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Historical Blood Serum Samples from Wilmington, North Carolina: The Importance of Ultrashort-Chain Per- and Polyfluoroalkyl Substances

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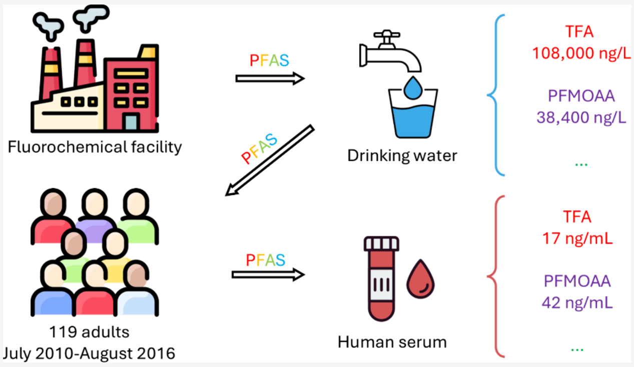

ABSTRACT: From 1980 to 2017, Wilmington, North Carolina (NC), residents unknowingly consumed high levels of PFAS through drinking water. Some of these PFAS are expected to have short biological half-lives, and current biological sampling will miss these exposures. To assess historical PFAS exposures, we (1) determined PFAS levels in serum samples of Wilmington, NC residents obtained during the years 2010–2016, (2) examined temporal trends of serum PFAS over this period, and (3) associated serum PFAS levels with PFAS concentrations in drinking water. We quantified 56 PFAS, including ultrashort-chain PFAS, in 119 adult human serum samples and 47 PFAS in water samples from 2017. Twenty PFAS were detected in >50% of serum samples; perfluoromethoxyacetic acid (PFMOAA) and trifluoroacetic acid (TFA) exhibited the highest median concentrations (PFMOAA: 42 ng/mL; TFA: 17 ng/mL). PFMOAA and TFA were also the dominant PFAS in Wilmington drinking water, accounting for 95% of quantifiable PFAS. The serum-to-water ratio was linearly correlated with "effective" PFAS chain length, indicating that PFAS with longer chain length and a sulfonic acid group are more bioaccumulative. While chain length is important for bioaccumulation, this study highlights that exposures to high concentrations of ultrashort-chain PFAS in drinking water contribute to their dominance in human serum.

KEYWORDS: *perfluoroalkyl acids (PFAAs), per- and polyfluoroalkyl ether acids (PFEAs), hexafluoropropylene-dimer acid (HFPO–DA, GenX), biomarkers of exposure, fluoropolymer manufacturing byproducts*

■ INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) form a large class of synthetic organic chemicals. Many PFAS are persistent, bioaccumulative, and mobile, and adverse health effects are associated with exposure to a number of well-studied PFAS.[1−4] PFAS are ubiquitous in environmental and biological matrices from across the globe,[5−7] and their concentrations are elevated near fluorochemical manufacturing facilities.[8−12]

Since the early 2000s, many countries and companies started the phase-out of long-chain perfluoroalkyl acids (PFAAs) (perfluoroalkyl carboxylic acids (PFCAs) with ≥7 perfluorinated carbons and perfluoroalkyl sulfonic acids (PFSAs) with ≥6 perfluorinated carbons) and their derivatives. The demand generated by the phase-out has been met by large-scale production of alternative PFAS, leading to the presence of per- and polyfluoroalkyl ether acids (PFEAs), short-chain PFAAs (PFCAs with 3–6 perfluorinated carbons and PFSAs with 4 to 5 perfluorinated carbons), and ultrashort-chain PFAAs (PFCAs with ≤2 perfluorinated carbons and PFSAs with ≤3 perfluorinated carbons) in drinking water near and downstream of fluorochemical facilities in the United States,[9,13] the Netherlands,[12,14] China,[15,16] and Germany.[17] Apart from fluorochemical manufacturing, sources of PFAS introduction into the environment include facilities that incorporate PFAS into consumer products (e.g., textiles) or use PFAS for manufacturing (e.g., semiconductor manufacturing, chrome plating).[5] Furthermore, PFAS releases occur during the use of PFAS-containing products, including the use of firefighting foams, as well as during the management of PFAS-containing waste (e.g., landfilling, incineration).[5] Atmospheric decomposition of refrigerants, such as hydrofluoroolefins, followed by subsequent wet and dry deposition, is an increasing source of trifluoroacetic acid (TFA).[5]

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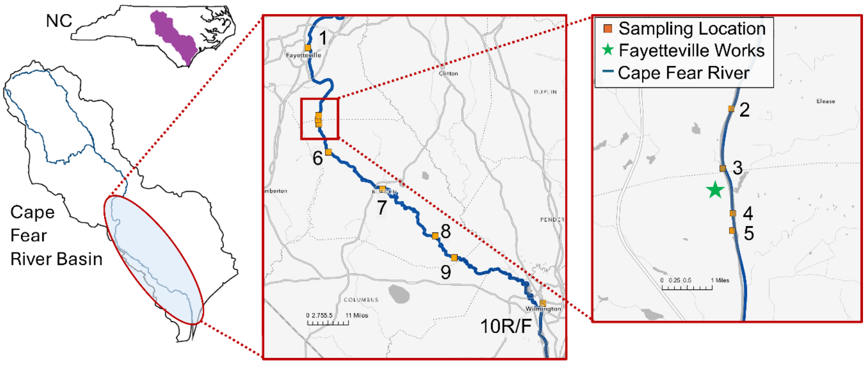


Figure 1. Water sampling sites along the Cape Fear River, NC, USA. The Wilmington drinking water treatment plant is at location 10R/F.

Ultrashort-chain PFAAs (e.g., TFA) and PFEAs [e.g., perfluoro-2-methoxyacetic acid (PFMOAA)] have been largely overlooked in previous studies because they are (1) not targeted by standard analytical methods and (2) thought of as being of lower concern[18] because shorter-chain PFAS typically exhibit shorter half-lives in the human body and thus lower bioaccumulation potential compared to long-chain PFAS.[19] With the development of analytical methods targeting ultrashort-chain PFAS, these previously overlooked compounds have been included in a few recent studies, where they were found to be the dominant PFAS in various environmental matrices as well as in human blood serum; as a result, they are now drawing increasing interest from government regulators, researchers, and residents of PFAS-impacted communities.[8,20,21]

In North Carolina (NC), US, a fluorochemical manufacturing facility (Fayetteville Works) located on the bank of the Cape Fear River (CFR) released PFAS through wastewater discharge and air emissions since 1980.[8] The wastewater discharge led to high levels of PFEAs in the CFR and in finished drinking water of over 200,000 residents living downstream of the facility in Wilmington, NC.[8−10] Many residents in Wilmington first became aware of PFEAs in their drinking water in early June 2017 after an article about the contamination was published in the Wilmington Star News.[22] In June 2017, the fluorochemical manufacturer agreed to capture process wastewater containing PFEAs,[23] after which the sum concentration of six PFEAs at a drinking water intake ~80 km downstream of the facility decreased rapidly.[8]

Characterizing human exposure to low-molecular-weight PFAS is challenging because of their short biological half-lives; when exposure is discovered, people make changes rapidly to lower exposure, making it difficult to capture biomarker levels, such as serum concentrations. One of the PFEAs discharged by the facility is hexafluoropropylene oxide−dimer acid (HFPO−DA, its ammonium salt is commonly known as "GenX"), which was detected at concentrations up to 4500 ng/L at the Wilmington drinking water intake in 2013.[9] When Kotlarz et al.[24] collected and analyzed 344 serum samples from Wilmington residents in November 2017 and May 2018, HFPO−DA was not detected, likely because HFPO−DA levels in drinking water dropped rapidly starting in mid-June of 2017 when discharge controls were implemented and/or many residents stopped drinking tap water or installed home filters after becoming aware of the drinking water contamination. The half-life of HFPO−DA in the human body is short (~81 h),[25] and HFPO−DA levels in the residents' serum likely dropped below the method reporting limit (MRL) by the time samples had been collected. Kotlarz et al.[24] lacked an analytical method to quantify PFMOAA levels in their serum samples. Understanding serum PFMOAA levels in Wilmington residents is important because 110,000 ng/L PFMOAA was measured in a 2015 CFR water sample collected at the Wilmington drinking water intake,[26] and the PFMOAA body burden of people drinking water with such high PFMOAA concentrations has not been assessed to date. Furthermore, information about TFA levels in water and serum samples of Wilmington residents has been lacking.

The principal aim of this study was to comprehensively characterize PFAS levels in samples of human blood serum collected in 2010−2016 from people living in Wilmington, NC; i.e., prior to the point in time when people became aware of the PFAS contamination; this information is critical to understanding past exposure. In this study, we quantified 56 PFAS, including 15 PFCAs with 1−17 fluorinated carbons, 10 PFSAs with 1−10 fluorinated carbons, 14 PFEAs, 2 diprotic acids, and 15 PFAA precursors. Using data for the 2010−2016 serum samples, temporal trends were investigated to assess how serum PFAS levels changed while Fayetteville Works was discharging high levels of PFAS into the CFR. For a subset of PFAS, results were also compared with previous studies[24,27] that reported results for serum samples collected 6−11 months after people had become aware of the PFAS contamination. Additionally, we measured PFAS concentrations in water samples that were collected in May 2017 from 10 locations along the CFR, including locations upstream and downstream of Fayetteville Works and Wilmington drinking water, and associated the drinking water PFAS levels with PFAS levels in human serum samples.

MATERIALS AND METHODS

Sample Information. Serum was obtained from 119 adults residing in Wilmington, NC (19 zip codes -28401, 28402, 28403, 28404, 28405, 28406, 28407, 28408, 28409, 28410, 28411, 28412, 28428, 28429, 28443, 28449, 28451, 28479, 28480) who contributed blood samples from July 2010 to August 2016 as part of the UNC Cancer Survivorship Cohort.[28] The participants were excluded from the cohort because they did not have a history of cancer; samples from these individuals remain part of the cohort biobank.[29] Information on patient use of pharmaceuticals containing fluorinated moieties was not available for our analysis; however, we do not have reason to believe that patients in this sample were more likely than the general population to use these agents. Serum samples were stored at −80 °C until 3 months prior to analysis, when they were moved to a −20 °C freezer.

CFR water samples from sites located upstream (3 sites, locations 1−3) and downstream (7 sites, locations 4−10) of Fayetteville Works were collected in May 2017 (Figure 1). Location 9 is the drinking water intake on the CFR for a drinking water treatment plant that serves Wilmington, NC (Location 10). At Location 10, both raw (Location 10R) and finished (Location 10F) water samples were collected. Water samples were collected in 250 or 500 mL high-density polyethylene (HDPE) bottles and archived at 4 °C until analysis in early 2025. We considered the possible conversion of precursor compounds to PFAAs, including ultrashort-chain PFAAs, but ruled out that such transformations were important based on the following lines of evidence: (1) as shown in Figure S5, TFA levels in water samples upstream of Fayetteville Works were orders of magnitude lower than in downstream water samples, ruling out substantial TFA precursor presence as a result of wastewater discharges upstream of Fayetteville Works and (2) total oxidizable precursor assay results suggested that precursor levels in wastewater from Fayetteville Works and in CFR samples impacted by Fayetteville Works wastewater were low.[26,30]

Analytical Standards. Names, abbreviations, CAS registry numbers, and sources of native PFAS standards and mass-labeled internal standards for quantifying PFAS in serum and water are summarized in Tables S1 and S2.

Analysis of PFAS in Serum. Fifty-six PFAS in six subclasses were quantified in serum (Table S1). Eleven ultrashort-chain and short-chain PFAS were analyzed in the Department of Civil, Construction, and Environmental Engineering at NC State University using a liquid chromatography and tandem mass spectrometry (LC-MS/MS) (1290 LC, 6495 triple quadrupole MS, Agilent Technologies, Santa Clara, CA) system equipped with a 50× 2.1 mm LC Column (Raptor Polar X, 2.7 μm, RESTEK, Bellefonte, PA) or a 3.0× 50 mm high-performance liquid chromatography (HPLC) column (InfinityLab Poroshell 120 EC-C18, 2.7 μm, Agilent Technologies, Santa Clara, CA). Acquisition methods for the 11 shorter-chain PFAS are summarized in Tables S3−S6. MRLs and results for quality control samples, including matrix spikes, are summarized in Tables S7−S11. The sample preparation method is provided in Text S1. Matrix interferences can lead to over-reporting of ultrashort- and short-chain PFAS concentrations in biological samples.[31,32] To minimize the likelihood of reporting false-positive results, we (1) analyzed blank samples prepared with calf serum, (2) analyzed human serum standard reference material (SRM) 1957 from the National Institute of Standards and Technology (NIST), and (3) for PFMOAA and perfluoropropanoic acid (PFPrA), confirmed their presence by high-resolution mass spectrometry (HRMS). TFA could not be confirmed by HRMS because the analytical column on the HRMS system (see the next paragraph) did not retain it. Calf serum blanks did not contain detectable levels of the targeted ultrashort- and short-chain PFAS. Peaks corresponding to some of the targeted ultrashort- and short-chain PFAS were observed in the SRM sample, but responses were below or near the MRL (Text S2). The presence of PFMOAA and PFPrA was confirmed by HRMS via the following lines of evidence: (1) retention time matching, (2) exact precursor mass matching the theoretical mass of the $[M\text{-}H]^-$ ion, (3) presence and ratio matches for $[M\text{-}H]^-$ and $[M+1\text{-}H]^-$ isotopes, and (4) exact mass product ion matching to an analytical standard (Text S2).

The remaining 45 longer-chain PFAS were analyzed in the Molecular Education, Technology and Research Innovation Center (METRIC) at NC State University using a liquid chromatography and HRMS (LC-HRMS) (Vanquish LC system coupled to an Orbitrap Exploris 240 [ThermoFisher Scientific]) system equipped with a Kinetex F5 100 Å analytical column (2.1 100 mm, Phenomenex, Torrance, CA).[33] The acquisition methods for analyzing the 45 longer-chain PFAS are summarized in Tables S12 and S13, and MRLs are listed in Table S14. The sample preparation method is provided in Text S3.

Analysis of PFAS in Water. PFAS concentrations in water were quantified using an LC-MS/MS system (1290 LC, 6495 triple quadrupole MS, Agilent Technologies, Santa Clara, CA). Each water sample was analyzed using four analytical methods that were optimized for the targeted PFAS. Detailed sample preparation methods can be found in Text S4. Acquisition methods are summarized in Tables S15−S18. MRLs and results for quality control samples are summarized in Table S19.

Statistical Methods. Statistical tests were conducted using R.[34] Average values were used for samples analyzed in duplicate. In the temporal trend scatter plots for compounds with detection frequencies (defined here as percentage of samples with PFAS concentration > MRL) > 50%, a fill value of the MRL divided by the square root of 2 was assigned to samples with PFAS concentrations < MRL.[35,36] We evaluated temporal trends using Spearman's rank correlation coefficients. *P*-values <0.05 were considered statistically significant. Correlations among PFAS serum concentrations were determined using Spearman's rank correlation coefficients and visualized using a heatmap with hierarchical clusters. Coefficients of ⩾ 0.70 were considered highly correlated. To calculate summed PFAS concentrations in serum samples, we summed the median concentrations for PFAS with >50% detection frequencies. Serum-to-water enrichment ratios (R_{SW}) were calculated by dividing the median PFAS concentration in serum samples by the corresponding PFAS concentration in finished drinking water.

■ RESULTS

Study Population. We obtained 119 archived serum samples from participants enrolled in the UNC Cancer Survivorship Cohort, collected between July 2010 and August 2016, with the majority (83%) collected in 2012−2015. Demographic characteristics of the 119 adults who contributed serum samples are summarized in Table 1. Samples were obtained from people ranging in age from 18 to 81 years, with the majority being male (61%) and White (88%).

PFAS in Serum. Among the 56 PFAS analyzed in the serum samples, 34 were detected in at least one serum sample. Ten [PFPrA, perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS), perfluorooctanesulfonic acid (PFOS), and PFMOAA] were detected in nearly all serum samples (≥98% detection frequency). Five PFAS [perfluoroheptanoic acid (PFHpA), perfluoro-3,5,7,9-butaoxadecanoic acid (PFO4DA), perfluoro-3,5,7,9,11-pentaoxadodecanoic acid (PFO5DoA), 5-(1,2,2,2-tetrafluoro)ethoxy-perfluoro-3-oxa-4-

Table 1. Demographic Characteristics of the 119 Adults Who Contributed Serum Samples, Wilmington, NC, 2010–2016

characteristic	number	percentage
age group (years)		%
18–49	12	10
50–59	24	20
60–69	21	18
70–81	17	14
missing value	45	38
gender		
female	47	39
male	72	61
education level		
some high school, high school graduate, or GED certificate	28	24
some college or technical school	26	22
college graduate (bachelor's degree)	36	30
postgraduate or professional degree	25	21
missing value	<11	<9
year of sample collection		
2010[a]	<11	<9
2011	<11	<9
2012	32	27
2013	33	28
2014	16	13
2015	18	15
2016	11	9

[a] Cell sizes <11 are suppressed to protect patient confidentiality.

methylpentanesulfonic acid (Nafion Byproduct 2), and N-methylperfluorooctanesulfonamido-acetic acid (NMeFOSAA)] were detected in 90–97.9% of serum samples (Table 2). Another five PFAS, including TFA, were found in 50–89.9% of serum samples (Table 2), and 14 were detected in <50% of the serum samples (Table S20).

The sum of median PFAS concentrations in serum was 99.5 ng/mL, with five PFAS accounting for 85% of the summed total. Among them, PFMOAA had the highest median concentration (42 ng/mL), comprising 42% of the summed total, followed by TFA (17 ng/mL), PFOS (14 ng/mL), PFOA (6.2 ng/mL), and PFPrA (5.4 ng/mL) (Figure 2). HFPO–DA was detected in only 20% of the serum samples with concentrations up to 15 ng/mL.

PFAS in Water. Concentrations of 47 PFAS were measured in water samples collected from 10 sites along the CFR, both upstream (locations 1–3) and downstream (locations 4–10) of the Fayetteville Works fluorochemical plant (Table S21). Twenty-eight PFAS were detected in at least one location. Concentrations of 19 of the 28 PFAS increased significantly (p-value <0.05) between locations 3 and 4 that bracketed the wastewater treatment plant effluent discharge point of the fluorochemical facility (Figure S5). Concentrations of the remaining nine PFAS, including the frequently studied PFOA, PFOS, and PFHxS, exhibited no significant change between locations 3 and 4 (Figure S6). The two largest concentration increases immediately downstream of the facility were observed for two ultrashort-chain PFAS, TFA, and PFMOAA, over 6,000,000 and 1,200,000 ng/L, respectively (Figure S5). Notably high increases were also observed for PFPrA (increased 109,000 ng/L), perfluoro-3,5,7-trioxaoctanoic acid

Table 2. Concentrations of PFAS Detected in More Than 50% of the 119 Serum Samples from Adults in Wilmington, NC, between 2010 and 2016, Organized by Subclass and Chain Length

			concentration (ng/mL)					
compound	MRL (ng/mL)	samples with PFAS conc. > MRL (%)	10th percentile	25th percentile	50th percentile	75th percentile	95th percentile	max
PFCAs								
TFA	10	77%	*	10	17	39	93	590
PFPrA	0.1	99%	0.73	2.2	5.4	16	38	47
PFBA	0.1	56%	*	*	0.12	0.21	0.32	10
PFHpA	0.05	97%	0.090	0.13	0.28	0.45	1.1	1.9
PFOA	0.05	100%	2.1	3.9	6.2	9.4	18	27
PFNA	0.05	100%	0.74	1.2	2.2	3.3	5.5	8.2
PFDA	0.05	100%	0.17	0.28	0.55	0.93	1.6	2.0
PFUnDA	0.05	98%	0.099	0.16	0.27	0.44	1.0	1.5
PFDoDA	0.05	51%	*	*	0.051	0.074	0.16	0.24
PFTrDA	0.05	78%	*	0.052	0.061	0.088	0.15	0.20
PFSAs								
PFBS	0.1	100%	0.21	0.26	0.35	0.47	0.65	0.96
PFHxS	0.05	100%	1.1	2.2	3.6	5.6	11	21
PFHpS	0.05	98%	0.19	0.40	0.67	1.0	1.7	2.3
PFOS	0.5	99%	4.9	8.8	14	21	37	49
PFEAs								
PFMOAA	0.1	99%	6.0	14	42	96	190	280
PFO3OA	0.5	74%	*	*	1.1	2.3	6.8	9.8
PFO4DA	0.5	90%	0.43	0.87	2.0	4.5	8.9	19
PFO5DoA	0.5	91%	0.54	1.3	2.6	6.4	15	21
Nafion byproduct 2	0.1	92%	0.11	0.20	0.74	1.5	4.4	8.3
PFAA precursor								
NMeFOSAA	0.1	93%	0.12	0.14	0.21	0.34	0.96	2.1

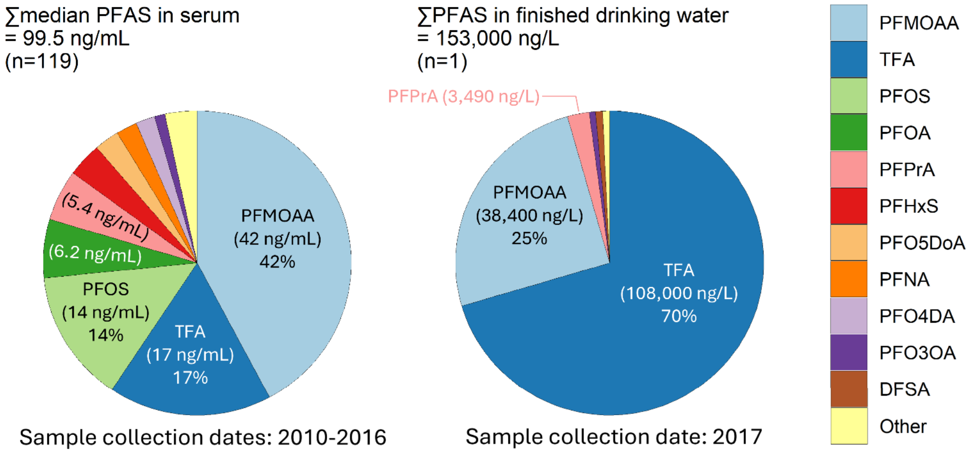
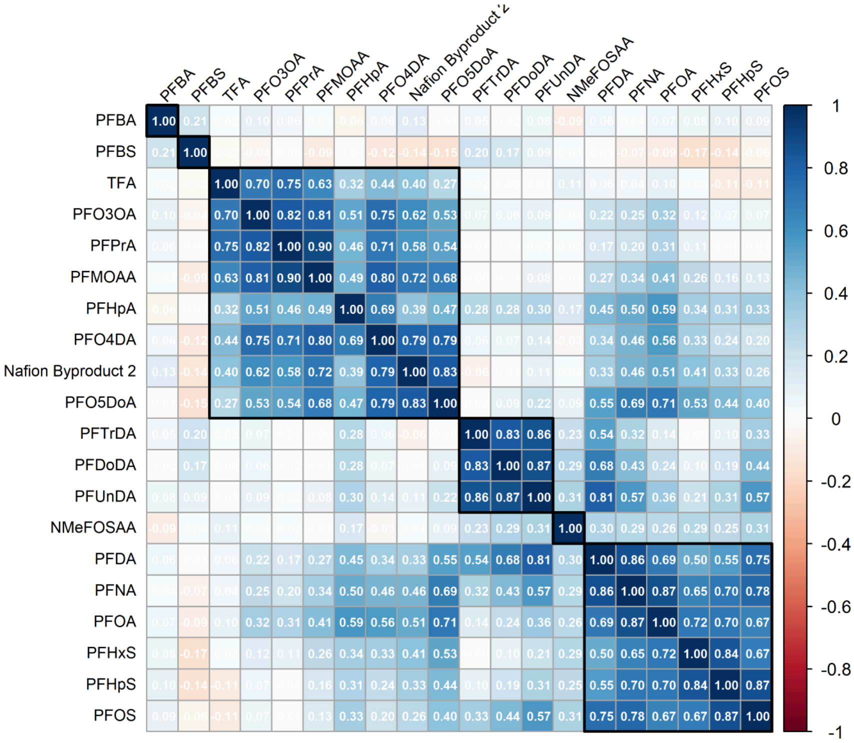


Figure 2. Percentage of PFAS concentrations (median) in serum samples (left). "Other" includes PFAS with individual contributions <1%. Percentage of PFAS concentrations in finished drinking water for Wilmington collected from Location 10F (right). "Other" includes PFAS with individual contributions <0.5%.



	PFBA	PFBS	TFA	PFO3OA	PFPrA	PFMOAA	PFHpA	PFO4DA	Nafion Byproduct 2	PFO5DoA	PFTrDA	PFDoDA	PFUnDA	NMeFOSAA	PFDA	PFNA	PFOA	PFHxS	PFHpS	PFOS
PFBA	1.00	0.21	0.02	0.10	0.06	0.03	-0.06	0.06	0.13	0.01	0.05	-0.02	0.08	-0.09	0.06	0.04	0.07	0.08	0.10	0.09
PFBS	0.21	1.00	0.02	-0.04	0.00	-0.09	-0.01	-0.12	-0.14	-0.15	0.20	0.17	0.09	0.04	-0.01	-0.07	-0.09	-0.17	-0.14	-0.06
TFA	0.02	-0.02	1.00	0.70	0.75	0.63	0.32	0.44	0.40	0.27	0.02	0.00	0.01	0.11	0.06	0.04	0.10	0.03	-0.11	-0.11
PFO3OA	0.10	-0.04	0.70	1.00	0.82	0.81	0.51	0.75	0.62	0.53	0.07	0.06	0.09	0.04	0.22	0.25	0.32	0.12	0.07	0.07
PFPrA	0.06	0.00	0.75	0.82	1.00	0.90	0.46	0.71	0.58	0.54	0.01	0.02	0.02	0.02	0.17	0.20	0.31	0.11	0.01	0.01
PFMOAA	0.03	-0.09	0.63	0.81	0.90	1.00	0.49	0.80	0.72	0.68	0.00	0.01	0.08	0.01	0.27	0.34	0.41	0.26	0.16	0.13
PFHpA	-0.06	-0.01	0.32	0.51	0.46	0.49	1.00	0.69	0.39	0.47	0.28	0.28	0.30	0.17	0.45	0.50	0.59	0.34	0.31	0.33
PFO4DA	0.06	-0.12	0.44	0.75	0.71	0.80	0.69	1.00	0.79	0.79	0.06	0.07	0.14	-0.03	0.34	0.46	0.56	0.33	0.24	0.20
Nafion Byproduct 2	0.13	-0.14	0.40	0.62	0.58	0.72	0.39	0.79	1.00	0.83	-0.06	0.01	0.11	0.04	0.33	0.46	0.51	0.41	0.33	0.26
PFO5DoA	0.01	-0.15	0.27	0.53	0.54	0.68	0.47	0.79	0.83	1.00	0.00	0.09	0.22	0.09	0.55	0.69	0.71	0.53	0.44	0.40
PFTrDA	0.05	0.20	0.02	0.07	0.01	0.00	0.28	0.06	-0.06	0.00	1.00	0.83	0.86	0.23	0.54	0.32	0.14	0.00	0.10	0.33
PFDoDA	-0.02	0.17	0.00	0.06	0.02	0.01	0.28	0.07	0.01	0.09	0.83	1.00	0.87	0.29	0.68	0.43	0.24	0.10	0.19	0.44
PFUnDA	0.08	0.09	0.01	0.09	0.02	0.08	0.30	0.14	0.11	0.22	0.86	0.87	1.00	0.31	0.81	0.57	0.36	0.21	0.31	0.57
NMeFOSAA	-0.09	0.04	0.11	0.04	0.02	0.01	0.17	-0.03	0.04	0.09	0.23	0.29	0.31	1.00	0.30	0.29	0.26	0.29	0.25	0.31
PFDA	0.06	-0.01	0.06	0.22	0.17	0.27	0.45	0.34	0.33	0.55	0.54	0.68	0.81	0.30	1.00	0.86	0.69	0.50	0.55	0.75
PFNA	0.04	-0.07	0.04	0.25	0.20	0.34	0.50	0.46	0.46	0.69	0.32	0.43	0.57	0.29	0.86	1.00	0.87	0.65	0.70	0.78
PFOA	0.07	-0.09	0.10	0.32	0.31	0.41	0.59	0.56	0.51	0.71	0.14	0.24	0.36	0.26	0.69	0.87	1.00	0.72	0.70	0.67
PFHxS	0.08	-0.17	0.03	0.12	0.11	0.26	0.34	0.33	0.41	0.53	0.00	0.10	0.21	0.29	0.50	0.65	0.72	1.00	0.84	0.67
PFHpS	0.10	-0.14	-0.11	0.07	0.01	0.16	0.31	0.24	0.33	0.44	0.10	0.19	0.31	0.25	0.55	0.70	0.70	0.84	1.00	0.87
PFOS	0.09	-0.06	-0.11	0.07	0.01	0.13	0.33	0.20	0.26	0.40	0.33	0.44	0.57	0.31	0.75	0.78	0.67	0.67	0.87	1.00

Figure 3. Spearman correlation heatmap for serum PFAS with >50% detection frequency in 119 people from Wilmington, NC, 2017–2019. Thick black borders around the three larger squares highlight hierarchical clusters.

(PFO3OA) (increased 35,500 ng/L), difluorosulfoacetic acid (DFSA) (increased 36,000 ng/L), 2-(1,2,2,2-tetrafluoroethoxy)perfluoroethanesulfonic acid (NVHOS) (increased 28,900 ng/L), PFO4DA (increased 11,600 ng/L), and HFPO–DA (increased 5870 ng/L).

Concentrations of these 19 PFAS decreased downstream of the facility as the distance from the facility increased (Figure S5), consistent with previous studies of this facility[26] as well as a fluorochemical facility in China.[21] Location 10 is a treatment plant providing drinking water for Wilmington, NC. In the finished drinking water, TFA dominated the PFAS signature (70% of the summed concentration of targeted PFAS; concentration: 108,000 ng/L), followed by PFMOAA (25%, 38,400 ng/L), PFPrA (2%, 3,490 ng/L), DFSA (1%, 1,130 ng/L), and PFO3OA (1%, 998 ng/L) (Figure 2). Other compounds each contributed <0.5% to the summed concentration of targeted PFAS. The HFPO–DA concentration in the finished drinking water was 288 ng/L. PFOA, PFOS, and PFHxS were detected at concentrations of 12.9, 15.9, and 7.0 ng/L, respectively.

Correlations between PFAS in Serum. For PFAS detected in >50% of serum samples, correlations between PFAS serum concentrations were visualized with a heatmap with hierarchical clustering (Figure 3). PFAS can be grouped into three main clusters with compounds in each cluster likely sharing similar sources.

Temporal Changes of PFAS Concentrations in Serum. Only one of the 20 PFAS with >50% detection rates in serum

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samples, Nafion byproduct 2, showed evidence of increasing concentrations from 2010 to 2016 (Figures 4 and S7). Nafion byproduct 2 was one of eight frequently detected compounds clearly associated with the fluorochemical production facility; the other seven (TFA, PFPrA, PFBA, PFMOAA, PFO3OA, PFO4DA, and PFO5DoA) exhibited no significant temporal trends (Figure 4). For the other 12 frequently detected PFAS, 10 showed significant temporal trends, consistent with decreasing PFAS serum levels over the July 2010 to August 2016 period (Figure S7). Most PFAS that showed decreasing concentrations over time belonged to the long-chain PFCA and PFSA subclasses, with PFHxS and PFHpS being exceptions (Figure S7). Overall, the results in Figures 4 and S7 highlight that time is a poor predictor of serum PFAS levels.

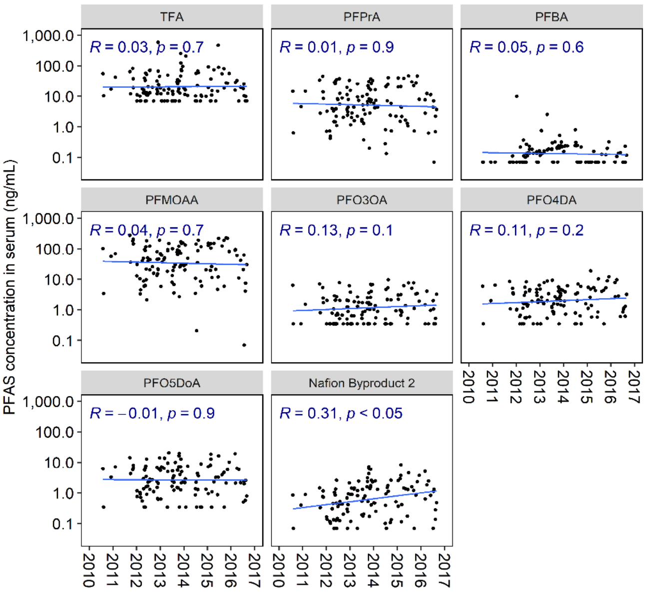

Figure 4. Temporal trends for ultrashort- and short-chain PFAS as well as PFEAs in serum over the study period (2010−2016). Only PFAS detected in >50% of the serum samples are shown. The blue text on each plot represents the coefficient and p-value of the Spearman correlation. Blue straight lines are linear trend lines.

For PFAS detected in 8−50% of serum samples, temporal trends of their detection frequencies were investigated. Detection frequencies of HFPO−DA and NEtFOSAA decreased from July 2010 to August 2016, while the detection frequency of 4:2 FTS increased in the study period (p-value < 0.05) (Figure S8). While HFPO−DA was detected overall in 20% of samples, it was most frequently detected in 2011 (83%, $n = 6$) and was not detected in any sample collected in 2014 and 2016. NEtFOSAA was detected in >50% of samples collected from 2010 to 2014 and decreased to around 20% in 2015 and 2016. Conversely, 4:2 FTS was not detected in any samples collected between 2010 and 2012 and started to be detected from 2013 (Figure S8).

Relationship between PFAS in Serum and Finished Drinking Water. PFAS were grouped into six categories based on their concentrations in finished drinking water and their detection frequencies in serum samples (Table S22). Fourteen PFAS were detected in both finished Wilmington drinking water (Location 10F) and in >50% of serum samples. The relationship between PFAS levels in serum and finished drinking water was studied by using the serum-to-water enrichment ratio (R_{SW}: median PFAS concentration divided by the finished drinking water concentration). R_{SW} was found to increase with increasing PFAS chain length, which is consistent with previous studies (Table S23).[19,37] One exception was the R_{SW} value for PFDA, which was lower than those for PFOA and PFNA, possibly because of the uncertainty associated with low PFDA concentrations in drinking water.

Moreover, the log-normal transformation of R_{SW} was positively correlated with PFAS chain length and the presence of a sulfonic acid headgroup, i.e., the number of fluorinated carbons plus 2.52 if the compound contains a sulfonic acid headgroup (Figure 5). The correlation was developed based on a group contribution method using structural features for 13 out of the 14 PFAS with available R_{SW} values. Nafion Byproduct 2 was excluded from the correlation because it contains two unique structural features that none of the other 13 PFAS contained (branched moiety and polyfluorinated moiety). Three structural features were extracted from the 13 PFAS: the number of fluorinated carbons, the number of ether oxygens, and the headgroup (Table S24). The headgroup parameter was assigned a value of 0 if the compound contains a carboxylic acid group and 1 if it contains a sulfonic acid group. A linear regression was developed between $\log_{10} R_{SW}$ and the three structural features. The number of ether oxygen atoms had an insignificant effect on $\log_{10} R_{SW}$. The contribution of a sulfonic acid group to $\log_{10} R_{SW}$ was found to be 2.52 times that of a fluorinated carbon. Consequently, the "effective" PFAS chain length was defined as the number of perfluorinated carbon atoms plus 2.52 if the compound has a sulfonic acid headgroup, and R_{SW} was log-linearly related to the "effective" chain length (Figure 5). The $\log_{10} R_{SW}$ values were normally distributed (Shapiro−Wilk test p-value > 0.05), and the linear regression exhibited homoscedasticity (Breusch−Pagan Test p-values >0.05).

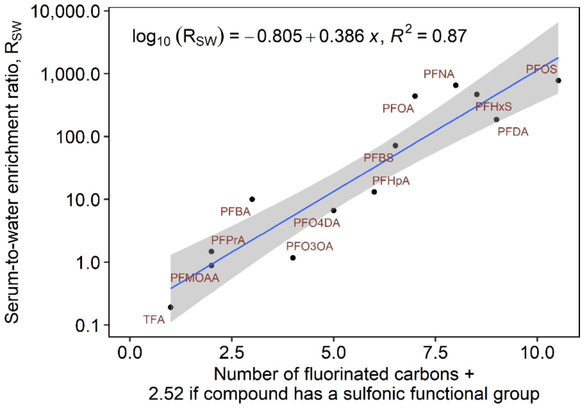

Figure 5. Correlation between serum-to-water enrichment ratio (R_{SW}) and "effective" PFAS chain length. The gray region represents the 95% confidence interval.

DISCUSSION

A key finding of this study is that TFA and PFMOAA exhibited the highest maximum and median serum concentrations among the 56 analyzed PFAS and were detected in the majority of serum samples that were collected over the 2010−2016 study period (77% detection frequency for TFA, 99% detection frequency for PFMOAA). Recent studies from Tianjin, China, Shandong, China, and Indiana, US, also found that ultrashort-chain PFAS such as TFA and PFMOAA can dominate the PFAS signature in human serum, but the previously detected serum TFA and PFMOAA concentrations were substantially lower than those observed here. The median TFA concentration in serum of adults living in Tianjin was 8.46 ng/mL (serum collected in 2017).[38] The median and maximum TFA concentrations in serum of 81 adults in Indiana were 6.0 and 77 ng/mL, respectively (serum collected in 2020).[20] Thus, median TFA levels in our study were approximately two to three times those reported in Tianjin and Indiana, while the maximum TFA level was approximately eight times that found in Indiana residents. The dramatically higher TFA serum levels in Wilmington residents were likely linked to the high TFA concentrations in drinking water. The TFA level in finished Wilmington drinking water in May 2017 (location 10F) was 108,000 ng/L, substantially higher than the median concentration found in Indiana (79 ng/L).[20] TFA levels in Tianjin's drinking water were not reported.[38]

The median PFMOAA level in the serum of residents living near a fluorochemical facility in Shandong was 12.9 ng/mL,[39] which is less than one-third of the median level we found in the serum of Wilmington residents. The PFMOAA level in the river near the fluorochemical facility in Shandong was much lower than that in the finished drinking water for Wilmington residents (an average of 5000 ng/L in the Xiaoqing River in Shandong[40] compared to 38,400 ng/L in finished drinking water of Wilmington).

Ultrashort-chain PFAS, such as TFA and PFMOAA, have been overlooked in many previous studies because of a lack of readily available analytical methods and the assumption that their half-lives in the human body are short. However, with extremely high intake from drinking water and/or other sources[20,41−43] and potential rapid uptake into human blood,[44] the body burden of ultrashort-chain PFAS can reach very high levels and may lead to potential adverse health outcomes. Based on a risk assessment conducted by the Dutch National Institute for Public Health and the Environment (RIVM), a TFA guidance level of 2200 ng/L was derived for drinking water.[45] The derivation considered liver toxicity as the endpoint and relied on a potency factor of 0.002 for TFA relative to the toxic potency of PFOA. The TFA level in Wilmington exceeded the Dutch guidance level by a factor of almost 50. Conley et al.[44] found PFMOAA had the same maternal and developmental toxicity endpoints as PFOA and HFPO−DA in Sprague−Dawley rats. Moreover, at a given maternal serum level, PFMOAA was 3−7 times less toxic than PFOA for most endpoints they investigated but slightly more potent than PFOA for increased maternal and pup liver weights. Considering that the median serum level of PFMOAA (42 ng/mL) was about 7 times that of PFOA (6.2 ng/mL) in Wilmington residents, PFMOAA could be an important toxicity contributor despite its ultrashort-chain structure.

HFPO−DA was detected in 20% of the serum samples with concentrations up to 15 ng/mL. HFPO−DA was not detected in serum samples collected in Wilmington, NC, in November 2017,[24] about 5 months after Fayetteville Works stopped process wastewater discharges into the CFR.[8] The only other two studies that detected HFPO−DA in human serum were from China and Canada. The detection rate of HFPO−DA in 977 serum samples collected in 2019−2020 from residents living near a fluorochemical facility in Shandong was 8.4%, and the maximum concentration was 0.65 ng/mL.[39] HFPO−DA was detected in 71% of serum samples collected in 2019−2020 from 224 people living in Toronto, Ontario, Canada, and the surrounding area; the geometric mean concentration was 0.45 ng/mL and the 95th percentile 16.76 ng/mL.[46] The higher detection frequency in the Canadian study compared to ours was likely linked to differences in MRLs (0.024 ng/mL,[46] 1 ng/mL here). While the source of GenX in our study was clearly linked to drinking water contamination from the upstream fluorochemical facility, GenX exposure sources were not mentioned in the Canadian study.[46]

Kotlarz et al.[24,27] determined levels of 10 legacy PFAS and 10 PFEAs in serum samples collected from Wilmington residents in November 2017 and May 2018 and found eight PFAS with >60% detection frequencies. A comparison between the concentrations of these eight PFAS in Kotlarz et al. and our study is presented in Figure S9. Concentrations of PFHpA, PFOA, PFNA, PFHxS, PFOS, and PFO4DA were comparable between the two studies, while Kotlarz et al. found higher levels of PFO5DoA and Nafion byproduct 2 in 2017−2018 than what we determined in our study from 2010 to 2016. From the temporal trends analysis (Figures 4 and S3, S4), we found that many PFAS, especially ultrashort-chain PFAS, short-chain PFAS, and PFEAs, exhibited unchanged concentrations or detection frequencies in human serum over the study period, suggesting that these compounds reached their steady-state serum levels. Many of these PFAS are associated with Fayetteville Works, highlighting that Wilmington residents and residents in other downstream communities were exposed to ultrashort- and short-chain PFAS as well as PFEAs from Fayetteville Works long before they were first measured in water.[47]

From the Spearman correlation analysis between PFAS serum concentrations, inferences about shared sources can be made for PFAS that cluster together (Figure 3). In the first cluster (leftmost cluster in Figure 3), two ultrashort-chain PFAS (TFA and PFPrA) and a short-chain PFAS (PFHpA) were grouped with four PFEAs (PFO3OA, PFO4DA, PFO5DoA, and Nafion Byproduct 2). Compounds in this cluster are closely linked to discharges from the Fayetteville Works fluorochemical plant located ∼80 km upstream of the drinking water intake for Wilmington, NC.[48] Concentrations of these compounds increased significantly downstream of the wastewater effluent location of Fayetteville Works (Figure S5). Although PFHpA shows a weaker correlation with the other PFAS in the cluster, it was identified as the most abundant among 10 targeted PFAAs discharged by Fayetteville Works in 2006.[49]

In the second cluster (middle cluster in Figure 3), long-chain PFAS with 10−12 fluorinated carbons [PFUnDA, perfluorododecanoic acid (PFDoDA), and perfluorotridecanoic acid (PFTrDA)] are grouped together with strong positive correlations ($\rho \geq 0.83$). A possible source for these compounds is the degradation of side-chain fluorinated polymers that may have been associated with historical wastewater discharges from textile plants in the headwater region of the CFR watershed.[50,51]

Finally, six frequently studied long-chain PFCAs and PFSAs—PFOA, PFNA, PFDA, PFHxS, PFHpS, and PFOS—are grouped together (rightmost cluster in Figure 3), showing moderate-to-strong correlations ($\rho \geq 0.50$). These compounds were detected at statistically similar concentrations in the CFR upstream and downstream of Fayetteville Works except for PFNA, for which levels were statistically different but the difference was small (∼0.5 ng/L). PFHpS was not detected in any of the collected water samples, likely due to its high MRL (20 ng/L). Nakayama et al. in 2006[52] investigated the levels of 10 PFAAs in the CFR basin and found the highest concentrations of PFOA, PFNA, PFDA, PFHxS, and PFOS upstream of Fayetteville Works. Therefore, the compounds in the third cluster were likely from a variety of sources that originated primarily upstream of Fayetteville Works.

We found the log-normal transformation of R_{SW} was positively correlated with the number of perfluorinated carbon atoms in PFAS plus 2.52 if the compound contains a sulfonic acid headgroup. Previous studies have also found that R_{SW} increases with an increasing PFAS chain length. However, absolute values of R_{SW} for individual PFAS in our study were generally higher than those in other studies (Table S23). One possible reason could be that PFAS concentrations in the drinking water sample were lower than average historical values. This may be the case for some compounds, such as GenX, for which an average value of 631 ng/L was reported[9] for samples collected in 2013 at location 9, while a value of 780 ng/L was reported[26] for a 2015 sample collected at location 9; in the 2017 sample reported here, the GenX concentration was 288 ng/L for finished water at location 10. Similarly, for PFMOAA, a concentration of 110,000 ng/L was reported[26] for a 2015 sample (Location 9), while the 2017 sample we analyzed had a concentration of 38,400 ng/L (location 10F). However, for many other PFAS, concentrations in the 2017 sample from location 9 were similar to, and in some cases higher than those reported in prior studies (Table S25).[9,26] Locations 9 and 10F are the intake and finished water for the drinking water treatment plant in Wilmington. Because the plant was not equipped with technology to remove PFAS, differences in PFAS levels between Locations 9 and 10F are small compared to concentration differences between sampling years (Table S25). If using the data for the 2015 water sample to calculate R_{SW}, the R_{SW} values of this study are closer to those for Fountain, Colorado, residents for compounds for which comparisons can be made (Table S23).[37] Variability in PFAS concentrations in the CFR can be a result of variations in streamflow, but the 2017 sample was collected under relatively typical streamflow conditions (Figure S10). In addition, PFAS concentrations in the river vary as inputs to the river vary. PFAS mass flows in the river varied greatly over a 3-month period in 2013.[9] It is likely, therefore, that PFEA inputs to the CFR may have been lower during the 2017 sampling campaign compared with those assessed in prior sampling campaigns. Exposure duration can also affect PFAS levels in serum,[53] which can cause differences in R_{SW} values between studies.

Comparing PFAS levels between serum and water samples, we found five PFAS (PFUnDA, PFDoDA, PFHpS, PFO5DoA, and NMeFOSAA) were not detected in the finished drinking water but in >50% of serum samples (Table S22). Median serum concentrations of these five PFAS ranged from 0.051 to 2.6 ng/mL. These PFAS are all long-chain compounds with 7–11 fluorinated carbons or, in the case of PFO5DoA, share structural similarities with long-chain PFAS. They were not detected in water but frequently detected in serum samples, indicating (1) residents had been exposed to these compounds through drinking water at a time prior to our water collection, and these compounds had not been excreted from their bodies at the time of serum sample collection, (2) these compounds are highly bioaccumulative such that even with a concentration that is not detectable in water, they accumulated to measurable levels in the human body, and/or (3) there were other sources of exposure.

DFSA had a relatively high concentration in the finished drinking water (1130 ng/L) but was not detected in any serum samples. Possible reasons could be (1) DFSA has a slow absorption rate and/or fast excretion rate from the human body due to its ultrashort-chain and diprotic structure and (2) the MRL for DFSA in our analytical method (10 ng/mL) was too high to detect this compound in the serum samples.

Implications. In this study, we determined concentrations of 56 PFAS in 119 human serum samples obtained from Wilmington, NC. These samples provided a unique opportunity to assess PFAS exposure levels before people knew that their water was contaminated. These data are fundamental for understanding past PFAS exposure in Wilmington, a highly impacted community where over 200,000 residents drank PFAS-contaminated water, many for several decades.

TFA and PFMOAA were the dominant PFAS in the serum samples, despite their likely short half-lives in the human body. Their dominance can be explained by (1) extremely high concentrations in finished drinking water (108,000 ng/L for TFA and 38,000 ng/L for PFMOAA in a May 2017 sample) and (2) rapid uptake into human blood, which may be facilitated by their small size.[44] While current TFA and PFMOAA levels have likely decreased substantially from those in the historical blood serum samples evaluated here as a result of mandated discharge controls at the upstream fluorochemical manufacturer, this study, along with other recent studies,[20,38,39] highlights the importance ultrashort-chain PFAS can play in determining the overall human PFAS burden. TFA levels in drinking water sources are increasing globally as a result of industrial point sources, degradation of precursors, and atmospheric photodegradation of refrigerants,[54] likely causing widespread increases in serum TFA levels. Furthermore, the PFMOAA levels found in this study, which exceeded PFOA levels by a factor of about 7, along with recent findings about the toxic potency of PFMOAA relative to PFOA,[44] suggest that PFMOAA may have been an important toxicity contributor. We need to better understand the toxicity of ultrashort-chain PFAS, such as PFMOAA, TFA, and PFPrA, not only individually but also in mixtures like those observed in this study. Studies need to be conducted to investigate the broader environmental prevalence and potential health effects associated with exposure to ultrashort-chain PFAS.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.5c08146.

Table S1: List of targeted PFAS; Table S2: List of internal standards; Tables S3 to S6: Acquisition method for analyzing 11 shorter-chain PFAS in serum; Tables S7 to S11: MRL and quality control for analyzing 11 shorter-chain PFAS in serum; Tables S12 to S13: Acquisition method for analyzing 45 longer-chain PFAS in serum samples; Table S14: MRL for analyzing 45 longer-chain PFAS in serum; Tables S15 to S18: Acquisition method for analyzing PFAS in water samples; Table S19: MRL for analyzing PFAS in water samples; Table S20: PFAS concentrations in serum samples; Table S21: PFAS concentrations in water samples; Table S22: Relationship between PFAS concentrations in water and PFAS detection frequencies in serum; Table S23: R_{SW}; Table S24: PFAS structure features; Table S25: PFAS concentrations in water in this and previous studies (XLSX)

Text S1: Sample preparation procedure for analyzing 11 shorter-chain PFAS in serum; Text S2: Procedures to minimize analytical interferences during serum sample analysis; Text S3: Sample preparation procedure for analyzing 45 longer-chain PFAS in serum; Text S4: Sample preparation procedure for analyzing PFAS in water samples; Figures S5 and S6: PFAS concentrations in water samples; Figures S7 and S8: Temporal trend of PFAS in serum; Figure S9: Compare PFAS serum concentrations to a previous study; Figure S10: Streamflow of CFR (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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