,11-pentaoxadodecanoic acid (PFO5DoA) and PFHpA. Samples representative of exposure to fluoroethers via this population's drinking water were not available because the source of contamination was shut off prior to the, and levels of fluoroethers have dropped substantially since then (Kotlarz et al., 2020; Hopkins et al., 2018; Sun et al., 2016; Zhang et al., 2019). Zhang et al. (2019) provides a snapshot of the presence of novel fluoroethers in the Cape Fear River prior to discharge control (Zhang et al., 2019).

2.4. Receptor modeling

The three multivariate receptor models used in this analysis (UNMIX, PMF, and PCA-MLR) were run for each cohort individually (Thurston and Spengler, 1985; Norris et al., 2007; Norris et al., 2014). The CO serum results were also stratified by water district of residence (PMF only) to evaluate if exposure contribution varied when residential water was sourced further from the primary water contamination source (i.e., AFFF released from Peterson Space Force Base). The model objectives are to identify the number of sources, composition of each source, and exposure contributions for chemical constituents in each sample. For each model, an analyte inclusion requirement of ≥50 % detection (above the limit of detection or LOD) within each cohort was used to ensure accuracy, and ½ the LOD was substituted for PFAS measurements below the LOD as established in similar works (Li et al., 2020; Qi et al., 2017; Xu et al., 2018). Other methods that can be used for censored environmental data are maximum likelihood estimation, survival analysis, and non-parametric approaches (Helsel, 2012). While substitution remains the most common, these approaches can be much more robust, especially when data with <50 % detects is included (US EPA, n.d.-a). With this requirement, 11 PFASs were included for CO, 8 PFASs were included for NC, and 5 PFASs were included for NHANES. PFASs included in the initial models and their abbreviations are displayed

in Table 1. Unmix and PMF both have built in methods to assess the robustness and stability of each model including bootstrapping, rotation, and assessment of influential points. Table 2 compares the assumptions across each model and selection criteria used for determining the most appropriate solution using each method (Reff et al., 2007; Thurston and Spengler, 1985; Norris et al., 2007; Norris et al., 2014). Multiple combinations of compounds and numbers of sources were tested where appropriate, and these criteria were compared across models to select the best model for source characterization.

Table 2
Model assumptions and selection criteria. The model assumptions differ in some places across the models. The selection criteria were used to select the final models from the multiple models that were tested.

Model assumptions	UNMIX[a]	PMF[b]	PCA-MLR[c]
Chemical mass balance	x	x	
Linear correlation			x
Normality			x
Source composition approximately constant	x	x	
Positive contributions	x	x	
Selection criteria			
High model R^2	x	x	x
High compound R^2	x	x	
High S/N	x	x	
User discretion on set of included compounds	x	x	x
Identification and adjustment of influential outliers	x	x	
Runs Fall Within IQR of Bootstraps	x	x	
No significant negative bias	x		
No errors, significant changes in Q, or significant swaps in sources during bootstrap-displacement		x	
No source swaps during bootstrap-displacement		x	
Q_{true}/Q_{exp} close to 1		x	
Close to 100 bootstrap runs for 100 feasible results	x		
Primary goodness-of-fit metric	S/N	Q	R^2

Abbreviations: PMF, positive matrix factorization; PCA-MLR, Principal component analysis-multiple linear regression; S/N, Signal to noise ratio; IQR, interquartile range.
[a] Norris et al., 2007.
[b] Norris et al., 2014.
[c] Thurston and Spengler, 1985.

2.5. UNMIX model

USEPA's UNMIX Model software version 6.0 was employed for this analysis (Henry, 2003; Norris et al., 2007). UNMIX assumes all concentrations are positive and species do not degrade or react with one another thus conserving mass, an appropriate assumption for many PFAAs, such as those presented in Table 1 (Hekster et al., 2003). These assumptions allow for a mass balance calculation using Eq. (1).

$$C_{ij} = \Sigma_{k=1}^n x_{in} f_{nj} + e_{ij} \qquad (1)$$

where C_{ij} is the concentration of the ith species in the jth sample, x_{in} is the ith species concentration from the nth source, f_{nj} is the contribution from the nth source to the jth sample and e_{ij} is the error term (Hopke, 2016; Miller et al., 1972; Henry, 2002; Reff et al., 2007).

Unlike other receptor models (e.g., PMF), UNMIX does not require additional inputs beyond chemical species and concentrations. UNMIX employs singular value decomposition to reduce dimensionality of the data space, normalizes the data, and uses an algorithm to identify "edges" in the data to distinguish sources (Henry, 2003). To determine the best convergent model, UNMIX largely relies on the minimum R^2 and the minimum signal to noise ratio (S/N) to determine results (Henry, 2003).

2.6. PMF model

An in-depth explanation of the PMF algorithm can be found in the PMF 5.0 handbook and Reff et al. (Reff et al., 2007; Norris et al., 2014) The algorithm underlying PMF differs greatly from UNMIX, and criteria for selecting one over the other in the event that the results disagree can be found in a review by Henry and Christensen, where they conclude that Unmix is more appropriate when edges in the data are distinct and PMF functions better when there are many zeros in the loading and score matrices (Henry and Christensen, 2010). Like UNMIX, the PMF model is based on the CMB equation (Eq. 1) (Hopke, 2000). Unlike UNMIX, the number of sources in a PMF solution are user defined. Thus, the user can test solutions with a varying number of sources to determine an appropriate number for optimizing the diagnostic criteria. Another practical difference between PMF and UNMIX is that PMF includes uncertainty estimates for each data point. The uncertainty estimates are used in an equation to identify the value of the parameter Q, a goodness-of-fit parameter:

$$Q = \Sigma_{i=1}^n \Sigma_{j=1}^m \left(\frac{e_{ij}}{\sigma_{ij}} \right)^2 \qquad (2)$$

where, σ_{ij} is the uncertainty of the jth species concentration in sample i, n is the number of samples, and m is the number of species (Reff et al., 2007). Three different Q values are generated: $Q_{expected}$ is equal to (number of non-weak, a user selected label, data values in the data set (X)) -(numbers of elements in the factor contributions (G) and factor profiles (F), taken together), Q_{true} is the goodness-of-fit parameter calculated including all points, and Q_{robust} is the goodness-of-fit parameter calculated excluding points not fit by the model, defined as samples for which the uncertainty-scaled residual is greater than four (Norris et al., 2014). Along with other criteria (Table 2) these calculation can, in part, be used to choose the best model. Depending on the data available, various equations may be used to estimate uncertainties (Reff et al., 2007). Uncertainty = 5/6*LOD is commonly applied and was used in this analysis (Reff et al., 2007; Henry and Christensen, 2010).

2.7. PCA-MLR

A comprehensive explanation of the use of PCA-MLR for source apportionment can be found in Thurston and Spengler (1985). The PCA-MLR model relies on the same underlying principles as the two models described above, however the PCA-MLR model does not rely on the CMB equation and therefore does not impose any positive constraints on the model. For the PCA, the data were normalized via log-transformation The PCA was run with varimax rotation and varimax factors with an eigenvalue >1 were used in the analysis as done in previous works on the subject (Thurston and Spengler, 1985; Hopke, 1982; Pio et al., 1991; Hu et al., 2018). Following identification of factors, MLR was employed where the factors identified in the PCA were modelled as independent variables and the sum of the measured pollutant concentrations was the dependent variable. The regression coefficients from the MLR were then used to determine the exposure contribution, in percent, of each source with the following equation:

$$i \, (\%) = 100 * \left(\frac{Bi}{\Sigma_i^n Bi} \right) \qquad (5)$$

where B_i is the beta coefficient for a given factor (Thurston and Spengler, 1985).

3. Results and discussion

The overarching goal of this study was to quantify source contributions of PFAS exposure in human serum in two highly exposed communities and assess the utility of three different source apportionment methods in human serum across these communities and a broader reference population (NHANES). UNMIX, PMF and PCA-MLR were run on CO and NC serum datasets with results shown in Figs. 1, 2 and 3. For the CO and NC cohorts, the UNMIX and PMF models described three to four major sources, while the PCA-MLR method characterized two sources. Each cohort contained at least one distinct source of contamination by these models, but none of the sources elucidated were similar across the two cohorts. This is expected due to the overall lack of similarity in likely PFAS exposure source across these two cohorts. The NHANES analysis did not result in feasible solutions or solutions that met diagnostic criteria (Table 2) for any of the three models (results not shown).

3.1. CO cohort: results

For the CO dataset, UNMIX and PMF resulted in similar 3-source solutions and PCA-MLR resulted in a 2-source solution (Fig. 1a/b).

PFNA, PFHpA and MeFOSAA were excluded from UNMIX due to high specific variance (variance due to error > 50 %). The final solution had an overall R^2 of 0.87, had a minimum S/N ratio of 3.24 and met all diagnostic fit criteria as shown in Table 2. Source 1 (CO-UNMIX-1) accounted for 57 % of the total mass and had high loadings of sulfonates including PFHxS, PFHpS, and U-PFOS. Source 2 (CO-UNMIX-2) accounted for 10 % of the total mass and had high percent contributions of PFDA and PFUdA. Source 3 (CO-UNMIX-3) accounted for 33 % of the total mass and had high percent contribution of PFPeS.

PMF produced a solution after investigation of 2 through 4-source solutions. PFUdA and PFDA were excluded from the final PMF solution due to a low R^2 for the observed versus predicted estimates. PFNA was flagged as weak due to a low R^2. While the 4-source solution had lower Q parameters, it displayed a high degree of rotational ambiguity (caused by multiple similar solutions being generated when the matrices are rotated) during F-Peak rotation and had comparable R^2 values across species. Therefore, the 3-source solution was used. This solution had a Q_{true}/Q_{exp} of 11.7 and a Q_{Robust} of 4761. More detailed comparisons of how these models compared can be seen in supplementary table 2. The model was selected using the diagnostic criteria and resulted in the following sources: Source 1 (CO-PMF-1) with high percent contributions of sulfonates including PFHxS, PFHpS and U-PFOS, made up 59 % of the total; Source 2 (CO-PMF-2), with a high percent contribution of PFNA made up 27 % of the total; and Source 3 (CO-PMF-3), with a high percent contribution of PFPeS, made up 14 % of the total.

The PCA model was run with all compounds other than PFHpA and MeFOSAA because inclusion resulted in decreased overall R^2 and decreased percent variance explained. Two eigenvalues ≥1 were found in the PCA,
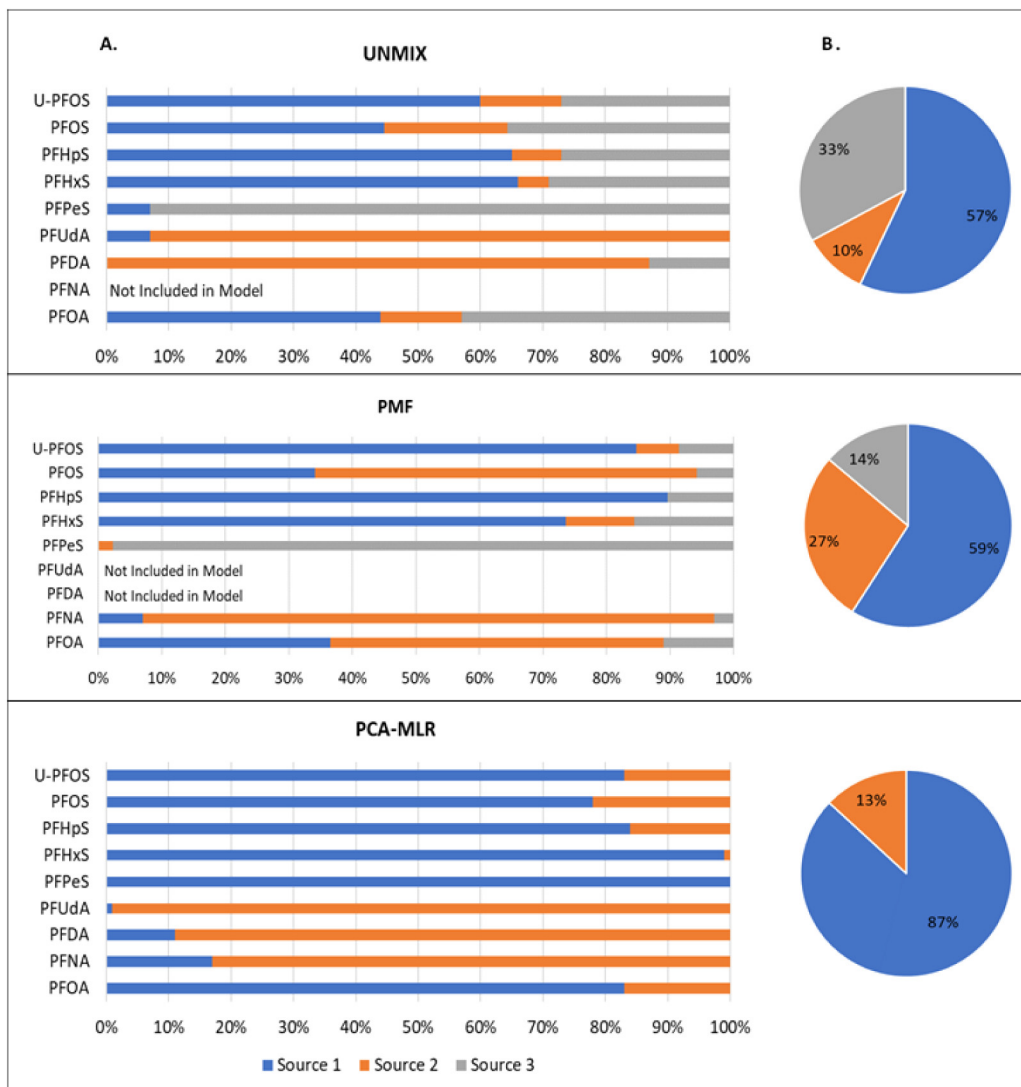
Fig. 1. A (Left Side) Source compositions for the CO cohort from three different models.
Source 1 is blue, source 2 is orange and source 3 is gray. Each bar shows how much of each compound was attributed to each source. B (Right Side) Exposure contributions for the CO from three different models. Source 1 is blue, source 2 is orange and source 3 is gray. The charts represent the amount of total exposure that each source was attributed to.
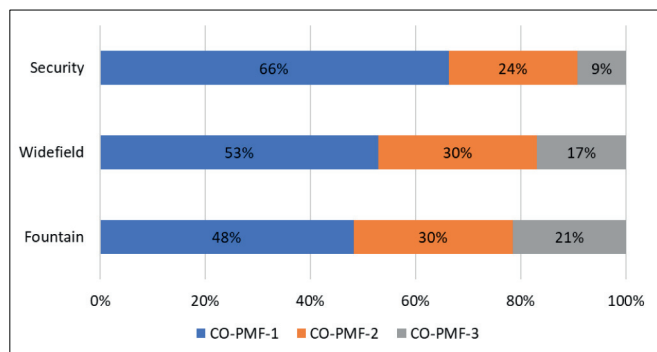

Fig. 2. Exposure contributions by water district of residence for CO cohort, results from PMF model. Source 1 is blue, source 2 is orange and source 3 is gray. Each bar shows how much of total exposure was attributed to each source for each water district.

which explained 76 % of dataset variation. When absolute principal component scores were fit in the MLR, source 1 (CO-PCAMLR-1) contributed 87 % and source 2 (CO-PCAMLR-2) contributed 13 % to the total solution identified by PCA-MLR, with a model R^2 of 0.94. CO-PCAMLR-1 had high percent contributions from the sulfonates and PFOA while CO-PCAMLR-2 had high percent contributions of PFNA, PFDA and PFUdA.

Following evaluation of source apportionment results for the CO cohort, PMF results were stratified by participant's water district of residence due to a clear north to south gradient of PFAS concentrations in the affected area (Barton et al., 2020; McDonough et al., 2021). PMF has the option to include a sampling site with data input so that different locations may be compared. Results indicate decreasing exposure contribution from CO-PMF-1 by water district with increasing distance from the known AFFF release site (Fig. 2). On average, CO-PMF-1 (the sulfonate dominated source) contributed 66 % of the exposure for the serum PFAS in Security water district participants, (i.e., participants living closest to drinking water contamination source); Widefield water district participants averaged 53 % contribution from CO-PMF-1; and Fountain water district participants (furthest from the contamination source) averaged a 48 % contribution from CO-PMF-1. Average exposure contributions from CO-PMF-2, the source with high percent contributions from PFNA, ranged

Fig. 3. A (Left Side) Source compositions for the NC cohort from three different models.
Source 1 is navy blue, source 2 is green, source 3 is yellow and source 4 is pink. Each bar shows how much of each compound was attributed to each source.
B (Right Side) Exposure contributions for the NC cohort from three different models.
Source 1 is navy blue, source 2 is green, source 3 is yellow and source 4 is pink. The charts represent the amount of total exposure that each source was attributed to.

from 24 % in Security to 30 % in Widefield and Fountain. Average exposure contributions from CO-PMF-3, the source dominated by PFPeS, ranged from 9 % in Security to 21 % in Fountain (Fig. 2).

3.2. CO cohort: potential exposure sources

The first exposure source characterized with each modeling approach (CO-UNMIX-1, CO-PMF-1 and CO-PCAMLR-1) had high percent contributions of sulfonates, specifically PFHpS, PFHxS, and U-PFOS for CO-UNMIX-1 and CO-PMF-1. The second source in all models had high percent contributions of longer chain carboxylates: PFDA and PFUdA for CO-UNMIX-1, PFNA in CO-PMF-2, and PFNA, PFDA and PFUdA in CO-PCAMLR-2. CO-UNMIX-3 and CO-PMF-3 had high percent contributions of PFPeS. PFOS and PFOA were not helpful in distinguishing sources despite being elevated in CO serum samples. However, PFOS and PFOA did contribute approximately 80 % of their mass to CO-PCAMLR-1.

Based on what is known about PFAS releases near the CO site, CO-UNMIX-1, CO-PMF-1 and CO-PCAMLR-1, representing 57 %, 59 % and 87 % relative overall source contribution, respectively, suggests that they are the AFFF-contaminated drinking water source (Barton et al., 2020; McDonough et al., 2021). Three important PFASs for this source (AFFF) across models were PFHpS, PFHxS and U-PFOS which were detected in raw drinking water samples taken in 2018 as part of the CO study (McDonough et al., 2021). Further, PFHpS and PFHxS are likely derived from AFFF, and have been found at high concentrations at other AFFF release sites

(Anderson et al., 2016; Backe et al., 2013). PFHxS has been found at elevated concentrations in the serum of firefighters exposed to AFFF (Rotander et al., 2015) and both PFHxS and PFHpS have been found in the serum of residents exposed to AFFF-contaminated drinking water, and in the associated raw water samples, in a Swedish community near a military installation (Xu et al., 2021; Li et al., 2018). While previous research from the CO site is the first to our knowledge to report U-PFOS in human serum (McCord et al., 2018), others have detected U-PFOS in AFFF-contaminated water and products as well as in the serum of mice dosed with AFFF (McDonough et al., 2021; McDonough et al., 2020; Barzen-Hanson et al., 2017).

The supposition that CO-PMF-1 may be identifying the AFFF-contaminated drinking water is further supported by the results of the stratified PMF analysis (Fig. 2). When stratifying by water district of residence, the exposure contribution by CO-PMF-1 decreases monotonically moving from the northernmost water district closest to the known AFFF source (Security) to the water district furthest from the known AFFF source (Fountain). This is consistent with the Barton et al. 2019 findings that water district of residence was a primary predictor of PFAS serum concentrations and McDonough et al. 2021 results showing that untreated water concentrations varied by water district in the same pattern (McDonough et al., 2021).

The second source described by all three models (i.e., CO-UNMIX-2, CO-PMF-2, and CO-PCAMLR-2), with exposure contributions of 10 %, 27 % and 13 %, respectively, had high percent contributions of the longer chain carboxylates, including PFNA, PFDA, and PFUdA. This source is not likely to be associated with drinking water, as these PFASs were either

not detected at all or were detected only at very low concentrations in the CO untreated water samples and concentrations did not vary significantly by water district (McDonough et al., 2021). Further, though only PFNA was included in the Barton et al. analyses, it was not associated with any drinking water-related variables, like many of the other PFASs (Barton et al., 2020). Based on market basket studies (i.e., where representative diets are characterized and daily intakes of contaminants or nutrients are estimated (Egan, n.d.)) conducted in several countries, it is plausible that this source should be attributed to diet (Domingo and Nadal, 2017). Long chain perfluorocarboxylates, such as those identified here, are more likely to accumulate in seafood (Dassuncao et al., 2018; Wang et al., 2019) and dairy products (Macheka et al., 2021) compared to shorter chain PFASs.

A third source characterized by the UNMIX and PMF models (i.e., CO-UNMIX-3 and CO-PMF-3) had relative contributions of 33 % and 14 %, respectively, and high percent contributions of PFPeS. Two potential candidates for this source are: 1) an AFFF-contaminated drinking water source, or 2) an outside exposure, such as consumer product exposure. It is difficult to assign this factor to a specific source as there is very limited literature on the use of PFPeS and it is not routinely measured in human serum. The Australian Department of Health found that PFPeS has been used in electroplating, antireflective coatings, carpet treatments, and is present in AFFF (Susmann et al., 2019). However, none of the sources the report cites specifically tested for PFPeS, rather they tested for PFHpS and PFHxS that are structurally similar but not identical. It is possible that PFPeS is related to the AFFF-contaminated drinking water given PFPeS was found in untreated drinking water samples at the CO Site (McDonough et al., 2021) as well as in the blood of residents exposed to AFFF-contaminated water in Sweden (Xu et al., 2020). Further, the PCA-MLR analysis only separated out two sources, with 100 % of PFPeS allocated to CO-PCAMLR-1 with the other sulfonates known to be derived from AFFF.

One potential issue with this interpretation is the lack of an expected trend for PFPeS (i.e., CO-PMF-3) in the stratified analysis (Fig. 2). Further, as presented in McDonough et al. 2021, in a second year of sampling at the CO site in a subset of the year one participants (N = 53 in year 2 [2019]; N = 213 in year 1 [2018]) there was no significant decline in PFPeS serum concentrations (McDonough et al., 2021). Over this one-year period, which took place after the water systems had mitigated the AFFF-contamination, other PFASs present in the contaminated drinking water (i.e., PFHxS, PFOS, PFOA, PFHpS and UPFOS) did decline significantly (p < 0.05). Given PFPeS is estimated to have a shorter elimination half-life than the other PFASs listed above, it follows that if drinking water was the primary source of PFPeS exposure and the drinking water exposure was remediated prior to the blood testing in 2018, a significant decline in serum concentrations would be expected (Xu et al., 2020). In fact, of 53 CO study participants, 16 (30 %) saw an increase in PFPeS from 2018 to 2019, with an average percent decline of only 11 % (Zhang et al., 2019). This, coupled with the fact that both CO-UNMIX-3 and CO-PMF-3 were similarly influenced by PFPeS, suggests that an additional source of PFPeS related to consumer product use may be present. In 2003, 3 M began using perfluorobutane sulfonic acid (PFBS) as a substitute for PFOS in their Scotchgard formulation which could result in PFPeS impurities in this newer mixture (AECOM, 2019). Indeed, in a 2020 exposure assessment conducted at a Michigan site contaminated by leachate from a landfill consisting of tannery waste contaminated with PFAS-containing Scotchgard, PFPeS was found in the serum of 86 % of participants (MDHHS, 2020). This finding is supportive of the hypothesis that the third source produced by UNMIX and PMF may be linked to a consumer product exposure source.

3.3. NC cohort: results

Results from the NC cohort are presented in Fig. 3. UNMIX and PMF both identified four-source solutions and PCA-MLR identified a two-source solution.

The UNMIX solution included all compounds. While excluding PFOA created a slightly better model solution based on a minimum S/N of 5.03,

it was included in the model due to its importance as a contaminant in the NC cohort; PFOA serum concentrations in Wilmington were significantly higher than the national average (Control, 2019). This solution had a minimum R^2 value of 0.92, a minimum S/N of 2.47, and met all diagnostic criteria (Table 2). Source 1 (NC-UNMIX-1) accounted for 3 % of the total mass and contained a majority of PFHpA. Source 2 (NC-UNMIX-2) accounted for 48 % of the total mass and contained the highest loadings of PFOS and PFNA, as well as high percentages of PFHxS and PFOA. Source 3 (NC-UNMIX-3) accounted for 22 % of the total mass and contained a high percentage of PFO4DA. Source 4 (NC-UNMIX-4) made up 28 % of the total mass and consisted of the highest levels of Nafion byproduct 2, PFO5DoA, PFHxS, and PFOA.

For the PMF analysis, multiple models with two to six sources were fit. PFHpA was flagged as weak due to a low S/N. The model with the highest Q_{robust} contained all compounds. The chosen model had the lowest Q_{true}/Q_{exp} (4.24), a relatively low Q_{Robust} (3478)), no rotational ambiguity and met all selection criteria in Table 2. More detailed comparisons of how these models compared can be seen in supplementary table 2. This model included all PFASs that were measured in ≥50 % of the participants. Source 1 (NC-PMF-1) made up 17 % of the mass and contained high loadings of PFHpA and PFO4DA. Source 2 (NC-PMF-2) made up 36 % of the total mass and contained high loading of PFOS and PFNA. Source 3 (NC-PMF-3) was 22 % of the total mass and contained the highest loadings of Nafion byproduct 2 and PFO5DoA. Source 4 (NC-PMF-4) was 25 % of the total and had high contributions of PFHxS and PFOA.

The PCA-MLR analysis was initially run with all chemicals. Because PCA-MLR does not have a way to control for negative concentration estimates, compounds with the most negative concentrations in each source were removed, until a solution that did not contain negative values was found. There were two eigenvalues greater than one and the final solution explained 86 % of the variance. The MLR had an R^2 of 0.95. The final solution contained two sources and did not include PFO5DoA and Nafion byproduct 2. Source 1 (NC-PCAMLR-1) comprised 34 % of the total mass and had the most PFHpA and PFO4DA. Source 2 (NC-PCAMLR-2) contributed 66 % of the total mass and had a majority of the PFHxS, PFNA, PFOA, and PFOS.

3.4. NC cohort: potential exposure sources

Several exposure sources (NC-UNMIX-3,4; NC-PMF-1,3; NC-PCAMLR-1) contained high percent-contributions of novel fluoroethers, specifically PFO4DA for NC-UNMIX-3 and NC-PMF-1, and Nafion byproduct 2 and PFO5DoA for NC-UNMIX-4 and NC-PMF-3. The model results also all share sources that contain high percentages of PFAAs (NC-UNMIX-1,2; NC-PMF-2,4; NC-PCAMLR-2), specifically PFHpA for NC-UNMIX-1; PFNA and PFOS for NC-UNMIX-2; and PFOA, and PFHxS for NC-PMF-4.

Based on what is known about the NC cohort, NC-UNMIX-3,4; NC-PMF-1,3; NC-PCAMLR-1, representing 22 % and 28 %, 17 % and 22 %, and 34 % exposure contribution, respectively, likely represent contributions from the Fayetteville Works Facility (Kotlarz et al., 2020; McCord and Strynar, 2019; Hopkins et al., 2018). This is consistent with the knowledge that Fayetteville Works is the only known source of the novel fluoroethers in the area (Kotlarz et al., 2020; McCord and Strynar, 2019; Hopkins et al., 2018). A high percentage of PFHpA–a PFAA –is also present in NC-PMF-1 and NC-PCAMLR-1. PFHpA was the dominant legacy PFAA measured in a 2006 wastewater discharge sample from Fayetteville Works (before methods for fluoroethers existed), and it was the second-most prevalent PFAA contributed by Fayetteville Works to the Cape Fear River based on samples collected in 2014 upstream and downstream of the facility (Sun et al., 2016; Strynar et al., 2015; Nakayama et al., 2007).

NC-UNMIX-1,2; NC-PMF-2,4; and NC-PCAMLR-2, representing 3 % and 48 %, 36 % and 25 %, and 66 % exposure contribution, respectively, all represent contributions from PFAAs. These PFAAs have been identified in the Cape Fear River and as contaminants of New Hanover County drinking water (Kotlarz et al., 2020; McCord and Strynar, 2019; Zhang et al., 2019; Wagner and Buckland, 2017). These model sources represent unidentified

drinking water sources of PFAAs that may be separated spatially or temporally from the exposures stemming from Fayetteville Works. PFAAs have been identified upstream of the Fayetteville Works facility, and analyses over time show these shifting from longer to shorter chain PFASs (Sun et al., 2016; Strynar et al., 2015; Nakayama et al., 2007).

PFHxS and PFNA were spread evenly across multiple exposure sources–NC-UNMIX-2,4 and NC-PMF-2,3,4–respectively. PFHxS had the lowest R^2 in the UNMIX results (0.72) which indicates a larger problem in the model's ability to characterize PFHxS exposure. PFNA, while primarily apportioned into NC-PMF-2,3,4, only contributed a small percentage to those sources–6 %, 5 %, and 6 % respectively–and the total contamination. PFNA is known to be present in food (such as fish) as discussed earlier (Domingo and Nadal, 2017; Dassuncao et al., 2018; Wang et al., 2019; Macheka et al., 2021; Susmann et al., 2019), and these models may have trouble disentangling background sources of contamination (i.e. sources that are not specific to those in highly exposed communities, such as non-local diet, that many people are likely exposed to at low levels), as evidenced by the lack of convergent results from the NHANES data. While Colorado has a very distinct and strong contamination source in the drinking water (AFFF), North Carolina may have multiple sources of water contamination, including several industrial wastewater discharges, AFFF, and runoff from fields, to which impacted biosolids are land-applied. Therefore, it is easier to determine potential additional sources beyond drinking water in the Colorado cohort.

3.5. Strengths

The major strength of this analysis is that all three receptor models found feasible solutions for PFAS sources in serum from two unique PFAS-exposed populations, the CO and NC cohorts, both of which have relatively small sample sizes (n < 350). Further, UNMIX and PMF produced fairly similar results within each cohort improving confidence in conclusions. The sources that were ascertained in each cohort are supported by the known contamination in each community and are corroborated by samples taken by other researchers of the contaminated water sources (Kotlarz et al., 2020; McCord and Strynar, 2019; Hopkins et al., 2018; Sun et al., 2016; Strynar et al., 2015; Nakayama et al., 2007).

This analysis allows for a more refined understanding than simple evaluation of inter-PFAS correlations. Because these models rely in part on the correlation between the concentrations of the chemicals, we explored how the receptor models improved on the interpretation of a Spearman's correlation (Supplementary Figs. 2–4). Spearman's correlations indicate general groupings of PFASs, but do not estimate the magnitude of individual or multiple PFAS contributions to potential sources. For example, in the CO dataset, it would be difficult to discern from Spearman's correlations anything beyond the already suspected fact that many of the sulfonates are associated with a common source. The analysis does not give an indication that PFPeS may be behaving differently or the degree to which PFOS and PFOA may be contributing to other sources beyond AFFF-contaminated drinking water. These results provide confidence that these models could be used in the future to help investigate sources of exposure using biological as well as environmental samples.

3.6. Limitations

Along with the strengths defined above these models also have limitations. Though successful at determining solutions in the CO and NC cohorts, the models did not result in informative or feasible solutions with a larger sample size (n = 1993) in the NHANES 2015 to 2016 dataset. The CO and NC datasets both included larger arrays of PFASs (11 and 8, respectively) than the NHANES dataset with only 5 PFASs. In this case, these receptor models may be limited by the number of chemicals, and they may only be effective for populations in specific regions that are highly exposed to distinct (and common) exposure sources, which the NHANES cohort were not (supplementary fig. 1). This was tested by running these models with the same 5 PFASs using the samples from the CO and NC

cohorts. These tests did not return feasible or informative results and resembled the results from the NHANES data (supplementary data 1). Because these models rely on the separation of compounds using different algorithms, a larger number of compounds may help in clustering the samples into different sources. This is especially true for UNMIX, which identifies sources by finding "edges" wherein a compound is not present in a specific source. Including more compounds increases the opportunities for edges in the model to arise.

In addition, the models had trouble effectively partitioning compounds that result from many exposure sources (such as PFOA and PFOS). Although PFOA was found in higher concentrations compared to the general population for both CO and NC (Supplemental Fig. 1), it likely originates from several sources. PFOA has been used in a variety of products for many years, making it difficult to identify its sources, especially in comparison to the novel fluoroethers observed in NC and the sulfonates associated with AFFF in CO.

A minor limitation worth noting is that in both cohorts, PCA-MLR found fewer sources than the other two models. Other studies that evaluated these three receptor methods have found that PCA-MLR is not always able to disentangle as many sources as the other two methods and may not be as effective at pulling out correlated sources (Qi et al., 2017; Qi et al., 2016; Zhang et al., 2012). This may be due to the lack of negative constraints, diagnostic criteria for model improvement, and/or bootstrapping. Conversely, UNMIX and PMF appear to be very sensitive to exposure contributions and may separate single sources into multiple sources.

It is important to be mindful that these receptor models were designed for use with environmental media, not a biological matrix like human serum. A key assumption of these models is that the contaminants do not degrade or react with one another. In the human body, some PFASs are excreted more quickly than others, may be absorbed at different rates or distributed differentially, and these models may not be distinguishing the exposure sources if toxicokinetic parameters vary drastically across individuals or different groups. These results represent a specific cross-section of time, which makes accounting for toxicokinetic differences challenging. To remedy this problem, Hu et al. separated participants by covariates that could affect toxicokinetics such as sex and age (Hu et al., 2018). When the participants in our study were separated into three groups–men, women, and children (North Carolina only)–the resultant models did not fit the selection criteria, were similar to the full models, or did not produce feasible results (Supplemental data 1). These results imply that these models, as is, may be unable to account for toxicokinetic differences between subpopulations. This could be due to a need for a larger sample size that makes up for the diminished power when separating the population into groups to account demographics that may affect toxicokinetics such as age and sex. There is also currently no way built into the model to account for individual toxicokinetic differences (Agency for Toxic Substances and Disease Registry (ATSDR), 2021; Norris et al., 2007; Norris et al., 2014). This problem may be ameliorated by modifications to the models that allow for covariates to account for such differences. The full models on the other hand produced feasible, interpretable results that met all selection criteria. This is probably not as major a limitation to this method as it may seem. The way PFASs, like all compounds, behave in the environment, as they do in the body, is also governed by a complicated suite of interacting variables (Agency for Toxic Substances and Disease Registry (ATSDR), 2021; *5 Environmental Fate and Transport Processes – PFAS — Per- and Polyfluoroalkyl Substances*, n.d.). The fate and transport of a compound after leaving a source can be affected by physical and chemical properties of the molecule itself, soil types, precipitation, etc. (US EPA, n.d.-b) These methods are agnostic to any variable that might affect the fate of a compound, whether it is in the environment or in the human body.

3.7. Implications and future research

The source apportionment models evaluated performed better in areas with distinct exposure sources and may not be useful in examining broader trends in the U.S. population. This is likely due to higher measured

concentrations and/or greater relative variation between PFASs in highly exposed populations. For the CO and NC cohorts, neither UNMIX nor PMF separated PFOA into separate sources effectively, possibly because PFOA exposure originates from multiple relatively small and/or overlapping sources.

This work found that UNMIX and PMF were able to extract three to four potential sources of PFAS exposure for both cohorts, while the PCA-MLR method identified two sources for each cohort. While PCA-MLR appeared to perform adequately, UNMIX and PMF were more successful at characterizing specific source groups in communities exposed to high concentrations of PFASs originating from specific contamination events. All three methods were unsuccessful in identifying specific exposure sources when the models were run with a dataset expected to be representative of background PFAS exposures in the U.S. (NHANES).

Though these three models can provide an idea of potential sources of PFAS exposure, they are limited in that they require educated judgment based on existing knowledge to identify sources. A potential preferred method of PFAS source apportionment would be CMB, however, for PFASs, CMB would require regionally specific source profiles for drinking water, dust, indoor air, diet, and consumer products. The collection and analysis of such samples would be time and resource intensive, and is often not feasible, especially in situations where investigators arrive after the exposure has taken place and the profiles may have changed since the initial exposure.

The results of UNMIX and PMF in both cohorts were similar enough that it was concluded that both models worked equally well. While UNMIX and PMF produce similar results, another consideration is usability. UNMIX's reduced requirement for user input leads to results that are easier for decision-makers to interpret. While UNMIX requires less user input, making it more usable and easier to interpret, it provides less control over the model and fewer ways to validate each iteration of the model than PMF. PMF contains a similar but much more robust method to compare different parameters in each model (including bootstrapping and rotation of these models) that is not present in UNMIX, while also letting you select the number of sources and select which species are weighted less in the model without removing them. This makes PMF more broadly useful and more adjustable to each specific situation. Overall, these models are probably most useful if used in tandem, as differences may reveal information about the exposure that would not be ascertained when using either separately. A more in depth look at the differences between these models can be found in Table 2, in their respective manuals, and across several publications (Hopke, 2016; Henry, 2002; Paatero and Tapper, 1994; Henry, 2003; Norris et al., 2007; Norris et al., 2014; Hopke, 2000).

Given the long half-life of many PFAAs (Li et al., 2018; U.S. Environmental Protection Agency, n.d.) and the relative lack of transformation or metabolism of PFAAs in the human body, these methods should be considered viable options for source apportionment of serum PFASs in populations that are not exposed to significant amounts of metabolically labile precursors. For PFASs with longer half-lives, serum concentration may be thought of as an integrated measure of exposure that represents both historical and current exposure, thus these methods may be able to disentangle past sources. It is important to keep in mind that PFASs that are quickly eliminated from the body (e.g., a short-chain PFAS like PFBS) often are not present in serum samples and would be excluded from source characterization. For these homologues, it is worth considering using urine and environmental media in parallel to serum for evaluation of other potential sources of PFAS exposure (Fuji et al., 2015; Olsen et al., 2009).

This work provides insight into the utility of applying models designed for environmental media for source apportionment of human serum. The approach could be applied in many epidemiological studies where data on environmental sources is lacking but biological samples are available. Understanding PFAS exposure contributions and how source contributions vary based on the specific exposure scenarios and profiles is essential to develop policies that are appropriately protective of public health, inform toxicity testing by identifying mixtures to test, and guide mitigation efforts by identifying the largest and most common contamination sources.

Abbreviations

PFAS	per and polyfluoroalkyl substances
NC	North Carolina
CO	Colorado
USEPA	United States Environmental Protection Agency
PMF	positive matrix factorization
PCA-MLR	principal component analysis-multiple linear regression; aqueous film forming foams
AFFF	perfluoroalkyl acid
PFAA	Perfluoro-n-heptanoic acid
PFHpA	Perfluorooctanoic acid
PFOA	Perfluorononanoic acid
PFNA	Perfluorodecanoic acid
PFDA	Perfluoroundecanoic acid
PFUdA	Perfluoropentanesulfonic acid
PFPeS	Perfluorohexanesulfonic acid
PFHxS	Perfluoroheptanesulfonic acid
PFHpS	Unsaturated perfluorooctanesulfonic acid
U-PFOS	Perfluorooctanesulfonic acid
PFOS	2-(N-Methylperfluorooctanesulfonamido)acetic acid
MeFOSAA	Perfluoro-2-{[perfluoro-3-(perfluoroethoxy)-2-propanyl]oxy} ethanesulfonic acid
Nafionbp2	Perfluoro-3,5,7,9-tetraoxadecanoic acid
PFO4DA	Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid
PFO5DoA	Nafion byproduct 2, Nafionbp2
S/N	signal to noise ratio

CRediT authorship contribution statement

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. DJW and KEB contributed equally.

Data availability

Data will be made available on request.

Declaration of competing interest

Acknowledgment

not necessarily reflect those of the Agency. EPA does not endorse any products or commercial services mentioned in this publication.

Appendix A. Supplementary data

Selected baseline characteristics of the CO and NC study populations; Model assumptions and selection criteria; Comparison of PFAS Concentration between the CO cohort, the NC cohort and the 2015–2016 NHANES cohort (U.S.); Spearman's Correlation Structures for CO, NC and NHANES datasets (PDF). Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2022.158842.

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