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Predictors of Emerging Per- and Poly-fluoroalkyl substances (PFAS) Exposure in Serum of U.S. Adults

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By

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Abstract

Predictors of Emerging Per- and Poly-fluoroalkyl substances (PFAS) Exposure in Serum of U.S. Adults

By Rachel Massingill

Per- and poly-fluoroalkyl substances (PFAS) are a diverse group of fluorinated organic compounds commonly used as lubricants, surfactants, stain- and fire-repellents and are applied to treat several types of flooring, furniture materials, and textiles. Legacy PFAS, including perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), are perfluoroalkyl acids (PFAAs) with 8 fluorinated carbons in their structure (C8) that were widely used until the early 2000s when global restrictions were imposed due to their environmental persistence and human toxicity. As a result, ultrashort-chain (C2-C3) and short-chain (C4-C7) PFAAs with less than 8 carbons in their structure were introduced as a safer and less-bio accumulative alternative. These shorter-chain compounds are now detected in human blood serum and the environment. Limited data is available characterizing ultrashort-chain and short-chain exposure pathways. In this study, we investigated whether sociodemographic factors, behavioral patterns, and housing attributes are associated with elevated levels of ultrashort- and short-chain PFAS in serum from 81 participants from Indiana, United States. Ultrashort-chain trifluoroacetic acid (TFA, C2) was the predominant PFAA in serum samples, and ultrashort-chain PFPrA was detected at levels comparable to long-chain PFAAs. Significant positive associations were observed between ultrashort-chain TFA and the 39 to 60-year-old age group (beta: 0.52, 95% CI [0.14, 0.91] and an apartment housing type (beta: 0.69, 95% CI [0.09, 1.29]). The high prevalence of ultrashort-chain compounds TFA and PFPrA indicates that the accumulation of ultrashort-chain alternatives has exceeded levels of long-chain PFAS in humans.

Predictors of Emerging Per- and Poly-fluoroalkyl substances (PFAS) Exposure in Serum of U.S. Adults

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INTRODUCTION

Per- and poly-fluoroalkyl substances (PFAS) are a diverse group of synthetic, fluorinated compounds that have been used in a variety of consumer and industrial applications since the 1940s.[1] These compounds possess strong carbon-fluorine bonds, making them durable substances with low surface tension and enhanced resistance to water, stains, oil, and fire.[2] Legacy PFAS, including perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), are perfluoroalkyl acids (PFAAs) with 8 fluorinated carbons in their structure (C8) that have been used extensively prior to the past two decades.[3] Their manufacturing and use have since been regulated due to their persistent environmental presence, tendency to accumulate in organisms, and toxicity to both wildlife and humans.[4] These C8 compounds were phased out in the early 2000s when production and use of PFOS were restricted under Annex B of the Stockholm Convention on Persistent Organic Pollutants, and in 2019, PFOA was targeted for global elimination under Annex A.[5] As a result, shorter-chain PFAAs with less than 8 carbons in their structure, were introduced into commerce and were marketed as a safer and less persistent alternative.[4] In recent years, studies have revealed that shorter-chain PFAAs exhibit bio-accumulative properties and have been detected in indoor and outdoor environments.[3] Compared to the longer-chain homologs, their short-chain structure and lower molecular weight allow for greater mobility with the potential to be transported over long distances.[4] Additionally, shorter-chain PFAS often demonstrate reduced efficacy compared to their long-chain counterparts and require higher quantities to maintain effectiveness standards, potentially leading to increased release of these chemicals into the environment.[6]

Exposure to ultrashort-(C2-C3) and short- (C4-C7) chain PFAS is of increasing public health concern. Numerous studies have identified these compounds in human blood serum and their toxigenic properties have been linked to significant health impacts on lipid metabolism, as well as reproductive, developmental, hepatic, and renal systems.[3] While studies have focused on quantifying the levels of ultrashort- and short-chain PFAS in samples such as dust and drinking water, their exposure pathways and relative contribution to biochemical uptake remain poorly understood. Absorption of PFAS can occur

through various routes of contact, including dietary and non-dietary ingestion, inhalation, and dermal absorption.[7] Previous research indicates that water intake is a significant pathway for legacy PFAS exposure, as conventional drinking water treatment processes fail to effectively remove these chemicals due to their resistance to chemical, physical, and biological degradation.[8,9] Similarly, multiple studies have suggested that ingestion and inhalation of indoor air and dust may constitute important PFAS exposure routes.[10] Dust consists of tiny particles and a complex mix of organic and inorganic compounds, enabling it to absorb and concentrate certain PFAS.[11] The indoor environment is an important potential source of PFAS exposure due to their widespread prevalence in household items. Specifically, PFAS are used as lubricants, surfactants, stain- and fire-repellents and are applied to treat several types of flooring (e.g. carpet, vinyl, hardwood, tile), furniture materials, and textiles (e.g. cloth, upholstery).[12,13] Because PFAS are applied to coat exterior surfaces, they can be directly released from materials and into the surrounding environment.[13] People spend a substantial amount of time indoors, resulting in frequent exposure to chemicals originating from household materials and products.[14] Understanding ultrashort- and short-chain PFAS exposure patterns in the indoor environment is crucial for identifying the main pathways of chemical uptake in humans and determining the extent to which residential sources contribute to the overall body burden.

In our previous study, we collected paired samples of residential dust and drinking water from households in Indiana, United States, and paired samples of urine and blood serum from residents of these homes (n = 81 participants). We quantified 47 PFAS in these samples.[3] Notably, we found that ultrashort- and short-chain PFAAs were the primary analytes detected in serum.[3] The results of our previous analysis indicate that ultrashort- and short-chain PFAS are ubiquitous in humans, however, the sources of these compounds remain largely unknown. In this study, we sought to determine upstream factors associated with elevated serum PFAS levels. Specifically, we investigated whether sociodemographic factors, behavioral patterns, and housing attributes are associated with elevated levels of ultrashort- and short-chain PFAS in serum. The analysis includes 9 long-chain, 5 short-chain, and 2 ultrashort-chain PFAAs, alongside information on 7 demographic factors and 9 housing characteristics extracted from participant

questionnaires. The goal of this analysis is to identify potential sources of exposure to emerging PFAS in residential indoor environments and to discern factors associated with increased risk of biochemical uptake of ultrashort- and short-chain PFAS.

METHODS

Sample Collection. The data for this analysis was obtained from study participants (n = 81) between August and December 2020 in the state of Indiana, United States. Individuals were recruited from the Person to Person (P2P) Health Interview Study cohort and provided written, informed consent prior to their involvement in the study.[15] Individuals residing in four Indiana counties, two urban (Marion and Lake) and two rural (Scott and Fayette) were eligible for participation. A structured questionnaire was administered to participants at the time of serum collection. We enrolled both men and women and collected data on their age, marital status, smoking habits, body mass index (BMI), and education level. We gathered information on housing and cleaning characteristics and patterns from each individual, including their home type, years of residence, home age, flooring and wallpaper type, water source, and vacuuming frequency.

Sample Analysis. Blood samples were collected from each participant and serum was separated via centrifugation upon delivery of samples to the laboratory. Serum samples were analyzed for 47 PFAS, including 23 PFAAs (3 ultrashort-chain, 6 short-chain, and 14 long-chain PFAAs) using liquid chromatographic and gas chromatographic-mass spectrometry.[3] We focused on the 23 PFAAs measured in serum for the purposes of this analysis.[3] The complete list of analytes and details of the analytical methods are described in the Supporting Information. We restricted our analysis to those analytes with a detection frequency (DF) greater than 50%. There were 7 PFAAs detected in <50% of samples, resulting in 16 PFAS included in downstream analyses. For all PFAS concentrations, values below the method detection limit (MDL) were imputed using MDL/2. Our PFAS analyte measurements maintained a right-skewed distribution and were logarithmically transformed (natural log) for statistical analysis.

Data Analysis. The distribution of demographic and housing characteristics in the study population was assessed using counts and percentages. Geometric means and their standard deviations

were calculated at selected percentiles to determine the distribution of serum PFAS. Spearman correlation coefficients were calculated to assess correlations between individual PFAS. To assess PFAS concentrations across demographic and housing attributes, we computed geometric means and deviations for each PFAS within distinct categories of demographic and housing variables. We then used linear regression to calculate beta estimates and 95% confidence intervals for the association between each demographic and housing attribute and individual, natural log transformed PFAS concentrations. We ran these models unadjusted, and then adjusted for age, body mass index (BMI), and education to account for potential confounding by these variables.

RESULTS

Study Population Characteristics. A summary of the population's demographic and housing characteristics (n = 81) is presented in Table 1. Participants' ages ranged from 25 to 88 years of age. Sixty-four percent of participants were female, 36% were male, and less than half were smokers (33%). Fifty-nine percent of participants were married, and most of the study population was Caucasian (80%). Twenty-seven percent of participants had attained a college education and 35% had some college experience. Over half of participants had a BMI classifying them as obese ($\geq$30 kg/m^2).

Forty-two percent of individuals had been living in their current residence for under 5 years. Eighty percent of households had carpeted flooring, while 20% had other flooring types (e.g. hardwood, tile, vinyl). Most residents used tap water as their drinking water source, with only 10% using private wells. Fifteen percent of households had vinyl wallpaper, and 69% of participants' homes were built more than 20 years ago. More than half of households had 3 or more occupants, and 60% of individuals spent an average of 8 hours or less away from home daily. Sixty-seven percent of participants resided in houses, the rest resided in apartments or mobile homes. Forty-three percent of households vacuumed most days, while over half did so less frequently (Table 1).

Table 1. Summary of Participants (n = 81) Demographic & Housing Characteristics.

DEMOGRAPHIC CHARACTERISTICS	N	PERCENTAGE %
AGE (YEARS)		
38 YEARS OR YOUNGER	27	33
39 – 60 YEARS OLD	28	35
61 OR OLDER	26	32
GENDER		
MALE	29	36
FEMALE	52	64
MARITAL STATUS		
SINGLE	48	59
MARRIED	33	41
RACE		
WHITE/CAUCASIAN	65	80
NON-WHITE	16	20
EDUCATION		
HIGHSCHOOL OR LESS	31	38
SOME COLLEGE	28	35
COLLEGE OR HIGHER	22	27
SMOKING		
SMOKER	27	33
NON-SMOKER	54	67
BMI (KG/M2)		
NORMAL WEIGHT (≤ 24.9)	27	33
OVERWEIGHT (25-29.9)	13	16
OBESE (30+)	41	51

HOUSING CHARACTERISTICS	N	PERCENTAGE %
FLOORING TYPE		
CARPET	65	80
NO CARPET (OTHERS)	16	20
MISSING	1	
HOME TYPE		
HOUSE	54	67
APARTMENT	18	22
MOBILE HOME/OTHERS	9	11
RESIDENCE BUILT		
BEFORE 1940	11	14
BETWEEN 1940-1969	20	25
BETWEEN 1970-1999	25	31
2000 OR AFTER	10	12
MISSING	15	19
VINYL WALLPAPER		
NO	62	77
YES	12	15
MISSING	7	9
PEOPLE LIVING IN HOME		
1-2 PEOPLE	38	47
3-4 PEOPLE	27	33
5-6 PEOPLE	16	20
YEARS AT RESIDENCE		
< 5 YEARS	34	42
5-10 YEARS	21	26
11-20 YEARS	9	11
21-30 YEARS	6	7
31+ YEARS	11	14
HOURS OUTSIDE OF HOME		
> 8 HOURS PER DAY	32	40
≤ 8 HOURS PER DAY	49	60
WATER SOURCE		
CITY WATER	73	90
WELL WATER	8	10
VACUUM FREQUENCY		
NEVER/SOME DAYS	43	53
MOST DAYS/EVERYDAY	36	44

PFAS Concentrations. We observed that ultrashort-chain PFAAs were detected in high abundance (Table 2). Specifically, TFA had the highest concentrations relative to all other PFAS (geometric mean 5.76 ng/mL), with notable variation between lower and upper bounds (2.18 and 14.71 ng/mL, respectively). PFPrA (geometric mean 0.95 ng/mL) was the second most abundant PFAA detected at levels consistently higher than those of short-chain PFAAs and comparable to levels of the legacy long-chain PFAAs. Concentrations of long-chain analytes including PFOS, PFOA, and

perfluorohexane sulfonic acid (PFHxS) (geometric means 1.53, 0.64, and 0.77 ng/mL, respectively) were detected at mean concentrations higher than the short-chain PFAS.

Table 2. Selected Percentiles, Geometric Mean, and Geometric Standard Deviation for PFAS Blood Serum Concentrations (ng/mL) among Participants (n = 81) in Indiana, U.S.

PFAS	Geometric Mean (Geometric SD)	5%	25%	50%	75%	95%
Ultrashort-chain (C2-C3)						
TFA	5.76 (2.12)	2.18	2.18	6.01	8.51	14.71
PFPrA	0.95 (1.71)	0.34	0.77	1.01	1.28	2.15
Short-chain (C4-C7)						
PFBA	0.18 (2.05)	0.06	0.14	0.19	0.24	0.43
PFBS	0.03 (5.47)	0	0.02	0.05	0.1	0.24
PFPeS	0 (4.19)	0	0	0.01	0.01	0.02
PFHxA	0.02 (4.19)	0	0.02	0.03	0.05	0.07
PFHpA	0.01 (4)	0	0.01	0.02	0.03	0.06
Long-chain (C6-C16)						
PFHxS	0.77 (2.8)	0.19	0.46	0.78	1.36	3.95
PFHpS	0.08 (3.42)	0.01	0.04	0.1	0.18	0.29
PFOA	0.64 (2.83)	0.16	0.41	0.63	0.97	3.09
PFOS	1.53 (3.26)	0.49	0.96	1.48	2.71	7.6
PFECHS	0.01 (3.4)	0	0.01	0.01	0.02	0.05
PFNA	0.19 (2.74)	0.08	0.15	0.21	0.31	0.59
PFDA	0.04 (2.92)	0	0.03	0.05	0.08	0.15
PFUdA	0.03 (3.51)	0	0.01	0.04	0.07	0.11
PFHxDA	0.01 (5.78)	0	0	0.02	0.05	0.1

Relationships among PFAS levels were examined using Spearman correlation coefficients (Figure 1). Strong correlations were observed among long-chain PFAAs. Legacy compounds PFOS and PFOA were also moderately correlated with other long-chain analytes. Ultrashort- and short-chain PFAAs exhibited slightly weaker correlations within their groups. Short-chain compounds perfluorohexanoic acid (PFHxA) and perfluoroheptanoic acid (PFHpA) were weakly correlated with multiple short-chain and long-chain analytes.

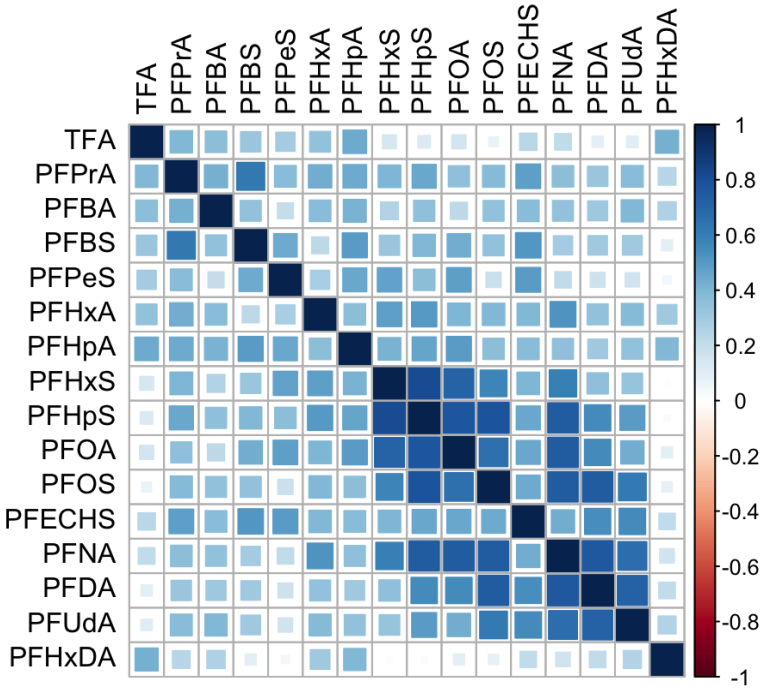

Figure 1. Correlation Heat Map showing the relationships between ultrashort-chain, short-chain, and long-chain PFAS.

Demographic Predictors. Geometric means (and geometric standard deviations) for each PFAS analysis were calculated across strata of demographic characteristics (Table 3, supplemental). Compared to individuals who were non-smokers, those who were smokers had slightly lower geometric means for ultrashort-chain PFAS. Participants who were married had higher geometric means for ultrashort-chain PFAA levels than individuals who were not married. Among those with some college education, the geometric means were predominantly lower across all analytes compared to participants who had a

college education and those with no college education. The geometric means for concentrations of long-chain PFAS in the oldest age group were double those in the youngest (Table 3, supplemental).

In unadjusted linear regression models, we observed that compared to females, males exhibited higher serum PFAS levels overall, and positive effect estimates were observed across all short-chain compounds. Positive significant associations were found for short-chain perfluorobutane sulfonic acid (PFBS) (beta: 0.84, 95% CI [0.09, 1.60]) and long-chain PFHxS (beta: 0.51, 95% CI [0.05, 1.28]). Additionally, we observed racial disparities where consistently higher PFAS serum concentrations were found among non-white participants compared to those who self-identified as white. Race was significantly associated with elevated short-chain perfluorobutanoic acid (PFBA) (beta: 0.40, 95% CI [0.02, 0.79]) in non-white individuals compared to whites, and for long-chain analytes perfluotoethylcyclohexane sulfonate (PFECHES), perfluoroundecanoic acid (PFUdA), perfluorohexadecanoic acid (PFHxDA) (beta: 0.98, 95% CI [0.35, 1.62], beta: 0.83, 95% CI [0.17, 1.5], and beta: 1.34, 95% CI [0.42, 2.26], for non-white versus white, respectively).

Body mass index, age, and education were identified as key demographic determinants of serum PFAS concentrations in our study population (Figure 2). Compared to individuals who were normal weight (BMI of $\leq$24.9 kg/m^2) participants who were obese (BMI 30 kg/m^2 or above) had higher levels of short-chain perfluoropentane sulfonic acid (PFPeS) and long-chain perfluorodecanoic acid (PFDA) (beta: 0.69, 95% CI [0.01, 1.38], and beta: 0.62, 95% CI [0.13, 1.11], respectively).

Compared to the youngest age group (38 years or younger), individuals between 39 to 60 years old had greater PFAS serum concentrations for all compounds except for short-chain PFPeS (Figure 2). A significant positive association was observed between ultrashort-chain TFA and the 39 to 60-year-old age group compared to those who were less than 38 years of age (beta: 0.52, 95% CI [0.14, 0.91]. Participants in the oldest age cohort (61 years or older) had the greatest effect estimates for all PFAS serum levels except for ultra-short-chain TFA, which was greater among those 39 to 60 years old.

Participants with a college education had higher levels of ultrashort-chain PFPrA, short-chain PFBA, PFBS, PFPeS, and PFHpA compared to individuals with no college education. However, these relationships were insignificant (Figure 2).

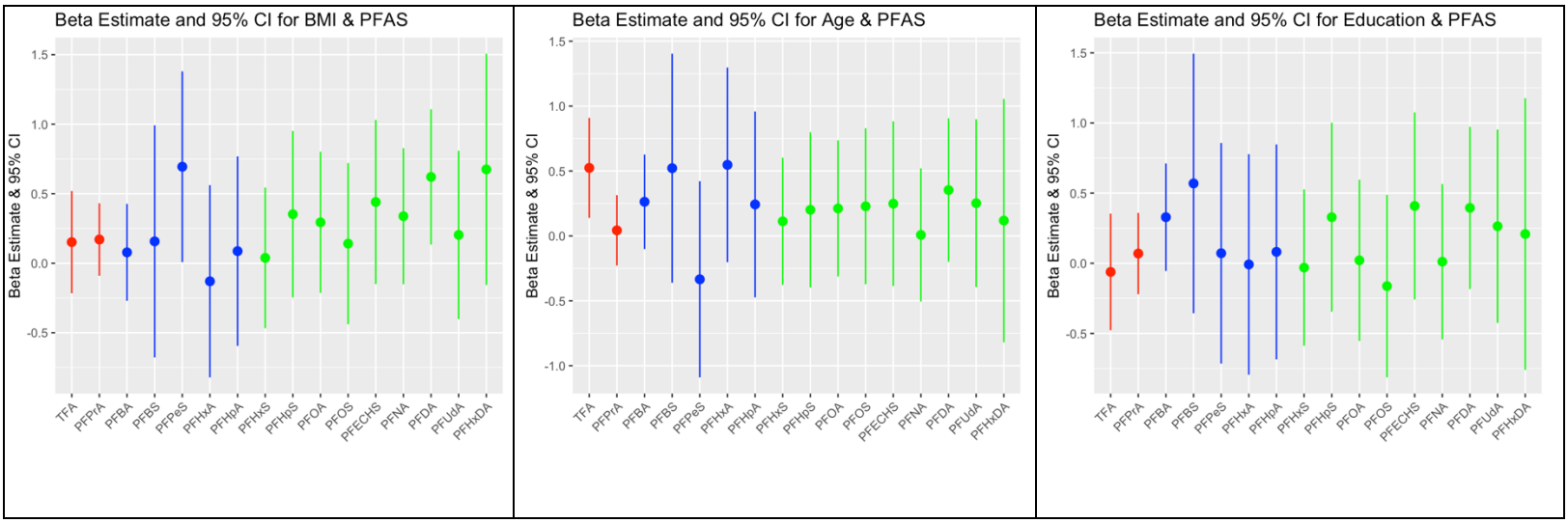

Fig. 2. Dot and whisker plots of the distribution of selected demographic variables (unadjusted) across log-transformed PFAS measurements (ng/mL) in blood serum. The lower and upper ends of the lines are the tails of the 95% confidence interval, and circles represent the beta estimate for covariates against each PFAS.

Ultrashort-Chain **Short-Chain** **Long-Chain**

Housing Predictors. Our study assessed the association between housing characteristics and levels of ultra-short and short-chain PFAS concentrations among U.S. adults, accounting for confounding variables such as age and educational attainment (Supplemental Table 5). While associations were broadly insignificant, notable trends were observed.

Participants who spent more time indoors exhibited higher concentrations of ultrashort-chain TFA and short-chain PFPeS. Compared to households with 5-6 occupants, those with 1-2 people exhibited higher effect estimates for ultrashort-chain PFPrA, and all other short-chain and long-chain PFAAs, except for PFBA and PFHxDA. Overall, homes with 3-4 residents had reduced serum PFAS

concentrations compared to larger residences, with the short-chain analytes PFBS and PFBA being particularly lower.

Our regression analysis revealed that individuals who vacuum frequently had predominantly higher PFAS serum levels across examined analytes. Negative significant associations were found between less frequent vacuuming and short-chain PFBA and long-chain PFNA (beta: -0.33, 95% CI [-0.65, -0.01], and beta: -0.48, 95% CI [-0.94, -0.01], respectively).

Participants from homes with vinyl wallpaper were found to have overall lower levels of ultrashort- and short-chain PFAAs and elevated concentrations of legacy compounds PFOA and PFOS. A positive relationship was observed for ultrashort-chain and short-chain PFAA concentrations in participants whose homes were constructed after the year 2000, except for short-chain PFPeS, which had a positive effect estimate for homes built between 1940-1969 and 1970-1999. Individuals residing in homes built between 1970 and 1999 had overall elevated serum measurements for long-chain PFAS. These relationships were not statistically significant.

In homes with carpeted flooring, participants had predominantly increased PFAA serum levels (Figure 3). Smaller effects were observed for ultrashort-chain TFA and short-chain PFBA, while larger increases in serum concentrations were evident among long-chain compounds. Particularly, the highest serum concentration estimates were for long-chain PFNA and PFUdA. These relationships were not significant, however, the type of flooring in homes may be confounded by housing type. Individuals living in mobile homes had negative effect estimates for ultrashort-and short-chain PFAS, except short-chain PFBS and PFPeS. The highest ultrashort- and short-chain serum levels were detected among those residing in apartments, and a positive significant association was found for ultrashort-chain TFA (beta: 0.69, 95% CI [0.09, 1.29]).

We observed prominent variations in PFAS serum measurements relative to the amount of time participants lived in their current residences. In our unadjusted model (Table 4, supplemental), individuals who lived in their homes for 31 years or more had substantially higher concentrations across all analytes, and significant positive associations were found for ultrashort-chain PFPrA and short-chain PFBA (beta:

0.41, 95% CI [0.06, 0.77] and beta: 0.50, 95% CI [0.02, 0.98], respectively). We assumed confounding by age considering participants who lived in their residence for longer were older, and therefore susceptible to increased exposure and bioaccumulation of PFAS throughout their extended lifespan. After adjusting for age and education, our analysis compared residence times (5 to 10 years, 11 to 20 years, 21 to 30 years, 31 or more years) to a reference of less than 5 years and produced positive effect estimates for ultrashort-chain and most short-chain and long-chain PFAAs (Table 5, supplemental). A significant positive relationship was observed between a residence time of 31 years or more and ultrashort-chain TFA (beta: 0.64, 95% CI [0.01, 1.28]. The beta estimates and 95% confidence intervals for PFAS concentrations among those with less than 5 years residence time compared to 31+ years are shown in Figure 3.

Concentrations of ultrashort-chain TFA and PFPrA, and short-chain PFBA and PFHpA were higher among participants using city water compared to those with well water as their water source. Additionally, individuals using well water had greater concentrations of long-chain PFAAs (Figure 3). In general, no significant differences were observed between well water and city water sources for the concentration of PFAS.

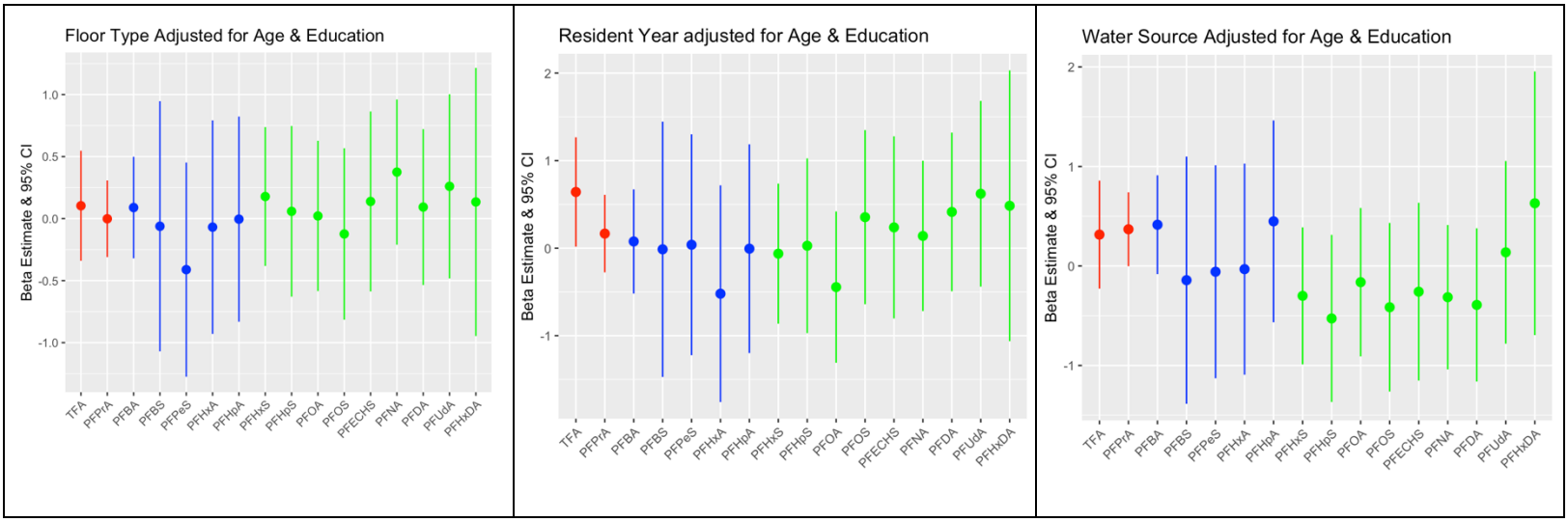

Fig. 3. Dot and whisker plots of the distribution of selected housing variables (adjusted for age and education) across log transformed PFAS measurements (ng/mL) in blood serum. The lower and upper ends of the lines are the tails of the 95% confidence interval, and circles represent the beta estimate for covariates against each PFAS.

Ultrashort-Chain **Short-Chain** **Long-Chain**

DISCUSSION

To the best of our knowledge, this is the first biomonitoring study to quantify the levels of ultrashort-chain PFAS in a U.S. population. We observed that ultrashort- and short-chain PFAS were highly prevalent in our population and ultrashort-chain TFA was the predominant PFAA detected in serum samples. Concentrations of long-chain PFAAs were expected due to their accumulation in human blood serum and historical widespread usage.[16,17] The distribution of many long-chain PFAAs were detected at levels below those of TFA and comparable to ultrashort-chain PFPrA levels. This evidence suggests a decrease in the prevalence of long-chain PFAS and accumulation of ultrashort-chain alternatives in humans. One other study from Tianjin, China reported similar findings, where TFA was detected at levels comparable to those of several long-chain PFAAs in human blood.[18] Our analysis suggests that an apartment housing type and an age of 39 to 60 years were significant predictors of ultrashort-chain TFA exposure.

Our analysis using Spearman correlation coefficients revealed strong correlations among long-chain PFAAs, suggesting a probable shared origin for these compounds.[17] This is consistent with the phase-out of long-chain PFAS in industrial and consumer applications, where these chemicals were often used together prior to their replacement by shorter-chain alternatives.[11] In contrast, weaker correlations observed among ultrashort- and short-chain PFAAs indicate a potential mixture of sources.[17] Short-chain compounds PFHxA and PFHpA were highly correlated with multiple short-chain and long-chain analytes, aligning with previous findings of strong positive correlations between these chemicals.[19,20] This may result from legacy compounds manifesting as impurities in fluorotelomer-based products.[11]

Our unadjusted model revealed significant differences in shorter-chain PFAS exposure by gender, race, body mass index, and age. Males exhibited significantly higher levels of PFAAs compared to females, aligning with previous studies suggesting that gender differences in PFAS serum levels could be due to higher rates of substance elimination in females during menstruation and gestation.[21] Race was significantly associated with exposure to short-chain PFBA and several long-chain analytes. While the majority of the study participants self-identified as white, the significant findings related to non-white individuals suggest that race may be a predictor of differential PFAS exposure. This association is potentially the result of underlying differences in geographic location, socioeconomic status, or lifestyle choices among self-identifying white and non-white individuals.

Body mass index was an important predictor of PFAS exposure, revealing increased overall PFAAs and significant associations for short-chain PFPeS and long-chain PFDA among individuals who were obese (BMI 30 kg/m2 or above) relative to those who were normal and underweight. This group also had higher geometric mean concentrations across a majority of PFAAs compared to individuals categorized as normal weight (BMI $\leq$ 24.9 kg/m^2) and overweight (BMI 25-29.9 kg/m^2). These findings resonate with recent epidemiological studies that emerging contaminants are associated with obesity through the promotion of adipogenesis (fat cell development).[22] Additionally, legacy PFAS have been linked to increased BMI, potentially due to higher exposure levels from sources such as food packaging of snacks and other processed food items.[23] Education was considered an indicator of socioeconomic

status, as previous studies found that higher socioeconomic levels correspond with increased PFAS serum detection.[24] Our analysis suggested that individuals with a college education had higher PFAS serum levels of ultrashort-chain PFPrA and multiple short-chain compounds.

Age was a prominent variable in this analysis due to the bioaccumulative properties of PFAS. The geometric means for concentrations of long-chain PFAS in the oldest age group (61 and older) were double those in the youngest (38 years and younger), reflecting historical usage patterns and prolonged exposure to legacy PFAS before manufacturing restrictions were imposed. A significant positive association was found between the 39 to 60-year-old cohort and ultrashort-chain TFA, further highlighting age-related differences in PFAS levels driven by changing exposure patterns over time. Younger age groups may be more likely to exhibit higher levels of ultrashort-chain TFA due to the increased use of newer consumer products manufactured with ultrashort-chain alternatives.

Although the majority of housing characteristics investigated in our study did not reach statistical significance, we identified trends that provide valuable insights into exposure patterns of emerging PFAS within residential indoor environments. We observed that households with fewer residents had predominantly higher levels of ultrashort- and short-chain PFAAs. This pattern may be partially explained by differences in household size. Larger households typically require more space, which could dilute the concentration of PFAS by allowing a wider spatial distribution of these compounds. Additionally, households with fewer residents have previously been associated with higher PFAS levels in household dust.[11] The presence of more people in a household has been linked to more frequent cleaning, which can reduce the build-up of PFAS by removing protective layers from the treated surfaces of consumer products. [11,25]

Our analysis showed that individuals who spent more time outside of their homes had greater levels of PFAS overall, suggesting potential workplace or outdoor environmental exposures as contributing factors. Notably, elevated concentrations of ultrashort-chain TFA were observed among participants who spent more time in their homes, which may be attributed to increased exposure to indoor

sources of TFA. This may indicate that the amount of time spent indoors serves as an indirect measure for certain indoor exposures influencing ultrashort-chain PFAS bioaccumulation.

Participants residing in homes with vinyl wallpaper exhibited higher serum concentrations of legacy compounds PFOS and PFOA. This observation may be related to the time period in which these homes were built, as vinyl wallpaper has become less popular over time. It is possible that homes with vinyl wallpaper were built before the phase-out of legacy PFAS, reflecting the historical use of these chemicals in building materials. Our questionnaire data revealed that a majority of individuals with vinyl wallpaper lived in homes built between 1970 and 1999, aligning with the era when legacy PFAS were commonly used in various construction applications. A similar trend was observed where elevated levels of long-chain PFAS were detected among individuals living in homes built between 1970-1999. This finding suggests a potential association between PFAS exposure and building materials prevalent during the 1970s to 1990s.

Numerous studies have shown that carpet is a prominent source of PFAS exposure due to the high concentrations of these substances found in carpet surface treatment products.[11] Additionally, carpets trap dust and indoor and outdoor pollutants more efficiently than hard surface floors such as hardwood or tile.[26] While carpet has been a primary focus of PFAS exposure studies, their significance relative to ultrashort and short-chain PFAAs is largely understudied. Our data showed that individuals with carpeted flooring had greater levels of long-chain PFNA, corresponding with findings from a previous study where greater concentrations of PFNA were found in carpeted homes.[20] Similarly, a positive significant association was observed for long-chain PFNA as well as short-chain PFBA in individuals who vacuumed their homes more frequently. In our previous study, we quantified PFAS in household dust and determined that less frequently vacuumed homes had PFAA concentrations up to three times higher than those vacuumed regularly.[3] The contradicting result in the current regression analysis may be explained by vacuuming-induced particle resuspension, which temporarily increases particulate matter in the indoor air through dust disturbance and mechanical stirring.[27] Although vacuuming reduces surface dust

contaminants, particle dispersion may lead to the inadvertent ingestion and inhalation of more dust particles, resulting in elevated PFAS serum concentrations over time.[27]

Water is considered an important source of PFAS exposure. These soluble compounds can penetrate natural and anthropogenic barriers to eventually reach drinking water sources, where common remediation methods do not adequately remove them.[28] Our analysis revealed that ultrashort-chain TFA and PFPrA, as well as short-chain PFBA and PFHpA, were higher in the serum of participants with city water as their water source. These results reflect the outcomes of previous studies where TFA, PFPrA, PFBA, and PFHpA were the prominent PFAAs detected in water sources.[28,29]

Our study had several limitations resulting from a small sample size, including reduced statistical power which induced a high degree of variability in our statistical analysis. Furthermore, our sample lacked generalizability to the broader population due to a small cohort limited in diversity and geographic coverage. Our study is also prone to recall biases, an inhere limitation of all self-reported data.

CONCLUSION

Our study is the first to report relationships between elevated concentrations of ultrashort-chain PFAAs in the blood serum of the U.S. population and demographic and housing characteristics. The results of this study suggest that shorter-chain compounds are accruing more prominently than their long-chain predecessors, and our data suggests that participants' home type, vacuum frequency, and length of residence are significant exposures associated with PFAS blood serum levels. Variations in PFAAs across housing variables indicate that residential factors can be important sources of exposure to shorter-chain PFAS in the indoor environment. Future research with a large sample size is needed to assess the relationships between ultrashort and short-chain PFAS serum concentrations and housing and behavioral characteristics.

PUBLIC HEALTH IMPLICATIONS

The results of our study indicate that ultrashort-chain and short-chain PFAS are accruing in human blood at levels greater than their long-chain predecessors. While shorter-chain compounds were marketed as a safe and less persistent alternative, it is evident that they possess bio-accumulative

properties, and the long-term human health effects of exposure remain largely unknown. In April of 2024, the Biden-Harris Administration finalized a national drinking water standard to reduce exposure to long-chain PFAS from contaminated drinking water.[30] Evidence that ultrashort- and short-chain PFAS are now ubiquitous in humans and the environment suggests that PFAS contamination will continue to be a prominent public health issue until further regulations are imposed.

SUPPORTING INFORMATION

The Supplemental Tables can be accessed at Supplemental Tables.

The complete list of analytes and details of the analytical methods for our previous study can be accessed at: Elevated Levels of Ultrashort- and Short-Chain Perfluoroalkyl Acids in US Homes and People.

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