TITLE: Incidence of per-and polyfluoroalkyl substances (PFAS) in private drinking water supplies in Southwest Virginia, USA

1. Kathleen Hohweiler* (*corresponding author*); Graduate Research Assistant; Biological Systems Engineering; 155 Ag Quad Lane; Virginia Polytechnic Institute and State University; Blacksburg, VA 24061-0303; khohwe1@vt.edu; ORCid: 0000-0002-0925-0191
2. Leigh-Anne Krometis; Associate Professor; Biological Systems Engineering; 155 Ag Quad Lane; Virginia Polytechnic Institute and State University; Blacksburg, VA 24061-0303; krometis@vt.edu; ORCid: 0000-0002-8602-1245
3. Erin J. Ling; Water Quality Extension Associate; Biological Systems Engineering; 155 Ag Quad Lane; Virginia Polytechnic Institute and State University; Blacksburg, VA 24061-0303; erinjling@vt.edu; ORCid: 0000-0002-7817-7979
4. Kang Xia; Associate Director of Virginia Agricultural Experiment Station, Director of the Center for Advanced Innovation in Agriculture, Professor; School of Plant and Environmental Sciences; 185 Ag Quad Lane; Virginia Polytechnic Institute and State University; Blacksburg, VA 24061-0303; kxia@vt.edu; ORCid: 0000-0002-4092-3403

Abstract

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made contaminants of increasing human health concern due to their resistance to degradation, widespread environmental occurrence, bioaccumulation in organ tissue, and potential negative health impacts. Private drinking water supplies may be uniquely vulnerable to PFAS contamination, as these systems are not subject to federal regulations and often include limited treatment prior to use. The goal of this study was to determine the incidence of PFAS contamination in private drinking water supplies in two counties in Southwest Virginia, USA (Floyd and Roanoke), and to examine the potential for reliance on citizen-science based strategies for sample collection in subsequent broader efforts. Samples for inorganic ions, bacteria, and PFAS analysis were collected on separate occasions by participants and experts at the home drinking water point of use (POU) for comparison. Experts also collected outside tap samples for PFAS analysis. At least one PFAS was detectable in 88% of POU samples collected (n=60), with an average total PFAS concentration of 23.5±30.8 ppt. PFOA and PFOS, two PFAS compounds which presently have EPA health advisories, were detectable in 13% and 22% of POU samples, respectively. Of the 31 PFAS compounds targeted, 15 were detectable in at least one sample. On average, each POU sample contained approximately 3.3 PFAS compounds, and one sample contained as many as 8 compounds, indicating that exposure to a mixture of PFAS in drinking water may be occurring. Although there were significant differences in total PFAS concentrations between expert and participant collected samples (Wilcoxon, alpha = 0.05), collector bias was inconsistent, and may be due to the time of day of sampling (i.e. morning, afternoon) or specific attributes of a given home. Future studies reliant on participant collection of samples appear possible given proper training, coordination, and instruction.

Keywords

PFAS; drinking water; household wells; citizen science; private drinking water

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are part of a large group of anthropogenic contaminants of increasing environmental and public health concern (Sunderland et al., 2019). These compounds were originally developed in the 1930s to make a variety of consumer products resistant to water, grease, and stains. As a result of their unique stable chemical structure and the hydrophilic nature of certain compounds, PFAS are mobile in various media including soil, water, and air, and are highly resistant to environmental degradation. Consequently, PFAS are commonly detectable in groundwater and soil across the globe (Brusseau et al., 2020; Newell et al., 2021). Both long-chain perfluoroalkyl acids (PFCAs; ≥ 7C and PFSAs; ≥ 6C) and their short chain fluorinated alternatives, such as PFBS and PFBA, are associated with a variety of negative human health effects, including diminished vaccine response, metabolic and immune disorders, liver disease, and kidney cancer (National Toxicology Program, 2016, Grandjean et al., 2012, Liu et al., 2018, Fenton et al., 2021, Shearer et al., 2021). Given frequent detection of PFAS in a variety of water sources, both treated and untreated, drinking water is currently suspected to be one of the major contributors of human exposure to PFAS compounds (Domingo & Nadal, 2019).

Regulation of PFAS in municipal drinking water supplies is rapidly evolving globally as monitoring programs intensify (Teymourian et al., 2021). US EPA released proposed National Primary Drinking Water Standards (NPDWS) Maximum Contaminant Levels (MCLs) and non-enforceable MCL goals (MCLGs) in early 2023 for six PFAS chemicals including PFOA, PFOS, PFNA, HFPO-DA, PFHxS and PFBS (US EPA, 2023, FRL 8543-01-OW). However, it is worth

noting that these advisories, and any eventual enforceable limits, only apply to municipal drinking water treatment systems. The US EPA does not have jurisdiction over the estimated 23 million private drinking water wells in use nationally. Hence, monitoring for standard and emerging contaminants in these households' drinking water is the full responsibility of the participant (Murray et al., 2021). In some cases, these wells can serve up to 15 service connections or 25 people and may still be considered a private system. Given the minimal oversight and treatment, these systems are considered uniquely vulnerable to contamination (Hernandez & Pierce, 2023; Lee & Murphy, 2020).

At present, the understanding of PFAS presence in private drinking water supplies and associated consumer risk is limited (Hu et al., 2016; Lee & Murphy, 2020). The most extensive national study examining PFAS in untreated groundwater used for drinking analyzed point of use (POU) samples submitted from across the US. At least one PFAS compound (out of 44 target compounds) was detectable in 20% of private wells (55/269 samples), with an average concentration of 8.2 ng/L (Smalling et al., 2023). However, this point of use study only analyzed PFAS in six Virginia wells, with none of these private well samples collected from the Appalachian region. Seventeen PFAS were observed at least once; the short-chain compounds, PFBS and PFHxS, and long-chain compound, PFOA, were most frequently detectable. These results are similar to those reported in an earlier examination of samples from the point of extraction of 254 private and public wells representing seven aquifer networks across the Eastern United States (McMahon et al., 2022). In this study, fourteen of the 24 targeted PFAS compounds were detectable at least once in ambient groundwater samples; at least one PFAS compound was detectable in more than half of the samples (54%; n=254) and more than two PFAS compounds were detectable in 47% of samples. The frequency of PFAS detections and

total (Σ) concentrations differed considerably between the study well networks with detection frequencies ranging from 3.7 to 92.9% for different aquifer systems. It is important to recognize that, unlike the Smalling et al. (2023) survey, the McMahon et al (2020) investigation collected groundwater well supply samples, not samples from the point of use, which may not be representative of consumer exposure, as PFAS levels may be impacted by treatment processes and distribution/premise plumbing.

Four geographically focused field-scale studies have examined exposure to PFAS in private well-dependent homes at the POU in the US. Mulhern et al. (2021) quantified the effectiveness of a commercially available POU, single stage, activated carbon block (ACB) filter in removing both long and short chain PFAS from 18 homes in North Carolina. All wells (n=18) and all samples contained at least one detectable PFAS compound prior to treatment; the majority (83%) of pre-treatment influent samples had at least one PFAS above the Minimum Reporting Level (MRL), ranging from 0.5-6.2 ppt (Mulhern et al., 2021). Although this study focused on quantifying the effectiveness of POU carbon activated filters in removing both short- and long-chain PFAS, it is important to note past research suggests the majority of private well systems commonly do not employ treatment devices (Smith et al., 2014). Additional community focused efforts have demonstrated that drinking water wells located in very rural or remote areas are not immune to PFAS contamination, though potential sources of groundwater contamination differ. Twelve PFAS compounds were detectable at least once in 25 private well water samples collected in southeast Alaska, with total PFAS concentrations as high as 120 ppt (Babayev et al., 2022). It should be noted that while the community targeted by this study was quite rural, there was a nearby supply airport. Alam et al (2022) recruited 68 citizen scientists to collect well water samples from outside spigots located near areas with land applied biosolids in Pennsylvania.

PFAS compounds were detectable in 73.5% of samples, with a maximum concentration of 80.4 ng/L (Alam et al., 2022)

Previous surveys of PFAS incidence in private drinking water systems in the United States have demonstrated that detectable levels and number of PFAS compounds are variable and warrant additional study in other locations. There is a particular need for more data on rural areas as well as standardization of collection methods. The present effort aims to identify potential incidence of PFAS compounds in private drinking water supplies in southwest Virginia, and to establish a standard participant sampling protocol that can be applied by researchers in larger future statewide PFAS investigations. Although many previous studies of private drinking water supplies for contaminants other than PFAS have relied on simple citizen science protocols to collect samples for later analysis in laboratories, established protocols for sample collection by professionals for PFAS testing are quite rigorous (e.g., (Michigan Department of Environmental Quality, 2021). Previous studies of PFAS in private wells have also primarily focused only on high-risk areas after a known PFAS environmental exposure or wells located in close physical proximity to legacy PFAS areas including airports and land applied with biosolids (Alam et al., 2022; Babayev et al., 2022; Mulhern et al., 2021). Our study recruited participants from one Virginia county designated as “higher risk” due to the presence of airports and unlined landfills and one county designated as “lower risk” as centralized point sources of PFAS have not been locally identified (Virginia Department of Health, 2021). At the time of submission, available data regarding PFAS incidence in private wells in Virginia, which serve approximately one-fifth of state residents, is limited to a few private well samples (5 samples) collected as part of the Smalling et al (2023) study. Given the limited data available on PFAS incidence in Virginia surface and groundwater supplies (Virginia Department of Health, 2021), the present study

represents an important first step in quantifying PFAS concentrations in Virginia drinking water, with significant implications for the ongoing management and regulation of emerging environmental contaminants in private drinking water nationally.

2. Methodology

2.1 Site Selection and Participant Recruitment

Participants were recruited through a partnership with the Virginia Household Water Quality Program (VAHWQP), a Cooperative Extension effort that provides low-cost water quality analysis and private water system education throughout the Commonwealth of Virginia (Benham et al., 2016). Potential participant households were recruited from Roanoke and Floyd County, VA as these communities are: within easy driving distance from the central Virginia Tech campus where the lab analysis was conducted; have a history of significant participation in past VAHWQP drinking water clinics; comprise similar underlying geologies (fractured bedrock); and represent high (Roanoke) and low (Floyd) PFAS contamination risks, respectively, as determined by the Virginia Department of Health Office of Drinking Water (VDH ODW) during their 2021 Phase 1 PFAS Study (Virginia Department of Health, 2021). Recruitment emails were sent to all past VAHWQP participants from the last three years (2020 – 2022) who cited their location as within the boundaries of Floyd and Roanoke counties. In exchange for participation in the sampling effort, households received a free standard VAHWQP home water analysis (i.e., for lead, bacteria, etc.) in addition to PFAS testing as an incentive. Participant selection, recruitment, and human subjects research were approved and overseen by the Virginia Tech Institutional Review Board (IRB #21-492). From the group of responding households (approx. 22 total), 10 households were selected in each county (n=20 total) for participation based on participant availability during set sampling times.

2.2 Sample collection

In order to examine potential bias introduced by participant sampling comparing to expert sampling, tap water from each of the twenty participating homes was sampled twice (Figure 1): once by experts (i.e., trained graduate students, Extension faculty, professors) following the Michigan Department of Environmental Quality PFAS General Sampling Guidance (e.g., specifying clothing, use of personal care products prior to sampling, etc.) (Michigan Department of Environmental Quality, 2021), and once by participants following simplified directions as described in Figure S-1 (Supplemental Information). All in-home sampling was completed between June and August 2022.

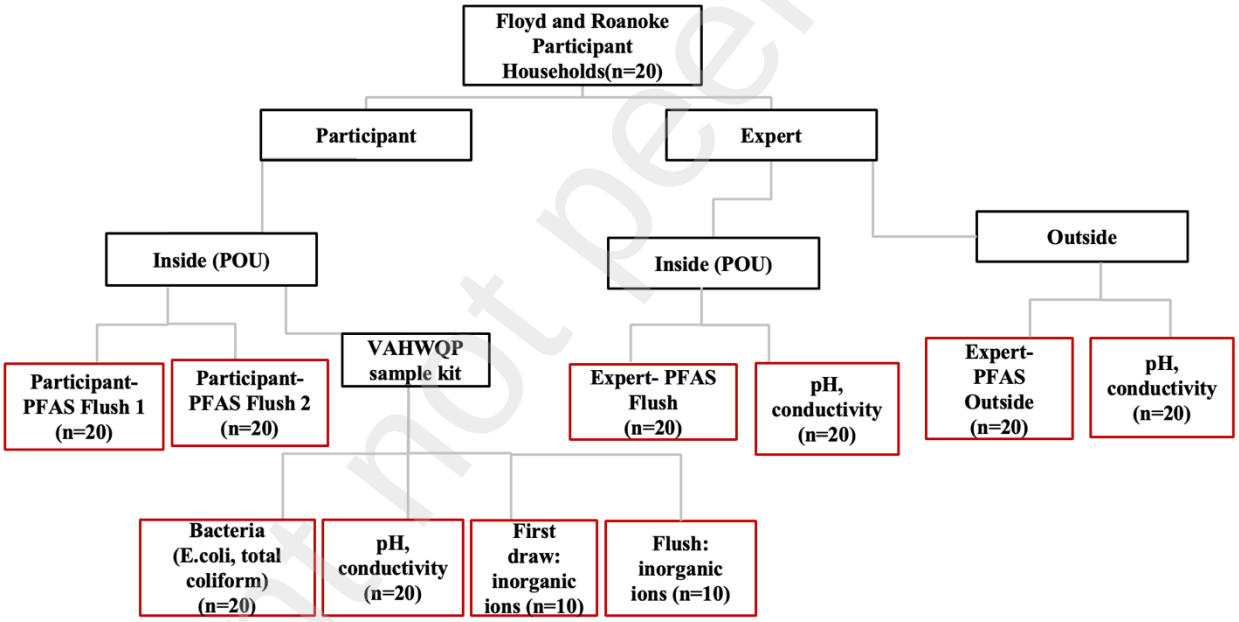

Figure 1. Scheme of sample collection by participant (Participant-PFAS Flush 1 and Participant-PFAS Flush 2) and experts (Expert- PFAS Flush and Expert- PFAS Outside) at each participating home.

Experts traveled to each participant's home in Floyd or Roanoke County at a pre-arranged date and time. Samples were collected inside the home at the point of use identified as the primary source of drinking water for the household (e.g., kitchen faucet), and outside of the

home at a well spigot or faucet depending on the configuration of the participant's plumbing system. Experts collected two samples at both locations: a sample for conductivity and pH analysis in an acid-washed 250 mL bottle and a sample for PFAS analysis in a 1 L high density polyethylene (HDPE) bottle that was washed, prepared, and stored according to Michigan DEQ guidelines (labeled as Expert- PFAS Flush and Expert- PFAS Outside in Fig. 1) (Michigan Department of Environmental Quality, 2021). Samples for PFAS analysis were immediately returned to a double Ziploc bag and put on ice in a separate cooler following collection.

Directly following expert sample collection, households were provided a home sampling kit that included six bottles for sample collection and sampling instructions (Supplementary Figure S-1). Residents of the same county were instructed to collect their tap water samples on a specified day, approximately a week after the initial expert home visit, from the same inside POU sampled by experts during the initial home visit. Per EPA home sampling guidelines typically used in Extension programming (EPA 815-F-18-022; US EPA, 2018), households were instructed to leave water stagnant in household plumbing overnight (e.g. not use the water for at least 8 hours) in order to obtain a first-draw sample for metals analysis, flush the pipes for 1 minute, and collect five additional samples (a first flush of 1 L sample for PFAS analysis (labeled as Participant- PFAS Flush 1 in Fig. 1), three subsequent 250 mL flush samples for analysis of metals, bacteria, and nutrients, and a fifth flush of 1 L sample for PFAS analysis (labeled as Participant- PFAS Flush 2 in Fig. 1). All samples for PFAS analysis were collected in pre-prepared 1 L HDPE bottles, and the participants were instructed to immediately return them to double Ziploc bags and seal following collection. Participants were instructed to place all samples into their home refrigerator prior to returning samples the same day to researchers at a pre-specified central drop-off point and time for transport back to Virginia Tech for processing.

In keeping with typical VAHWQP sample clinic procedures (Benham et al., 2016), household sampling occurred on a single pre-arranged day for each county.

2.3 Participant Surveys

In addition to providing home water samples, participants also completed two household surveys: a standard VAHWQP survey consisting of 18 simple multiple choice and short answer questions addressing perceived water quality, water use, known household plumbing characteristics, and alternate drinking water sources (Supplementary Figure S-2); and a PFAS-specific survey consisting of 24 multiple choice and short answer questions regarding the household's water treatment system, sociodemographic characteristics, product use, and prior PFAS awareness (Supplementary Figure S-3). Responses were coded into Microsoft Excel and matched to home water quality results via an alphanumeric lab code included on surveys and collection bottles for each sampling kit.

2.4 Analytical Methods

The samples collected by households and experts were analyzed for PFAS on an Agilent LCMS 6490 Triple Quad System according to US EPA Standard Methods 533 and 537.1 via direct injection (Shoemaker & Tettenhorst, 2009) within a week of initial collection. As a result of quantification limits on the LCMS machine and analytical standard availability, the quantification limits for PFOA, PFOS, and PFBS was 25 ppt; the quantification limit for all other target analytes was 5 ppt. Positive detections below the lowest analytical standard were quantified via extrapolation of the established calibration curve, as regulatory limits at the time (2022) were below quantification levels (Teymoorian et al., 2023). In order to increase the amount of quantifiable data available for comparison to health advisories, extrapolated values were used in subsequent statistical analyses; however, these quantification limits are noted, and

values detectable below quantification limit (BQL) values are denoted as such via asterisks in all Figures.

In addition to samples for PFAS analysis, separate samples were collected for standard analyses offered by VAHWQP. These samples were analyzed in the laboratory within 12 hours of collection for: total coliform bacteria and *E. coli* via the Colilert defined substrate method (IDEXX, Westbrook, MN; Standard Method 9223B), pH and conductivity via an Oakton benchtop pH/conductivity/TDS meter (Cole Parmer, Vernon Hills, IL, USA), metallic cations (Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Ba, Pb, U) via ICP-IMS process as described in Standard Methods 3030D and 3125B (APHA/AWWA/WED, 1998); and nitrate and fluoride via Standard Methods 4500-NH and 300.0, respectively (APHA/AWWA/WEF, 1998).

2.5 Statistical Analysis

All statistical analyses were conducted in RStudio version 12.0 +353 (RStudio, Boston, MA). After confirming a non-normal data distribution via the "qqnorm" command in the "ggplot" data package, the Wilcoxon Signed Rank Test was used to determine potential differences between total PFAS concentrations in participant and expert collected samples with significance defined at an alpha value of 0.05. Potential significant relationships between values of the most commonly detectable PFAS and inorganic ions of human health concern were identified via a Spearman's correlation matrix.

2.6 Communication to Participating Households

In keeping with the mission of VAHWQP and Virginia Cooperative Extension (VCE), all participating households received copies of their water quality results via mail or email, depending on preference. Standard water quality measures (i.e. coliform, lead) were compared to

existing EPA regulatory guidelines for municipal systems per standard VAHWQP communication (Benham et al., 2016). Because both the analytical and regulatory landscape for PFAS is rapidly evolving, households received separate letters outlining their PFAS results (example provided per Supplementary Figure S-4). It is worth noting that in these examples, results are benchmarked against the 2022 Health Advisory limits in keeping with the regulatory landscape at the time of sample collection.

3.0 Results

3.1 Household System Design, Demographics, and PFAS Product Use

A total of 19 participants completed the standard VAHWQP/Extension survey describing basic system characteristics and perception of in-home water quality. Based on participant reported information on well age and depth, the wells sampled in this study were constructed between 5 to 69 years ago with average age of 24 years, and their depth ranges from 210 to 500 feet with an average of 325 feet deep. Most participants reported household systems were built with plastic piping (79%) though 40% indicated they also had some copper piping. Most homes (65%) reported using some kind of water treatment, reporting the usage of sediment filters (58%) and/or water softeners (32%). Other reported in-home treatment included ultraviolet light devices (16%), acid neutralizers (11%), reverse osmosis (5%), iron removal (5%), and carbon filters (5%). In keeping with previous research in the region (Patton et al., 2020), the most common reported water treatment devices target removal of aesthetic contaminants (i.e. taste, smell) rather than health based contaminates, such as lead or bacteria.

Although all homes answered at least some of the questions in the supplemental PFAS survey detailing household demographics and product use, only 18 of the 20 homes responded to all survey questions. On average, each household included 2.5 individuals (i.e., 55 reported

household members across 20 homes), and 86% self-reported as white. Over half of the households (55%) included at least one member with a post-college degree (i.e., MS, PhD, etc.), and almost half (45%) reported an annual household income over $98,000. These demographics are in keeping with past reports on program participants (Smith et al., 2014).

The most common "daily" usage products likely to contain PFAS identified by participants were nonstick cookware and cosmetics (both 37%). Households also reported "occasional" use of waterproofed clothing (i.e., Gore-Tex) (79%), aluminum foil (63%), sunscreen (58%), microwave popcorn (47%), and microwave meals (26%). Less than half of the participating households recalled hearing about PFOA/PFOS in the national news (44%) and only two households had heard about PFAS in state or local news (11%). These rates are surprisingly low, given increasing media and public attention as a result of the US EPA's interim health advisories and a publicized discharge of PFOA by DuPont in the mid-Ohio Valley (Herrick et al., 2017). Three participants (16%) included notes at the end of their survey stating that they did not know what PFOS/PFOA were, even though they had volunteered for a study focused on these contaminants. Some participants (39%) reported having seen products advertised as "PFOA/PFOS free", though the majority (89%) indicated that the designation of a product as "PFOA/PFOS free" would influence their decision to buy it.

3.2 Bacteria and Metal Cations in Samples

For standard/incentive VAHWQP/Extension program samples (n=20), none were positive for *E. coli*, and less than one-third (30%) were coliform positive (average coliform concentration = 560 MPN/100mL for positive samples). Fecal indicator bacteria are the most common priority pollutant observed in private system samples (Allevi et al., 2013; Patton et al., 2020) and very few of these systems included disinfecting treatment, so detection in some

samples was not surprising. Interestingly, one of the homes that submitted total coliform-positive samples employed a treatment system rated to remove bacteriological contaminants (i.e., UV light). Overall, bacteria contamination was lower than in similar research studies on private wells in Virginia (Allevi et al., 2013; Smith et al., 2014), which may reflect the success of participants' previous engagement with Extension programming (Benham et al., 2016).

No samples collected from any home in the study exceeded the MCLs for metals (e.g., lead, arsenic) (Table S-1, Table S-2). More than 80% (82.5%) of in-home water samples contained lead (max observation = 10.2 ppb) in either the first draw or flush sample (n=40), which is notable as the US EPA MCLG for lead is zero (i.e. not detectable). Samples from two homes in Floyd and one home in Roanoke County exceeded the Treatment Technique recommendations for copper, which could indicate an issue with corrosive source water. Aluminum, iron, and manganese were detectable in samples at values exceeding recommended taste and aesthetic levels (SMCL) in homes in both counties. 70% of homes in Roanoke and 20% of homes in Floyd County yielded samples that exceeded the SMCL for sodium. Increased consumption of sodium via consumption of well water can be an issue for those on a low sodium diet or with chronic medical problems such as hypertension or high blood pressure (Thompson et al., 2022). Sodium is frequently used in water softeners, which were use in one-third of homes, and is a naturally occurring cation often associated with wells drilled in areas with bedrock consisting of sandstone, limestone, dolomite, and shale, commonly sodium-bearing rocks, all of which are present in the two counties where the samples were collected (Virginia Department of Health, 2021).

3.3 PFAS incidence in Point of Use (POU) samples

At least one PFAS compound was detectable in at least one indoor POU drinking water sample in 19 of the 20 participating homes (i.e., all but Home #1, Figure 2). Of the total 60 POU samples collected, 88% contained at least one PFAS. The average Σ PFAS concentration in POU samples across both study counties was 23.5 ppt, with a maximum observation of 160 ppt in a single sample.

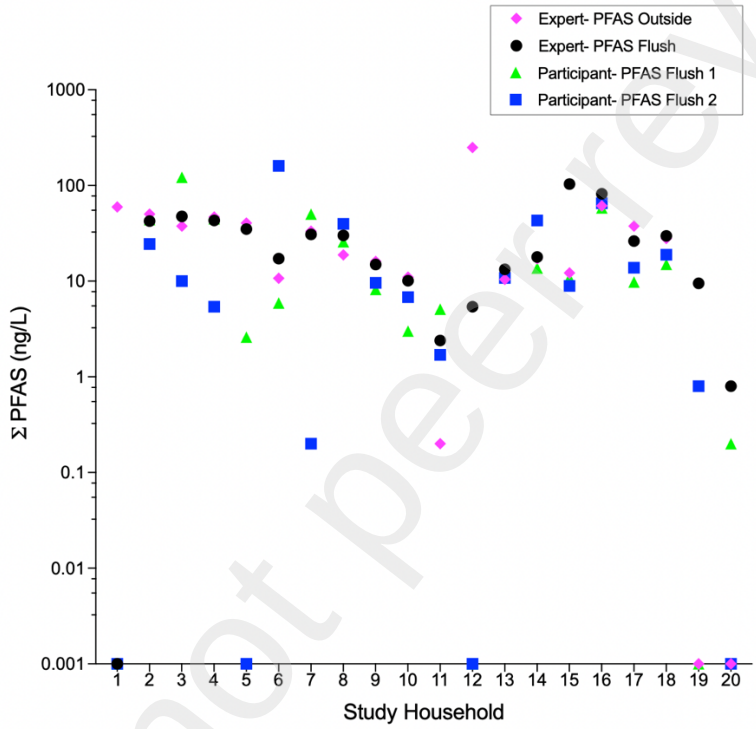

Figure 2. Σ PFAS concentrations in each of the study households for all four samples per home: Three POU samples (Two collected by the expert, one at the POU and one at an outside spigot (Expert- PFAS Flush and Expert- PFAS Outside) and two collected by a participant at the point of use (Participant- PFAS Flush 1 and Participant- Flush 2).

Short-chain PFAS, though only accounting for 8 of the 31 target PFAS in analysis, were responsible for 65% of detections in the POU samples (n=60; Supplementary Fig. 5-6). PFBS, which is currently associated with an US EPA proposed MCL of 2,000 ppt, was detectable in a majority (62%; 37/60 samples) of POU samples, though the average concentrations were lower

(3.99 ppt in Roanoke and 1.07 ppt in Floyd). Gen-X, also currently included as a target in US EPA guidelines, was not detectable in any sample. At present, the US EPA is recommending a Hazard Index (HI) to assess the human health risk associated with mixtures of four PFAS compounds, which combines detections of two short-chain PFAS (GenX and PFBS) with the detection of two long chain PFAS, PFHxS and PNFA, as a proxy for overall health risk. Although PFHxS (10 POU samples, 16.67%), PFBA (29 POU samples, 48.33%) and PFBS (37 POU samples, 61.67%) were detectable, PFNA and GenX were not detectable in any sample, and no POU samples in this study were in violation of the proposed Hazard Index of 1.

Of the 17 long chain PFAS analyzed, 9 were detectable in at least one sample. The most commonly detectable long chain compound was ADONA (42%). The two long chain PFAS compounds associated with current US EPA proposed MCLs, PFOA and PFOS, were detectable in 13% and 22% of samples, respectively. Though observed concentrations were relatively low (average values: 7.4±6.87 ppt and 3.1±4.02 ppt), the current recommended US EPA MCLG is zero, i.e. no detection.

Only three POU samples yielded a Σ PFAS value that exceeded the 2016 US EPA Lifetime Health Advisory of 70 ppt (160 ppt, 103.2 ppt, and 81.8 ppt) primarily consisting of PFPeA, PFBA, and PFOA. These three detections represent three separate homes, but it is worth noting that in every case, each home also submitted a POU sample with a Σ PFAS ≤ 70 ppt. This reflects intra-home variability and potential time-dependent fluctuations in PFAS at the POU. For example, one of the three homes with Σ PFAS ≤ 70 ppt had an initial expert POU sample reporting a Σ PFAS value of 103.2 ppt. However, the additional samples collected a week later from yielded Σ PFAS concentrations of 10.8 ppt and 8.90 ppt, which were below the overall study average of 23.5 ppt. In the case of the home with a POU sample containing Σ PFAS of 160

ppt, the initial expert sample measured 17.2 ppt Σ PFAS and the first participant flush sample collected one week later had a Σ PFAS value of 5.90 ppt. It was the second participant flush sample, collected only minutes following the first participant flush sample, that measured 160 ppt Σ PFAS.

3.4 PFAS Occurrence at the Outside Tap

In addition to POU samples, PFAS samples were collected by the expert team from an outdoor tap or spigot. In total, 90% (18/20) homes had at least one PFAS in their outside tap sample with an average Σ PFAS concentration of 37.2 ppt (Supplementary Figure S-9). Samples collected from outside taps in Floyd County had nearly twice the average Σ PFAS concentration (55.4±77.43 ppt) as compared to those collected from homes in Roanoke County (30.5±19.43ppt), which was surprising given that Roanoke County was designated as "high risk" and Floyd as "low risk" for PFAS contamination in finished municipal drinking water by the Virginia Department of Health (Virginia Department of Health, 2021). These detections may indicate the presence of environmental or geological factors contributing to PFAS presence in Floyd if the outside taps represent pre-treatment and pressure tank water. However, given the heterogeneity of indoor plumbing layouts, these high detections might also represent changes throughout the premise plumbing, i.e., the presence of garden hoses or well caps that may be sources of PFAS. The overall study maximum Σ PFAS detection was from an outdoor tap in Floyd County (249.5 ppt; included the study maximum detection of PFBS at 202.5 ppt). Observed outside tap sample Σ PFAS concentrations were not significantly correlated to either inside tap sample Σ PFAS concentrations or coliform concentrations, suggesting there may not be any interaction with surface soil in the case of a cracked well cap.

3.5 Numbers and Types of PFAS

As evidenced by the Hazard Index approach proposed by US EPA (FRL 8543-01-OW, US EPA 2023), simultaneous exposure to mixtures of different PFAS compounds is of increasing concern. In this study, 66% of analyzed POU samples contained ≥ 3 PFAS compounds. The average sample contained over 3 separate PFAS compounds (Figure 3, Supplementary Figures S5-8). The average number of distinct PFAS compounds per sample was similar for Floyd and Roanoke Counties (3.4 and 3.2, respectively). The maximum number of PFAS compounds detectable a was 8 in a single POU sample collected from a Roanoke County home.

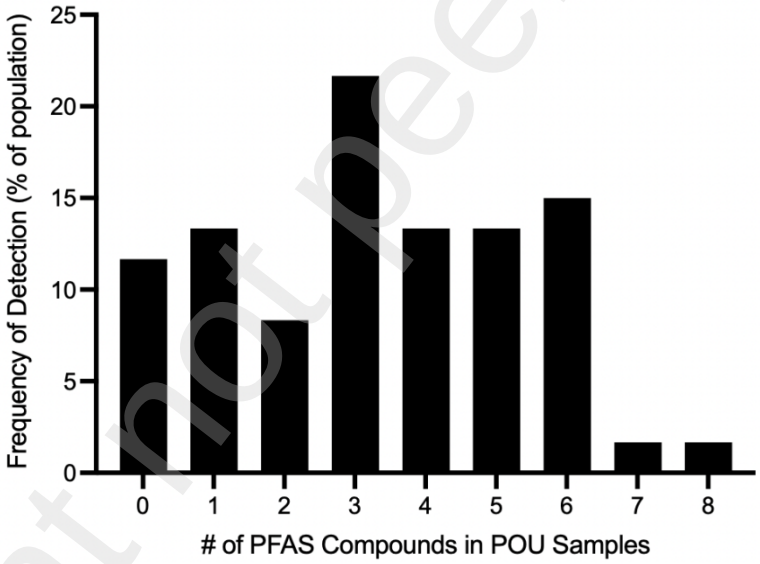

Figure 4. Histogram showing the % frequency of detection of the number of PFAS compounds in POU samples (n=60).

The average number of PFAS in a POU sample was 3.3 while the average in outside samples was 3.4. The types of PFAS within samples were quite unique (Supplementary Figures S5-8), though some general patterns were observed. Short chain PFCA, PFBA, and short chain PFSA, PFBS, were detectable together in 21 POU samples (n=60). Long chain PFAS and their

known replacement short chain alternatives were similarly detectable together in in the POU samples. For example, PFOS and its short-chain replacement, PFBS, were detectable together in 10 of the 60 total POU samples. PFOA also co-occurred with various other PFCAs including PFHxA and PFBA, although seemingly less frequently. Unlike PFOS + PFBS, PFOA + PFBS were detectable together in only one sample and PFOA + PFHxA were detectable together in 4 samples.

A Spearman's Correlation Matrix was used to examine the strengths and directions of relationships between concentrations (i.e., magnitudes) of PFAS compounds in all analyzed samples (n=80; 60 POU samples and 20 samples from outside taps; Figure 5). Among relationships of various PFAS, high detections of PFHxA and PFBS were indicative of higher overall Σ PFAS concentrations in samples. Relatively strong positive correlations were observed between PFOS and PFHxS (r_s =0.66) and PFOS and PFOA (r_s=0.63), (i.e., higher concentrations of PFOS were likely to correlate to higher concentrations of PFOA and PFHxS) This is not surprising, as PFHxS and PFOS both have similar atomic structures: fully fluorinated carbon backbones with sulfonic acid head groups, with differing numbers of carbons (8 and 6, respectively). PFOS and PFOA are both considered metabolites of other PFAS but are no longer in active production in the US and are highly resistant to environmental degradation (Martin et al., 2010).

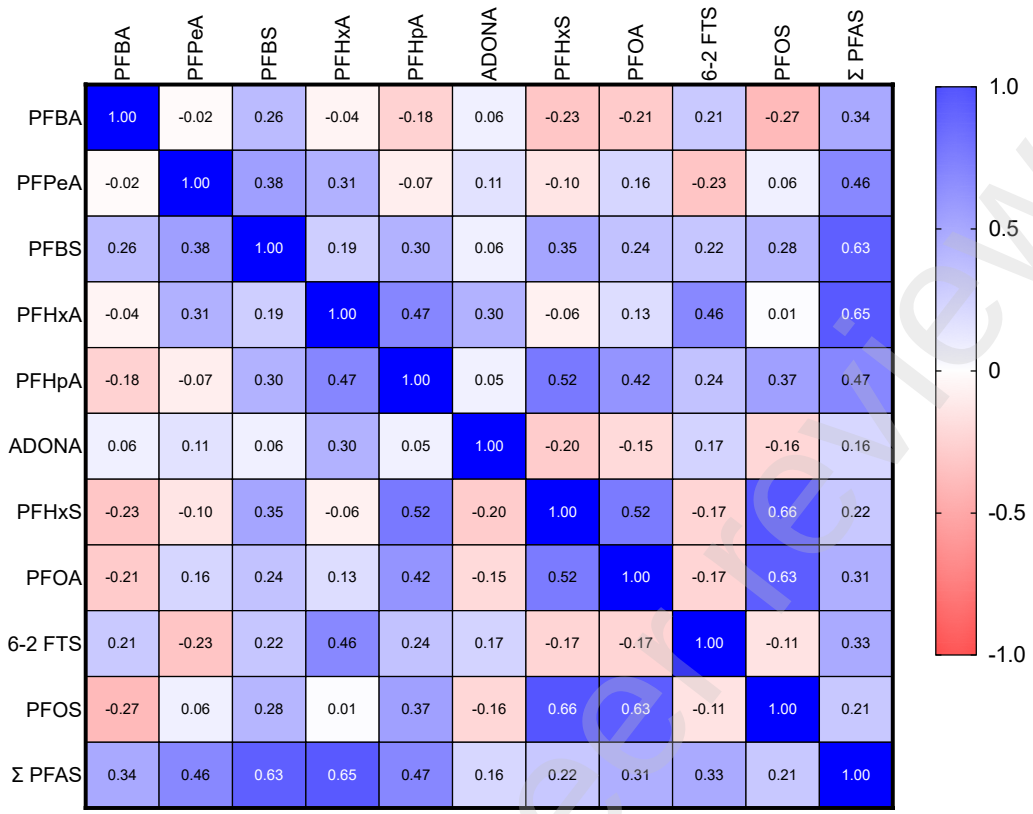

Figure 5. Spearman's Correlation Matrix showing strength and direction of relationships between various detectable PFAS across all tested samples (n=80). A perfect positive association of ranks would be assigned a r_s of +1.

3.6 PFAS and Metallic Cation Detection

As patterns of metallic cations may be associated with specific plumbing and/or source water characteristics, potential relationships between these and Σ PFAS are explored in Figure 6. While well-established relationships between corrosivity markers (Pb and Cu, r_s=0.79) were present, there were few relationships between Σ PFAS and other inorganics. Σ PFAS at the POU and Cl^- were weakly positively correlated (r_s=0.46) for POU samples, as were Σ PFAS and SO_4^{2-} (r_s=0.45) in samples from outside tap. These observations do echo those reported by McMahon et al. (2020): in their analysis of 254 groundwater samples collected in the eastern United States, concentrations of Cl^- and SO_4^{2-} were significantly higher in samples containing PFAS detections

than in samples with no detections, leading the authors to hypothesize that higher concentrations of Cl^- and other divalent anions could compete with PFAS for sorption sites on minerals, making them more mobile. Although the present study examined POU drinking water rather than source groundwater, given the few treatment systems present, geochemistry would be expected to play a role in PFAS detection. McMahon et al. (2020) also hypothesized that the reductive dissolution of manganese and iron oxides in more anoxic groundwater could reduce the PFAS sorption capacity of solids and subsequently mobilizing PFAS in the environment. However, in the case of this study, only very weak inverse correlations between Σ PFAS and total Fe (r_s=-0.26) and Σ PFAS and total Mn (r_s=-0.13) in the tested water samples were observed.

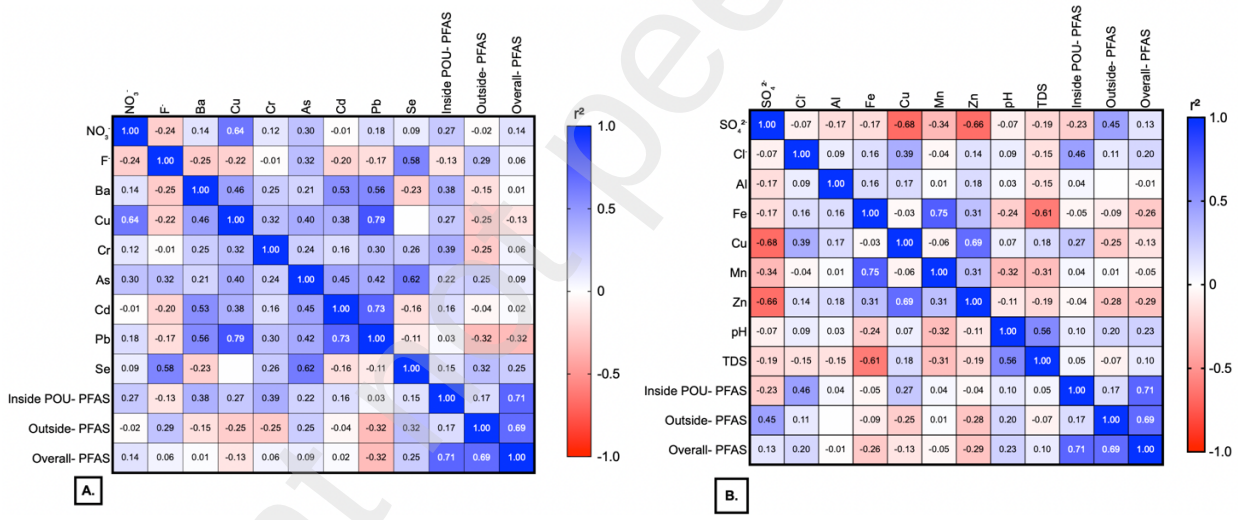

Figure 6. Spearman's correlation matrixes illustrating the strength and direction of statistical relationships between average concentrations of inorganics with SMCLs (a) and MCLs (b), compared to average Σ PFAS concentrations.

3.7 Comparison of expert and participant samples

Previous PFAS studies have both employed citizen scientists to collect their own drinking water samples for analysis (Alam et al., 2022; Mulhern et al., 2021; Smalling et al., 2023) as well as teams of experts (McMahon et al., 2022). Instructions for the sampling of groundwater from state agencies can include strict requirements for field technicians regarding sample preparation, field clothing, and personal care product usage to reduce the potential for sample contamination. However, the level of strictness regarding sample preparation and collection are generally not practical for citizen scientists. Assessment of the potential for sampling bias between trained experts following strict state mandated protocols and citizen participants using simplified easy to follow protocols for sample collections for PFAS testing had not yet been formally explored.

In this study, when comparing the expert collected POU samples and the first participant collected flush sample (Participant- PFAS Flush 1) (Fig. 1), mean ranks of expert samples were significantly higher Wilcoxon Signed Rank Test (alpha= 0.05). However, when expert collected POU samples were compared to the second participant collected samples (Participant- PFAS Flush 2, Fig. 1), the converse was true: the mean ranks associated with the Σ PFAS in the participant samples were significantly higher. This difference in directionality suggests that there is no predictable, consistent bias between participant (citizen science) and expert collected samples, i.e. citizen participants are as well well-equipped as experts to collect their own drinking water samples for PFAS analysis give sufficient instructions and coordination. Perhaps more compellingly, this result also suggests further questions regarding the flushing behavior of PFAS compounds in private drinking water systems that are worthy of further investigation, which will be discussed further in the subsequent section.

4.0 Discussion

The goal of this project was to study the incidence of PFAS in private drinking water supplies in two Southwest Virginia counties, Floyd and Roanoke, and to determine if it was possible to employ citizen science initiatives for water sample collection for PFAS testing in subsequent, larger sampling efforts. Overall, 88% of POU samples (n=60) contained at least one PFAS, with an average Σ PFAS concentration of 23.5± 30.8 ppt, indicating that the occurrence of long and short chain PFAS in samples from private drinking water systems is observable. The rate of detection is more than three times that reported in the 2021 VDH study of Virginia municipal drinking water systems (24% of samples positive, i.e. 15/63). However, it is worth noting that in keeping with regulatory needs, analytical methods are advancing rapidly and reducing detection limits (Teymourian et al., 2021). The VDH study did not list the value of their practical quantitation level (PQL) and their samples were analyzed after undergoing solid phase extraction which would test PFAS in the water and those initially adsorbed on minerals in the water. Our study relied on direct sample injection of supernatant after centrifuging a sample at high speed to separate water and the mineral solids in the sample. The role that dissolved minerals play in the sorption of PFAS in untreated groundwater used for drinking has not been fully explored and is deserving of future attention to further advance method development to best quantify actual consumer exposure (Hellsing et al., 2016).

Almost half of the PFAS compounds targeted by the UPLC-MS/MS analysis (15/31) were detectable at least once from participants' private drinking water systems. Two thirds of samples (53/80) contained ≥ 3 PFAS in mixture. Laboratory studies have demonstrated that complex mixtures of specific PFAS, including PFHxS, GenX, PFNA, and PFBS, may have additive toxic effects (Fenton et al., 2021). An examination of patterns of PFAS detection in groundwater wells along the east coast of the US noted that mixture of different PFAS occur

frequently, with ≥ 3 PFAS occurred in > 80% of samples but the dominant PFAS varied between sampled well networks (McMahon et al., 2022). The present study echoes these findings as the POU sample contained on average of 3.3 PFAS and the sample from outdoor taps contained on average 3.4 PFAS.

Given the frequent detections of PFAS at the POU, it is worth noting that this study confirmed past work noting that drinking water treatment in homes supplied by private systems, particularly treatment targeting priority contaminants, is still uncommon. Although 65% of participants (n=18) reported using some kind of treatment device, the most common treatments are targeted to address aesthetic, rather than health-based, issues and include sediment filters or water softeners. Although field research on PFAS removal at the POU in private systems is limited, Mulhern et al. (2021) reported that activated carbon filters were effective in removing short and long chain PFAS from homes located in areas with drinking water systems known to be contaminated with GenX. However, only one out of the 20 homes in this study reported employing a carbon filter for treatment. Although more extensive research is required to confirm consistent PFAS removal under the variable water chemistries typical of private wells in baseline conditions, it is worth noting that activated carbon filters could, depending on their rating, also concurrently reduce lead and other inorganic ion concentrations in participant drinking water (Mulhern et al., 2021; Patton et al., 2023).

Multiple past examinations of private well water have relied on citizen science for sample collection, i.e. actual participants collect samples from their point of use (Allevi et al., 2013; Patton et al., 2023; Pieper et al., 2015; Smith et al., 2014). Given the ubiquity of PFAS in household products and the stringent collection protocols recommended for PFAS sample collection, there was some concern that inadvertent sample cross contamination would occur if

samples were collected by participants without following strict EPA protocols rather than expert researchers. While differences between expert and participant samples were present and significant, the directionality was not consistent: Σ PFAS concentrations in expert collected samples were significantly greater than the first draw POU sample collected by participants, and significantly less than the flushed POU sample collected by participants. This likely reflects changes in water quality associated with the time of sample collection: expert samples were generally collected at mid-day or the early afternoon, while participant samples were collected first thing in the morning. Further research is required to determine whether plumbing materials (e.g. Teflon-based tape or paste) might contribute to observed fluctuation of PFAS concentrations with time of the day for POU samples.

In addition to the potential effects related to time of collection, the study does include additional key limitations that should be acknowledged. As an initial investigation, samples were collected in a relatively small area with similar underlying geology; future efforts should explore the potential influence of well depth, aquifer materials, and ecoregion on PFAS detection. The average participant was relatively affluent, in keeping with previous private water system studies reliant on similar programming (Smith et al., 2014). Including more diverse households in future efforts targeting private systems will allow examination of economic and sociodemographic factors as predicting factors for PFAS contamination at the POU in keeping with recent examinations of patterns of PFAS detection at community water systems (Liddie et al., 2023). Finally, connecting sociodemographic and water quality data also will permit explicit consideration of the economic feasibility of potential treatment options.

5. Conclusions

Results from this work indicate that the occurrence of PFAS compounds from the point of use of private drinking water supplies is quite common (88% of total samples, n=80). Though the average concentration detectable was relatively low (23.5±30.8 ppt), it is worth noting that the composition of PFAS in the tested samples was variable and complex, demonstrating both inter and intra home variability. Considering the frequent detection and co-detection of various short chain PFCAs and PFSAs in drinking water supplies, it is clear there is a need for further research determining the human health risks associated with the chronic consumption of complex PFAS mixtures through drinking water. Although reliance on participant collected samples did not appear to result in predictable bias, variability between expert and participant samples, as well as between samples collected at indoor POU and an outdoor spigot, suggest that premise plumbing or other system factors may be contributing to PFAS concentrations at the POU, which should be further investigated. Finally, participants reported very little PFAS awareness, which suggests a need for continuing and improved education for those reliant on private drinking water supplies.

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