

# Investigating Exposure to Perfluoroalkyl Substances (PFASs) in Indoor Environments

by

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## Executive Summary

Poly and perfluoroalkyl substances (PFASs) are a class of chemicals used as stain and water repellents in a variety of consumer and industrial products, including in firefighting foams, textile coatings, cosmetics, and in paper coatings. PFASs are typically classified into two groups, precursor compounds and stable end products, which have differing chemical properties and human exposure pathways. The stable end products, including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), have long half-lives in the environment and are formed by the degradation of precursor molecules. In contrast, precursor compounds like fluorotelomer alcohols (FTOHs) and polyfluoroalkyl phosphates (PAPs) have short half-lives and can more readily degrade to stable compounds like PFOA via hydrolysis and oxidation. However, the U.S. has recently phased out the use of PFOS and PFOA in industrial and consumer applications, and manufacturers are responding by substituting lower carbon chain compounds (e.g. C4 and C6) in their products which are presumably less bioaccumulative and toxic. The implications of these manufacturing shifts on exposure pathways are yet to be researched in the literature.

Recent studies have indicated that these compounds are present in human tissues around the world. PFASs have been detected in breast milk and blood, and can cross the placenta in pregnant women. Both the precursors and stable metabolite compounds are associated with a variety of adverse health effects, including immunotoxicity and endocrine disruption. Thus, it is important to discern relevant exposure pathways for PFASs and the behaviors that increase or decrease exposure. While dietary exposure is predicted to be a primary pathway of exposure, inhalation and hand to mouth transfer of these compounds are relatively understudied. The goal of this study was to explore novel routes of indoor exposure to potential PFAS precursors and to determine if personal behaviors are associated with serum PFAS levels.

A cohort of 40 adults from the Duke community was recruited to participate in this study. Participants that lived off campus were recruited using emails, flyers, and word of mouth. The study cohort included 26 females and 11 males, and ages ranged from 22-34. Personal exposures were measured by collecting household dust, hand wipe, silicone wristband, and serum samples, and personal behaviors were analyzed via questionnaires. Non-volatile PFAS compounds were analyzed using negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS), and volatile PFASs were quantified with positive chemical ionization gas chromatography mass spectrometry (GC-MS). Statistical analyses employed in this study include Spearman correlation tests within and between exposure matrices, Kruskal-Wallis rank sum tests, and generalized linear models.

Six compounds (PFHxA, PFHxS, PFOA, PFNA, PFOS, and PFDA) were detected with greater than 50% detection frequency in the cohort. Males had higher serum levels of these compounds compared to females, and PFHxS and PFOS were detected in the highest concentrations in the cohort overall. A variety of personal behaviors also were associated with serum PFAS levels. Frequent consumption of microwaveable meals or microwave popcorn was associated with higher serum levels of certain compounds, particularly PFOA. The use of water filtration devices was associated with body burdens of PFASs, where the use of water filtration was correlated with lower PFOA levels but higher PFHxA levels in serum. Additionally,

cleaning behaviors such as high dusting frequency were significantly associated with lower serum PFHxA and PFHxS levels in the cohort.

After adjusting for sex, hand washing was also significantly associated with serum PFAS levels. PFDA, PFHxS, and PFNA were all higher in participants that washed their hands less frequently in multivariate analyses. Water filtration and dusting frequency continued to be significantly associated with internal PFAS doses after adjusting for sex, while microwaveable meal intake and vacuuming frequency were marginally significant.

In general, precursor compounds (FTOHs, diPAPs) were detected more frequently than persistent end products in the house dust, hand wipes, and silicone wristbands. While there were no general correlation trends between the levels of PFASs in dust, hand wipes, and wristbands with the serum PFAS levels, compounds in the hand wipes and wristbands were highly correlated to each other. Additionally, the dust, wristbands, and hand wipes were dominated by C6 PFAS precursors over C8 compounds, indicating that manufacturing phase outs are influencing exposure in the household.

This report makes several key points:

- Individuals are ubiquitously exposed to perfluoroalkyl substances based on the high detection frequencies of PFASs in serum samples.
- Males had higher body burdens of PFASs compared to females, potentially due to behavioral differences in some behaviors or shorter serum half-lives in women.
- Personal behaviors, specifically dusting frequency, hand washing, and water filtration were significantly associated with serum PFAS levels after adjusting for sex.
- The dominance of 6 carbon precursor molecules over longer chain compounds indicate that manufacturing shifts are altering human exposure to PFAS chemicals.

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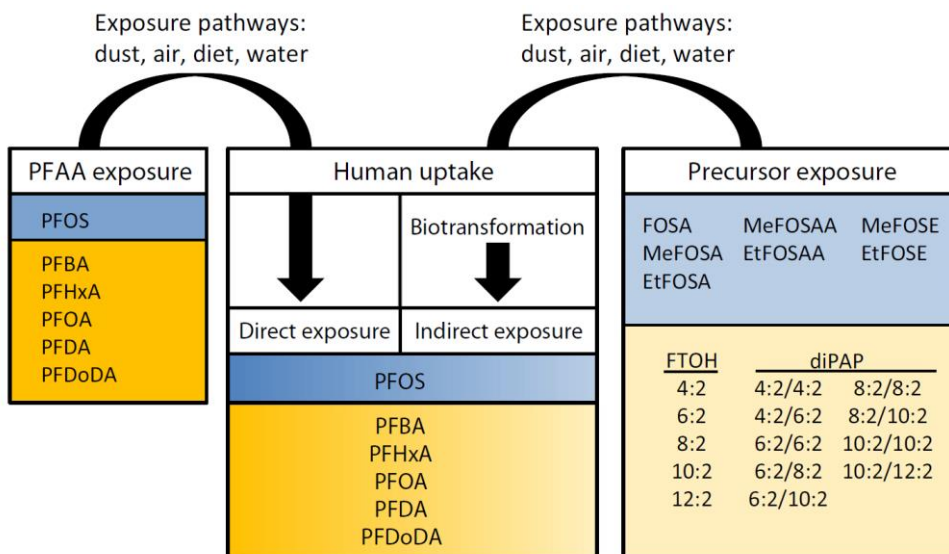
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## Introduction

Poly and perfluoroalkyl substances (PFASs) are extensively used in a variety of industrial processes and consumer products around the world. These compounds represent a large group of chemicals with similar physicochemical properties, with a base structure of a fluorinated hydrophobic carbon chain attached to numerous hydrophilic heads. This results in unique properties like chemical and thermal stability, good surfactant properties, and low surface free energy (Fromme et al., 2009). As a result of their physicochemical properties, these compounds are able to repel both moisture and oil and are commonly used in carpets, clothing, and cooking utensils as stain, water, or grease repellents (Zheng et al., 2012; Stahl et al., 2011). Due to the combination of hydrophilicity from polar functional groups and hydrophobicity from the fluorinated backbone, PFASs have exceptional surface performance and are also widely used as polymerization aids, surfactants, and in fire-fighting foams (Fujii et al., 2013). Overall, PFASs have a variety of useful properties and have been synthesized since the 1960s, eventually becoming extensively utilized in a variety of industries and products.

While the overarching term ‘PFAS’ has been applied to chemicals used in a variety of industrial and consumer products, there are specific classes of these compounds with different functional groups that influence their fate and persistence in the environment. Of interest in this study are three types of perfluoroalkyl chemicals: the perfluoroalkyl acids (PFAAs), fluorotelomer alcohols, and polyfluoroalkyl phosphoric acid esters (see Table A1 for names and structures in Appendix A). Perfluoroalkyl acids include carboxylic, sulfonic, sulfinic, phosphonic, or phosphinic acid functional groups attached to a carbon-fluorine backbone (Buck et al., 2011). Common examples of perfluoroalkyl carboxylic acids include perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA), which denote eight-chain and six-chain carbon backbones, respectively. Similarly, perfluorooctanesulfonic acid (PFOS) and perfluorohexanesulfonic acid (PFHxS) are perfluoroalkyl sulfonic acid analogues. PFOS and PFOA are the most extensively researched PFAS compounds in the scientific literature, due to their persistence in the environment, ubiquitous human exposure, potential toxicity, and high production volume (Buck et al., 2011). These acids are typically directly emitted into the environment during manufacturing processes or indirectly formed by degradation and metabolism of precursor molecules in both the environment and in living organisms (Butt et al., 2014) (see Figure 1).

Alternatively, fluorotelomer alcohols and polyfluoroalkyl phosphates two classes of PFAS precursor molecules. These PFAS precursors can break down to form the aforementioned perfluoroalkyl acids. Polyfluoroalkyl phosphates (monoPAPs, diPAPs) are surfactants used in a variety of paper contact coatings and household products that have been detected in wastewater and sewage sludge, and break down to form fluorotelomer alcohols by hydrolysis (Butt et al., 2014). Fluorotelomer alcohols (FTOHs) are typically present in end products as impurities from their use during manufacturing of fluorotelomer acrylates, and have been found in various environmental samples and in air as a result of previous PFAS degradation (Buck et al., 2011). In general, the FTOHs are quickly oxidized in the environment and in organisms to stable end products including the perfluoroalkyl carboxylic acids (Butt et al., 2014).



**Figure 1.** Precursor degradation and direct routes of exposure to PFAS substances (adapted from Gebbink et al., 2015).

These types of compounds, along with other PFASs, have triggered increased scientific interest due to their widespread distribution, exposure, and potential toxicity to humans and the environment. PFASs have been measured in human blood worldwide, with the highest levels typically detected in industrialized areas (D'eon and Mabury, 2007). Interestingly enough, PFASs and their precursors have been found in the Arctic Circle, specifically in soil, sediment, wildlife, but also in the human blood and breast milk of individuals living in that remote area (Butt et al., 2010). The presence of PFAS compounds in remote areas is indicative of transport pathways like oceanic currents or atmospheric deposition which result in global environmental contamination. Global transport has been confirmed by observations of wet and dry deposition

of PFASs in North America, Asia, and Europe (Dreyer et al., 2009; Dreyer et al., 2010). In addition, elevated PFAS levels are detected in human blood, most notably in the U.S., Sweden, China, Catalonia, Japan, Germany, Brazil, Colombia, Belgium, Italy, Poland, India, Malaysia, and Korea (Ericson et al., 2008). The prevalence of PFASs in the environment and high usage of treated products in industrialized countries indicate that ecosystems and humans in these areas are at risk for exposure and, consequently, negative health impacts.

Due to the global presence of PFASs in various environmental media, humans are exposed to these compounds through a variety of pathways, both directly and indirectly. PFASs have been detected in fish, meat, animal products, and plants, therefore ingestion of contaminated food is one major direct exposure route (Stahl et al., 2011). PFOS concentrations above the limit of detection were measured in canned vegetables, potatoes, eggs, sugar, fish, and preserves, with over 10 other types of PFASs detected in potato products alone (Stahl et al., 2011; Ericson et al., 2008). Drinking water may also be a considerable source of exposure if the water is contaminated by PFASs (Stahl et al., 2011; Hölzer et al., 2008). Measurements from a Mid-Ohio Valley population that was exposed to drinking water contaminated with PFOA showed not just high serum concentrations, but also evidence that exposure to perfluoroalkyl compounds during pregnancy was associated with decreased birth weight in full-term infants (Darrow et al., 2013). Similarly, evaluation of surface water in the Cape Fear River Basin in North Carolina revealed detectable levels of target PFASs in every sample taken (Nakayama et al., 2007). Despite measurements of PFASs in food, it is not clear if direct exposure via ingestion of metabolites in foodstuffs, indirect exposure by precursors in food paper packaging, or other pathways are driving levels of PFAS compounds in human blood (Ericson et al., 2008).

Besides food and water, another possible route of exposure to PFASs may be through inhalation of contaminated air or exposure to contaminated dust. Outdoor air measurements reveal high concentrations of the more volatile PFASs, including FTOHs, perfluorooctane sulfonamidoethanols (FOSEs), and perfluorooctane sulfonamides (FOSAs), especially near PFAS manufacturing facilities (Fromme et al., 2009). In general, higher concentrations of PFASs were found in urban areas compared to rural locations, with total mean concentrations of FTOHs ranging from 11 to 165 pg/m<sup>3</sup> (Fromme et al., 2009). Though outdoor air can contain significant levels of PFASs, indoor air has been shown to contain certain PFASs whose levels can exceed that of outdoor air by a factor of 20 (Shoeib et al., 2005). Volatile FTOHs in

workplace air samples have been linked to serum PFOA levels, indicating that inhalation of contaminated indoor air may be a significant route of exposure to humans (Fraser et al., 2012). As indoor dust concentrations have been shown to follow PFASs in indoor air, accidental ingestion of contaminated dust can also contribute to overall human exposure to PFASs (Shoeib et al., 2005).

Regardless of the source of exposure, PFAS contamination in humans is evidenced by detectable levels of these man-made chemicals in various tissues. In humans, certain PFASs are likely to accumulate in blood and breast milk, and serum half-lives of stable compounds are estimated to range from 2.4 years (for PFOA) to 4.3 years (for PFOS) (Russell et al., 2015; Olsen et al., 2012). Once in the blood, these chemicals are biologically active, either undergoing metabolism to form acid end products or inducing effects in the exposed organism by binding to proteins (Stahl et al., 2011). The chronic toxicity of PFHxA has been studied in rats, and researchers have discovered liver and papillary (kidney) necrosis in high dosages (200 mg/kg/day) but no evidence of carcinogenicity (Klaunig et al., 2015). In contrast, PFOA is a confirmed carcinogen in rats, producing a tumor “triad” in the liver, testis, and pancreas common with agonists of the peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) (Klaunig et al., 2012). While PFOA can activate PPAR $\alpha$  in humans, rats, and mice, rodent PPAR $\alpha$  agonism is much more sensitive than human induction, therefore this PFAS is not suspected to cause liver cancer in humans (Klaunig et al., 2012). This is supported by earlier cellular toxicity testing in human liver cells, which confirmed that short chain PFASs (PFBS and PFHxA) do not generate reactive oxygen species (ROS) or DNA damage at concentrations up to 2000 $\mu$ M (Eriksen et al., 2010). Of the longer chain PFASs studied for genotoxicity, PFOA and PFOS generated very modest amounts of ROS, and PFNA caused minor DNA damage at a cytotoxic concentration, suggesting that these endpoints are not relevant to adverse effects of PFASs in liver cells (Eriksen et al., 2010).

One area of increasing concern with perfluoroalkyl chemicals is their potential to act as endocrine disruptors. Precursor molecules like the diPAPs and FTOHs have been linked to significant decreases in male hormones, including androgens, testosterone, androstenedione, and DHEA, and 8:2 diPAP is associated with increased cortisol levels in human adrenal cells (Rosenmai et al., 2013). PFOA, mono and diPAPs, as well as FTOHs also caused statistically significant increases in estrone in this study, with 8:2 FTOH also causing increased levels of

17 $\beta$ -estradiol (Rosenmai et al., 2013). PFASs have also shown to interact with thyroid hormones both *in vivo* and *in vitro*, with PFOS specifically acting as a thyroid hormone agonist (Ren et al., 2014; Weiss et al., 2009).

Despite these suggestive results, the interactions that PFASs have with the human body as a whole are less straightforward. According to Gilliland and Mandel, PFOA possibly exacerbates the liver's response to obesity and xenobiotics (1996). PFOS and PFOA have been linked with thyroid disease in the adult U.S. population (Melzer et al., 2010). The ability of PFASs to be transferred to babies before birth through the placenta and after birth through breast milk is concerning, and could indicate the possibility of PFASs acting as developmental toxicants (Olsen et al., 2009; Llorca et al., 2010; White et al., 2011). Epidemiological data are mixed, with some studies indicating that PFOA exposure during pregnancy is associated with lower birth weights, birth lengths, and abdominal circumferences (Fei et al., 2007; Fei et al., 2008) while other studies show no impact on gestational indices in PFOA-exposed women (Nolan et al., 2009). Later in life, serum PFAS levels have been associated with increased prevalence of attention deficit/hyperactivity disorder in young teens (Hoffman et al., 2010). In general, the epidemiological data suggest that PFASs may be altering hormone-related health outcomes in humans.

As toxicity data grows in the literature, the increased level of concern surrounding PFOS, PFOA, and other long-chain perfluoroalkyl substances has led to their phase-out and removal from products in the U.S. (Wang et al., 2013). PFOS was voluntarily phased out by its sole manufacturer, 3M, in 2000, while PFOA has been the target of EPA's 2010/2015 voluntary Stewardship Program for removal from the chemicals market (Wang et al., 2013). In response, manufacturers are shifting to shorter-chain PFASs, in the hope that they will be less bioaccumulative in both humans and in the environment (Wang et al., 2013). While these steps are good, the effects of perfluoroalkyl substances in the environment and in humans are still present due the amount of stain repellent products still in use and the ability of these compounds to linger in both wild animals and humans for many years (Stahl et al., 2011; Guo et al, 2012).

Given the recent shifts in manufacturing trends, it is necessary to better characterize the relative importance of the exposure pathways for both persistent and degradable PFASs in humans and assess if concentrations of certain compounds are changing in these pathways in response to regulatory modifications. Precursor exposure research has historically been limited,

and no research to our knowledge has been done to estimate PFAS exposure through hand wipes or silicone wristbands (Alves et al., 2014). Additionally, there is a lack of knowledge around what behaviors and personal characteristics are associated with serum levels, nor what steps could be taken to reduce one's own exposure. The overall aim of this study was to explore novel routes of indoor exposure to PFAS precursors in a population of young adults, and to determine the types of personal behaviors that may be associated with serum PFAS levels.

## **Materials and Methods**

### *Study Design*

Prior to recruitment and sampling, all protocols were approved by the Institutional Review Board through the Office of Research Support at Duke University. Young adult volunteers were recruited from the Duke community in the summer and early fall of 2015 using emails, study fliers, and word of mouth. Eligible participants were at least 18 years of age and lived off campus, and a total of 40 participants were recruited in the convenience sample. All participants gave informed consent prior to providing any information or samples. The study was divided into two time periods to facilitate efficient sample collection. Initially, surveys were administered and dust samples were taken during house visits, and then wristband, hand wipe, and serum samples were collected during an on campus visit.

### *Questionnaires*

During each home visit, a brief questionnaire was provided to collect information on demographics and personal behaviors (see Appendix C for a copy of the questionnaire). Participants provided information on a variety of personal characteristics, including age, sex, race, height, weight, marital status, and education level. Personal behaviors were also examined, and included frequency of hand washing, vacuuming, dusting, and fast food or microwaveable meal consumption. Questions regarding personal care product use and possession of stain-repellant clothing or furniture were also included in the questionnaire. Each participant's average time spent in specific microenvironments, such as indoors, outside, in public buildings, or on public transportation, was also recorded in the questionnaire. The distribution of answers for

each question was assessed and responses were grouped into high or low categories based on these natural distributions for further statistical analyses.

### *Home Visits*

Dust and questionnaire samples were first collected during a scheduled home visit. A dust sample was collected from each house, based on the room each participant testified to spending the most time in. The dimensions of this room were measured prior to sampling. Dust samples were collected by the same researcher at each home (to minimize differences in sampling techniques) using a Eureka Mighty-Mite vacuum cleaner (Model 3670) and crevice tool attachment. Dust samples were isolated before entering the vacuum bag by a cellulose extraction thimble (Whatman International, Maidstone, UK) inserted between the hose tubing and the crevice attachment and held in place with a rubber O-ring. The entire floor surface area of the room (or its equivalent) was vacuumed, and the vacuum operator drew the crevice tool across the top of all surfaces to collect as much dust as possible. Once vacuuming was complete, the cellulose thimble was removed, wrapped in foil, and sealed in a plastic bag at -20°C until analysis. All dust collection steps were performed with nitrile gloves to minimize collector contamination.

### *Laboratory Visits*

Within two weeks of the dust collection, participants agreed to visit our research laboratory to provide a wristband, hand wipe, and blood sample. Precleaned silicone wristbands were distributed to a subset of participants (n=25) five days prior to their laboratory visit, and participants were instructed to leave the wristbands on for the entire 5 days, including during showering and sleeping activities. Upon entering the lab, blood samples (~14mL) were collected by a trained phlebotomist via venipuncture and collected in a serum separator tube. These blood samples were immediately set on ice to clot for one hour, centrifuged for 5 minutes at 3500 rpm, and frozen at -20°C until analysis. The serum was then sub-sampled into 1 mL aliquots for separate PFAS and albumin analyses (stored in plastic cryovials). Serum albumin was analyzed at the Duke University Health System (DUHS) Clinical Laboratories using spectrophotometry techniques. In brief, serum samples were reacted with bromocresol purple (BCP) to form a

colored product, which was then measured for absorbance at 600 nanometers using a Beckman Coulter Unicel DxC 600/800 System (Beckman Coulter, 2015).

Wristbands were collected by the researchers, wrapped in foil, and sealed in a plastic bag and frozen at -20°C until analysis. Three pre-cleaned wristbands that were wrapped in foil were used as field blanks. Hand wipe samples were collected by soaking sterile gauze wipes in ~3 mL of methanol and wiping the surface of each participant's hands from fingers to wrist, top to bottom, twice, then wipes were wrapped in foil, sealed in plastic bags, and frozen at -20°C until analysis. This process was performed by the same researcher for each participant to keep the technique as uniform as possible throughout the sample collection process. Three hand wipes were opened, soaked in methanol, and then wrapped again to serve as field blanks. Nitrile gloves were worn during all wristband, hand wipe, and serum sample collection.

### *Chemicals*

Calibration, internal standard mixtures, and individual recovery standards were purchased from Wellington Laboratories (Guelph, ON, Canada). Formic acid, ammonium hydroxide and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO). Oasis HLB columns were purchased from Waters Corporation (Milford, MA). The Luna C18(2) (2.5 µm, 50 x 2 mm) analytical column was purchased from Phenomenex (Torrance, CA, USA). Methanol and water were HPLC grade (Burdick & Jackson, Honeywell, Morris Plains, NJ). Envi-Carb columns (1 mL, 100 mg) were purchased from Supelco (Bellefonte, PA). Mini-UniPrep vials (0.2 µm) were from GE Healthcare Life Sciences (Marlborough, MA). The serum method was validated using Standard Reference Material 1957 (National Institute of Standards and Technology, 2016).

### Sample Analyses

#### *Serum*

Serum samples were analyzed for perfluorinated carboxylates (C4-C10 PFCAs) and perfluorinated sulfonates (C4, C6, C8 PFSA) only. Extraction methods were modified from Liu et al. (2015). Samples were thawed, then 1 mL of serum was transferred to a 15 mL polypropylene centrifuge tube and spiked with the internal standard mixture (6 ng of MPFAC-

MXA). The samples were acidified with 4 mL of 0.1 M formic acid, then vortexed and sonicated for 15 minutes. Extracts were cleaned and concentrated using solid-phase extraction (SPE) techniques with Oasis HLB columns (60 mg, 3 ml). Briefly, the SPE columns were preconditioned with 2 mL methanol followed by 2 mL of 0.1 M formic acid. The sample was loaded and the tube was rinsed (3x) with 500  $\mu$ L of 0.1 M formic acid. The SPE column was rinsed with 2 mL of 0.1 M formic acid followed by 1 mL of 1%  $\text{NH}_4\text{OH}$  in water. Analytes were eluted with 1.0 mL of 1%  $\text{NH}_4\text{OH}$  in methanol and reduced to near dryness under a gentle nitrogen gas stream in a 40°C water bath. Extracts were reconstituted in 500  $\mu$ L of methanol, transferred to a cryovial and kept at -20°C until analysis.

#### *Hand Wipes and Wrist Bands*

Hand wipes and wrist bands were placed into 50 mL glass tubes, spiked with the internal standard mixture (MPFAC-MXA,  $^{13}\text{C}_4$ -6:2 diPAPs &  $^{13}\text{C}_4$ -8:2 diPAPs,  $^{13}\text{C}_4$ -6:2 FTOH &  $^{13}\text{C}_4$ -8:2 FTOH), and extracted with sonication in 10 mL methanol. This fraction contained the PFSAs, PFCAs and diPAPs. The methanol was decanted into a clean tube, the process was repeated twice more and the extracts combined. The hand wipes and wrist bands were then extracted using 10 mL ethyl acetate (3x) following a similar procedure to create the FTOH fraction. Both fractions were reduced in volume to 1 mL under a gentle nitrogen gas stream.

Samples for FTOH analysis were cleaned with florisol SPE columns. Briefly, columns were conditioned with 5 mL of methanol followed by 3 mL of hexane. The extracts were loaded and the column eluted with 4 mL of hexane that was discarded. Sample vials were rinsed with a small volume of ethyl acetate and FTOHs were eluted from the SPE column with 10 mL of ethyl acetate. These eluates were collected, blown down to near dryness, and reconstituted in hexane.

#### *House Dust-PFCAs, PFSAs, diPAPs*

Extraction methods for PFCAs, PFSAs and diPAPs were adapted from De Silva et al. (2012). Before analysis, dust samples were sieved to <500  $\mu\text{m}$  using soil sieves and then weighed on a mass balance. Approximately 0.100 g of sieved dust was weighed and transferred to a 15 mL polypropylene centrifuge tube. The internal standard mixture (12 ng of MPFAC-MXA, 30 ng of  $^{13}\text{C}_4$ -6:2 diPAPS &  $^{13}\text{C}_4$ -8:2 diPAPs) was spiked into the sample and 5 mL of methanol was added. The tubes were vortexed, covered and left overnight at room temperature.

The following day the tubes were sonicated for 15 minutes and centrifuged for 5 minutes at 4000 g. The methanol layer was transferred to a clean tube, the extraction was repeated and methanol layers combined. The extracts were reduced in volume to approximately 1 mL under nitrogen gas in a 40°C water bath and then cleaned using Envi-Carb SPE columns. Briefly, the column was pre-conditioned with 3 mL of methanol which was discarded, then the sample was loaded, the tube was rinsed with 1 mL of methanol, and the column eluted with an additional 1 mL of methanol. The eluents were reduced to ~1 mL, transferred to cryovials and stored at -20°C until analysis. Finally, samples were spiked with  $^{13}\text{C}_8$ -PFOA to quantify the internal standard recovery.

#### *House Dust-FTOHs*

Extraction and clean-up methods for FTOHs were adapted from Xu et al., 2013. Methods were similar to those for the PFCAs, PFSAs and diPAPs but with the following exceptions. The internal standard mixture was 50 ng of  $^{13}\text{C}_4$ -6:2 FTOH &  $^{13}\text{C}_4$ -8:2 FTOH and the extraction solvent was MTBE:acetone. Further, the Envi-Carb columns were pre-conditioned with 5 mL of MTBE and eluted with 4 mL of MTBE. Final eluates were blown down to near dryness, reconstituted with 1 mL of ethyl acetate and stored at -20°C until analysis. Finally, samples were spiked with  $^{13}\text{C}_2$ -8:2 FTOH to quantify the internal standard recovery.

#### Instrumental Analyses

##### *PFCAs, PFSAs, diPAPs*

Extracts were analyzed for PFCAs, PFSAs and diPAPs using negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS) techniques. Chromatography was achieved under gradient conditions using a Luna C18(2) column (50 x 2.0 mm, 2.5  $\mu\text{m}$  particle size, Phenomenex, Torrance, CA) preceded by a SecurityGuard Polar-RP (4 x 2.0 mm) guard cartridge. The mobile phases were methanol and water (modified with 2 mM ammonium acetate), flow rate was 300  $\mu\text{L}/\text{min}$ , the injection volume was 10  $\mu\text{L}$ , and the column oven was 40°C. Initial conditions were 75:25 water:methanol, increasing to 50:50 in 0.75 min, to 40:60 in 1.0 min, 5:95 in 2.75 min, and held for 0.5 min before decreasing to initial conditions in 0.75 min. Data were acquired under multiple reaction monitoring conditions using optimized

parameters (see Table B1 in Appendix B). Analyte responses were normalized to internal standard responses as described in Table B1.

### *FTOHs*

Extracts were analyzed for FTOHs using positive chemical ionization mode gas chromatography mass spectrometry (GC-MS) operated in single ion monitoring mode (SIM). The SIM ions are given in Table B2. Analytes were separated using a DB-WAX column (30 m x 0.250 mm, 0.25  $\mu$ m film thickness, Agilent, Santa Clara, CA). The carrier gas was helium and the injection volume was 2  $\mu$ L. The initial oven temperature was 60°C, held 1 min, increased at 5°C/min to 75°C, increased at 10°C/min to 130°C, then 50°C/min to 240°C. Analyte responses were normalized to internal standard responses as described in Table B2 (in Appendix B).

### *Quality Control*

Several types of field and laboratory blanks were used to keep quality control high. Sodium sulfate was used as a laboratory blank for dust during the extraction and analysis process. Three clean gauze pads served as field blanks for the hand wipe samples. Similarly, three pre-cleaned silicone wristbands were field blanks for the wristband samples. For the blood, 5 laboratory blanks of bovine serum were utilized for quality control. Serum, hand wipe, wristband, and dust samples were blank-corrected using the average field (wristband and hand wipe) or laboratory (dust and serum) blank measurement. The method detection limits (MDLs) for each type of sample were calculated as three times the standard deviation of the corresponding blanks. Serum samples were normalized to the average extraction volume (1 mL), and dust samples were normalized to each sample's extraction mass.

### *Statistical Analyses*

Summary statistics were calculated for all PFASs with a detection frequency greater than 50% in the various sample matrices, and the distributions of the data were assessed for normality using histograms, Q-Q plots, and Shapiro-Wilk tests (see Appendix D). PFAS levels did not meet the conditions for normality so non-parametric methods were used for statistical analyses or PFAS values were  $\log_{10}$  transformed for GLMs. PFAS measurements <MDL were assigned a value equal to MDL/2 for statistical analyses. Serum analytes are reported in values of ng/mL

serum, hand wipe and wristband analytes are reported as total mass in ng, and dust compounds are reported as ng/g dust.

Summary statistics for demographic information were calculated. Again, data was assessed for normality using histograms, Q-Q plots, and Shapiro-Wilk tests. Height and weight met conditions for being normally distributed, but BMI and age were found to be right-skewed. ANOVAs were run to determine the interaction between height and weight with gender. Due to the non-normal distribution of most data, other analyses were assessed using nonparametric statistical tests (e.g. Spearman correlation tests). Kruskal-Wallis rank sum tests were run to determine if survey data were associated with PFAS levels. Survey responses were dichotomized (e.g. high or low) for univariate analyses. Due to the possibility of confounding by sex, multivariate models were analyzed using  $\log_{10}$  transformed serum concentrations and examined in relation to sex and various personal behaviors.

The study cohort was compared to the most recent publicly available National Health and Nutrition Examination Survey (NHANES) data, collected by the Center for Disease Control and Prevention (CDC), to assess differences between the two sample groups' serum levels. Laboratory and demographic datasets for the 2011-2012 survey cycle were downloaded from the CDC website (Centers for Disease Control and Prevention, 2012). Summary statistics were calculated for all PFASs with a detection frequency greater than 50% in the NHANES 2011-2012 survey cycle, and the distributions of the data were assessed for normality. NHANES PFAS levels did not meet the conditions for normality and thus nonparametric tests were used for correlations (e.g. Spearman tests). Comparisons between the NHANES and Duke cohorts were ran using multivariate regression models on  $\log_{10}$  transformed data, and included variables such as sex, age, and cohort source. All statistical analyses were run using R (2015). Statistical significance was set at  $p < 0.05$ .

## **Results**

### *Population Characteristics*

Forty individuals consented to participate in the study and provided a dust sample and questionnaire responses; however, only 38 of the 40 participants provided a hand wipe sample and 37 provided a serum sample. The complete cohort included 27 females and 13 males. Ages

ranged from 22 to 34 years old, with a geometric mean of 25.59 years (+/- 3.2 years). BMI values for each individual were calculated based on self-reported height and weight values, with BMI ranging from 18.55 kg/m<sup>2</sup> to 37.59 kg/m<sup>2</sup>. The geometric mean for BMI was 23.23 kg/m<sup>2</sup> (+/- 4.24). Spearman correlation coefficients were calculated based on self-reported demographic variables, with no significant degree of intercorrelation found between age and any other factor. Weight and BMI were significantly correlated, which was expected. Sex and height were assessed using a one-way ANOVA and males were found to be significantly taller than females ( $p < 0.001$ ). Descriptive statistics of demographic variables can be seen in Table 1.

**Table 1.** Population characteristics (n=40).

<b>Statistic</b>	<b>Height (cm)</b>	<b>Weight (kg)</b>	<b>BMI</b>	<b>Age</b>
Min	149.86	45.36	18.55	22
Max	193.04	108.87	37.59	34
Mean	170.75	69.12	23.55	25.78
Median	170.18	64.86	22.4	25
St. Dev.	10.07	15.01	4.24	3.2
COV	0.059	0.22	0.18	0.12
IQR	12.7	17.92	4.89	4.25
GM	170.47	67.66	23.23	25.59

### *PFASs in Serum*

Six types of PFASs were measured in serum and had adequate detection frequencies (>50%) (See Table 2). These included PFHxA, PFHxS, PFOA, PFNA, PFOS, and PFDA. Several (PFHxS, PFOA, PFNA, and PFOS) were present in all of the serum samples, while PFHxA and PFDA were present in 83.8% and 97.3% of the samples, respectively. Geometric means for these six analytes ranged from 0.14-9.23 ng/mL serum (Table 2). PFHxA was present in serum in the lowest concentrations of all six analytes, with a range of 0.01-1.00 ng/mL and a geometric mean of 0.14 ng/mL. In contrast, PFHxS and PFOS were present in the blood in much higher concentrations. PFHxS values ranged from 3.27-24.28 ng/mL serum, with a geometric mean of 9.23 ng/mL serum. PFOS values ranged from 0.39-31.35 ng/mL serum, with a geometric mean of 4.96 ng/mL serum. Geometric means of the PFASs were different for males and females, with females reporting lower levels of PFASs for all six analytes. Males averaged 1.75 ng/mL more serum PFASs than females across geometric means.

**Table 2.** Levels of PFCs in serum, hand wipes, wristbands, and house dust.

Congener	Serum (ng/mL), n=37				Hand Wipes (ng), n=38				Wristbands (ng), n=25				Dust (ng/g), n=35			
	MDL	% Detect	GM	Range	MDL	% Detect	GM	Range	MDL	% Detect	GM	Range	MDL	% Detect	GM	Range
PFBA					0.91	0	NA	NA	3.82	4.0	NA	ND-5.41	0.16	34.3	NA	ND-135.35
PFPeA	0.18	0	NA	NA	0.30	2.6	NA	ND-0.42	0.50	8.0	NA	ND-1.00	0.09	37.1	NA	ND-5.40
PFHxA	0.03	83.8	0.14	ND-1.00	0.22	10.5	NA	ND-0.77	0.32	28.0	NA	ND-1.39	0.02	100	7.24	1.19-73.86
PFHpA	0.07	32.4	NA	ND-0.30	0.03	63.2	0.05	ND-0.33	0.36	40.0	NA	ND-1.38	0.05	37.1	NA	ND-20.50
PFOA	0.06	100	1.57	0.30-4.07	0.41	21.1	NA	ND-1.42	1.81	12.0	NA	ND-2.47	0.12	48.6	NA	ND-84.43
PFNA	0.02	100	0.67	0.23-4.02	0.16	44.7	NA	ND-0.63	0.15	64.0	0.25	ND-1.61	0.03	28.6	NA	ND-28.26
PFDA	0.04	97.3	0.28	ND-1.60	0.07	29.0	NA	ND-0.37	0.45	12.0	NA	ND-1.47	0.16	0	NA	NA
PFBS	5.57	2.7	NA	ND-6.24	1.33	5.3	NA	ND-6.66	41.40	28.0	NA	ND-2443.50	2.03	0	NA	NA
PFHxS	0.05	100	9.23	3.27-24.28	0.04	29.0	NA	ND-0.32	0.93	48.0	NA	ND-61.67	0.16	40.0	NA	ND-116.10
PFOS	0.06	100	4.96	0.39-31.35	0.16	42.1	NA	ND-3.47	1.42	4.0	NA	ND-3.34	0.21	31.4	NA	ND-114.15
6:2 FTOH					0.40	97.4	19.32	ND-618.83	0.79	100	190.44	25.0-996.25	21.0	91.4	312.37	ND-48170.50
8:2 FTOH					0.48	47.4	NA	ND-256.70	0.23	100	14.64	1.78-109.69	54.4	14.3	NA	ND-1702.54
6:2 diPAP					0.09	100	3.36	1.08-96.73	0.15	100	3.80	0.44-30.33	0.21	100	250.95	26.48-2818.18
6:2/8:2 diPAP													1.59	94.3	72.94	ND-440.70
8:2 diPAP					0.04	100	0.93	0.14-116.00	0.60	64.0	1.08	ND-41.99	1.23	34.3	NA	ND-245.73

Abbreviations: % Detect, percent detectable. NA, not available, ND, not detected.

### *Albumin Levels*

Because some PFAS levels are known to bind with high affinity to proteins, we measured serum albumin in 36 of the serum samples (one sample did not have sufficient volume). The arithmetic mean of the normally-distributed albumin samples was 4.6 g/dL (+/- 0.4 g/dL). The normal range of albumin for males and females above age 17 is 3.5-4.8 g/dL (Duke University Health System, 2016). Albumin levels were not significantly correlated to any of the PFAS levels measured. However, there was a suggestive correlation between perfluorohexane sulfonate (PFHxS) and albumin, with a Spearman rho of 0.24 ( $p = 0.08$ ), indicating a possible association between blood PFAS levels and protein concentration. Given these results, the PFAS levels were not normalized to albumin for further analyses.

### *Comparison with NHANES Data*

The study cohort was compared to publicly available NHANES data to assess differences between the two sample groups' serum levels and create a context for exposure to PFASs. The most recent NHANES data with serum PFAS data was the 2011-2012 survey cycle (Centers for Disease Control and Prevention, 2012). After limiting the age range of the NHANES data to 20-35, the number of NHANES participants was 460. The gender split of the NHANES data was fairly equal, with 231 females and 229 males. Serum levels of five PFAS analytes in the NHANES cohort ranged from 0.06-116.00 ng/mL (Table 3). Geometric means of these PFASs ranged from 0.20 ng/mL (for PFDA) to 5.37 ng/mL (for PFOS). Correlations between analytes in the NHANES cohort were assessed, and all of the PFASs present were significantly positively related to each other. Significant correlation coefficients ranged from 0.27-0.71 (Table 4).

**Table 3.** Levels of PFASs in serum from NHANES Survey Cycle 2011-2012.

<b>Serum</b> (ng/mL), n=460			
<b>Congener</b>	<b>% Detect</b>	<b>GM</b>	<b>Range</b>
PFOA	99.6	1.85	ND-43.00
PFNA	99.1	0.83	ND-10.25
PFDA	84.1	0.20	ND-3.09
PFHxS	98.5	1.04	ND-36.50
PFOS	99.8	5.37	ND-116.00

**Table 4.** Spearman correlations between PFASs in NHANES cohort (n=460).

	<b>PFHxS</b>	<b>PFOA</b>	<b>PFNA</b>	<b>PFOS</b>	<b>PFDA</b>
<b>PFHxS</b>	1				
<b>PFOA</b>	0.64***	1			
<b>PFNA</b>	0.42***	0.60***	1		
<b>PFOS</b>	0.67***	0.62***	0.62***	1	
<b>PFDA</b>	0.27***	0.48***	0.71***	0.60***	1

\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$

The two sample groups were then compared using generalized linear models on  $\log_{10}$  transformed outcome data. Model variables included age in years, gender, and cohort source (see Table 5). The NHANES cohort and females were used as reference groups, and coefficients were exponentiated to facilitate interpretation of the results. For categorical variables, exponentiated coefficients represent the multiplicative change in the serum concentration relative to the reference group, and for continuous variables, coefficients are reported as per unit change. Age was not a significant explanatory variable for serum concentrations of PFASs, but was close to significant for PFOA ( $10^{\beta} = 0.99$ ,  $p = 0.10$ ). In contrast, sex was a significant explanatory variable in the models for all five PFASs, with males having serum levels ranging from 1.15 to 2.13 times those of females. Cohort source also proved significant for two of the compounds, PFDA and PFHxS, with the NHANES cohort having much lower levels than the Duke cohort. For PFDA, Duke participants contained 1.46 (95% CI: 1.15, 1.85,  $p < 0.01$ ) times as much analyte in their serum than NHANES participants. The difference between the cohorts was even more pronounced for PFHxS, where the Duke cohort contained 10.22 (95% CI: 7.80, 13.61,  $p < 0.001$ ) times as much compound as the NHANES cohort.

**Table 5.** Regression analyses for predictors of serum PFASs across cohorts.

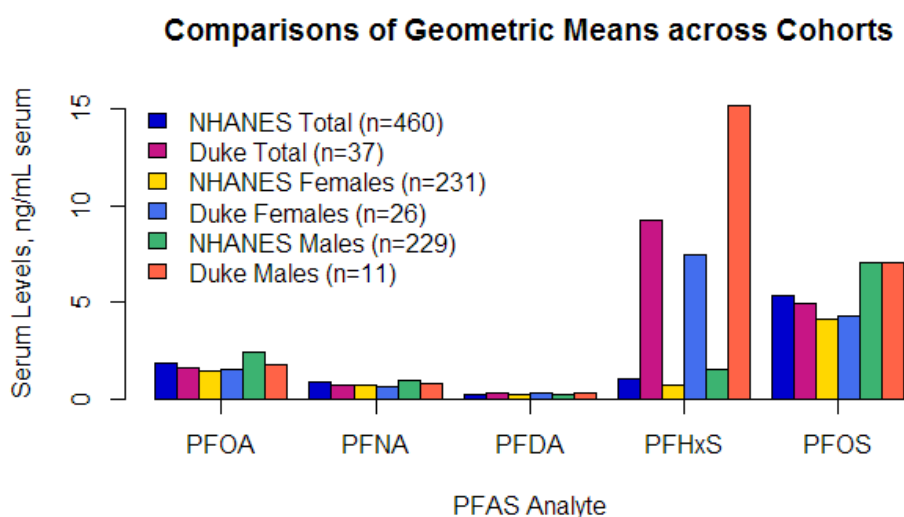
Predictor	PFOA		PFNA		PFDA		PFHxS		PFOS	
	Coefficient (95% CI)	P Value	Coefficient (95% CI)	P Value	Coefficient (95% CI)	P Value	Coefficient (95% CI)	P Value	Coefficient (95% CI)	P Value
<b>Age (years)</b>	0.99 (0.98, 1.00)	0.10	1.00 (0.99, 1.01)	0.91	1.00 (0.99, 1.02)	0.70	0.99 (0.98, 1.01)	0.48	1.00 (0.99, 1.02)	0.76
<b>Sex</b>										
Female	Reference	-----	Reference	-----	Reference	-----	Reference	-----	Reference	-----
Male	1.63 (1.46, 1.82)	< 0.001	1.33 (1.19, 1.48)	< 0.001	1.15 (1.01, 1.30)	< 0.05	2.13 (1.83, 2.48)	< 0.001	1.71 (1.49, 1.96)	< 0.001
<b>Cohort</b>										
NHANES	Reference	-----	Reference	-----	Reference	-----	Reference	-----	Reference	-----
Duke	0.92 (0.74, 1.14)	0.45	0.86 (0.70, 1.05)	0.14	1.46 (1.15, 1.85)	< 0.01	10.22 (7.80, 13.61)	< 0.001	1.03 (0.79, 1.34)	0.82

Exponentiated beta coefficients represent the multiplicative change in the serum concentration relative to the reference group for categorical variables, or per unit change for continuous variables (age).

The differences between the cohorts were also explored using charts and bar plots. Since sex was a significant explanatory variable in the model, each cohort was categorized by gender and geometric means were calculated (see Table 6). As confirmed by the GLM, females in both the NHANES and Duke cohorts had lower geometric means than males in their respective groupings. This trend was further visualized in the following bar plot, where differences between cohorts and sex for PFHxS are easy to see (Figure 2).

**Table 6.** Geometric means of PFASs in serum by sex and cohort.

Congener	Duke Serum (ng/mL)		NHANES Serum (ng/mL)	
	Males (n=11)	Females (n=26)	Males (n=229)	Females (n=231)
PFOA	1.73	1.50	2.39	1.44
PFNA	0.79	0.62	0.96	0.72
PFDA	0.32	0.26	0.21	0.18
PFHxS	15.19	7.48	1.53	0.71
PFOS	7.02	4.28	7.03	4.10



**Figure 2.** NHANES and Duke cohort comparisons of geometric means.

### *Serum Questionnaire Results*

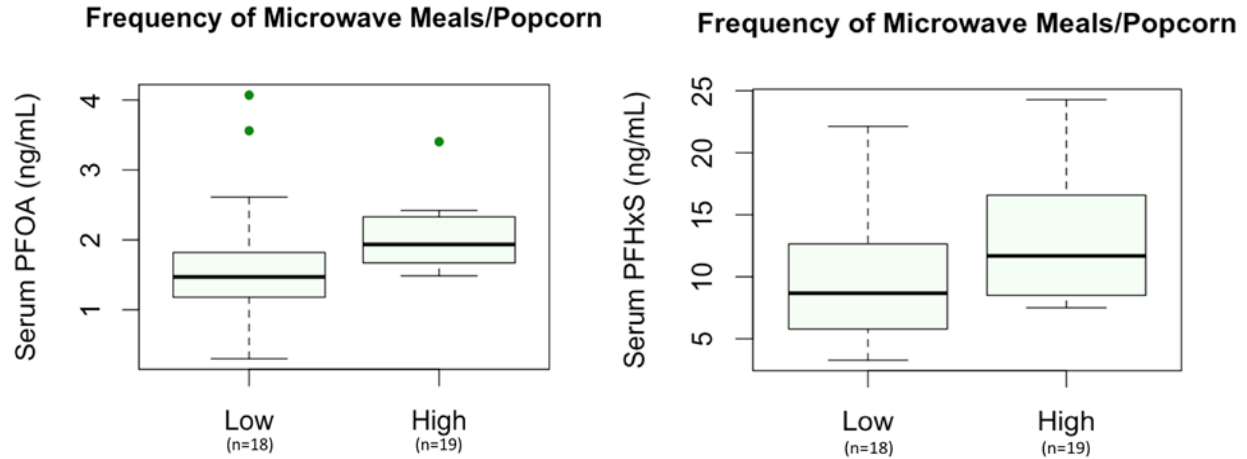
#### *Univariate Analyses*

The survey administered contained questions regarding various personal behaviors that could influence possible exposure to fluorinated compounds. Kruskal-Wallis rank sum tests

were run to determine if there were significant differences between the two categories for each question in relation to serum PFAS concentrations. Interestingly, different PFASs appeared to have different possible exposure routes and related personal behaviors. It should be noted that there were a number of questions/categories that could not be assessed due to uniformity in responses by a majority of the participants. These included: the possession of waterproof/stain-repellent clothing, time spent inside, time spent at home, age of home, age of furniture, frequency of fast food consumption, daily water ingestion, use of disposable water containers, use of reusable water containers, and vacuuming frequency.

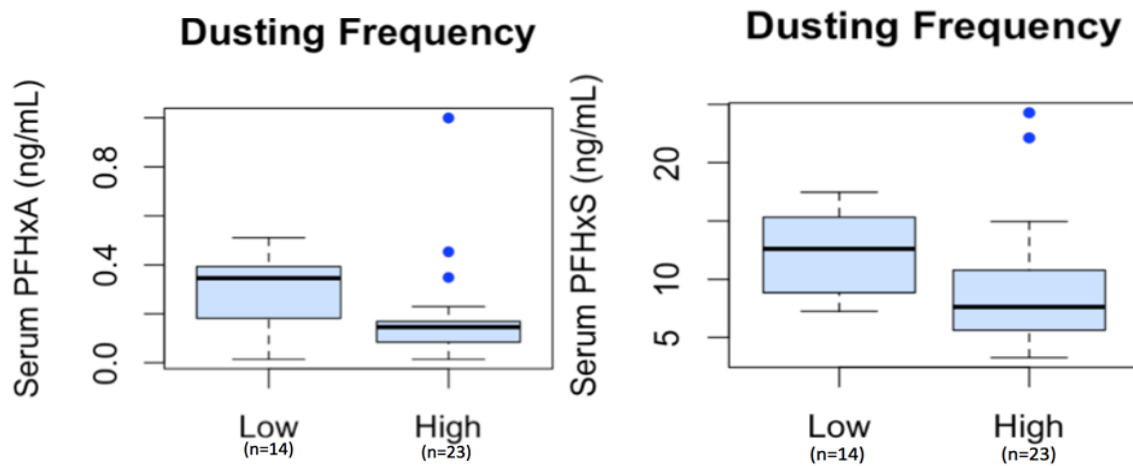
There were several personal behaviors that were significantly associated with serum concentrations. Decreased PFDA levels were associated with more frequent hand washing, although it was not quite statistically significant ( $p = 0.07$ ). This trend between frequent hand washing and decreased serum levels was seen with other PFASs, although it was not statistically significant. Participants that smoked in the past had higher serum levels of PFOA and PFNA compared to participants who had never smoked; however, these results were not statistically significant ( $p = 0.08-0.095$ ). People who wore makeup more frequently had higher levels of PFDA, which was marginally significant ( $p = 0.07$ ). Due to the lack of variation in response from males, only females were included in analyses for makeup use.

Consumption of certain foods or liquids were significantly associated with some of the PFASs in serum. Individuals who ate a relatively high amount of microwaveable meals or microwave popcorn had a general trend of higher levels of serum PFASs compared to those who consumed them less frequently. PFOA ( $p = 0.02$ ) in particular was found to be significantly higher (20% higher) in those that ate microwaveable food frequently. PFHxS was also higher (by 15%) in those that consumed this food type frequently as well, although it was not quite statistically significant ( $p = 0.09$ ) (see Figure 3). In addition, individuals who indicated that they used some sort of water filtration generally had lower levels of some PFASs. PFHxA levels were higher ( $p = 0.06$ ) in participants who indicated that they used some sort of water filtration system or device, while PFOA was higher in the blood of people who did not use water filtration ( $p = 0.09$ ).



**Figure 3.** Boxplots of serum PFAS levels for low and high microwave meal consumption.

Cleaning behaviors were also assessed for significant differences in serum PFAS concentrations. Individuals who fell into the group of high dusting frequency had, in general, lower serum levels of PFASs compared to those who dusted less frequently (see Figure 4). Serum PFHxA and PFHxS were significantly different between the two groups, while PFOA and PFNA were marginally significant ( $p = 0.009-0.1$ ). Those that dusted infrequently had 52% higher PFHxS and 77% higher PFHxA serum levels compared to those that dusted frequently. Though only two individuals answered that they had their carpets deep cleaned, there was a significantly higher serum concentration of both PFOA ( $p = 0.045$ ) and PFNA ( $p = 0.057$ ) in these individuals. In addition, PFHxA was marginally significant ( $p = 0.1$ ), and was lower in those who answered that they had not had their carpets deep cleaned.



**Figure 4.** Significant associations between dusting frequency and serum PFAS levels.

### *Multivariate Analyses*

Multivariate models were run to examine the influence of multiple variables on serum PFAS levels. Handwashing was found to be the most significant factor in relation to serum PFAS levels (see Table 7). After accounting for sex, PFDA, PFHxS, and PFNA were all significantly higher in those that washed their hands less frequently ( $p < 0.05$ ), with PFOA and PFOS being marginally significant ( $p < 0.1$ ) in this trend. Water filtration and sex were also significantly associated with PFHxA and PFOA serum levels ( $p < 0.05$ ). Interestingly, participants that used water filtration had 2.22 times as much PFHxA but 30% lower PFOA in their serum compared to those that did not use filtration devices (see Table 7). In addition, the influence of microwave meals/popcorn intake, sex, and vacuuming frequency were marginally associated with PFHxS serum concentrations ( $p = 0.06$ ). After accounting for sex, participants that ate microwave meals or popcorn less frequently had serum PFHxS levels 25% lower ( $p = 0.06$ ) and serum PFOA levels 30% lower ( $p = 0.09$ ) than those who ate these foods more frequently. Dusting frequency was another category with significant results after accounting for differences due to sex. PFHxS levels were 40% times higher in the serum of those participants that dusted infrequently ( $p < 0.01$ ). There was also a suggestive trend for PFDA and PFNA, indicating that dusting rarely in the household was associated with higher body burdens of PFASs ( $p < 0.1$ ).

**Table 7.** Multivariate analysis of personal behaviors and PFAS levels in serum adjusted for sex.

Predictor	PFDA		PFHxA		PFHxS		PFNA		PFOA		PFOS	
	Coefficient	P Value	Coefficient	P Value	Coefficient	P Value	Coefficient	P Value	Coefficient	P Value	Coefficient	P Value
<b>Dusting Frequency</b>												
Low Frequency	2.64	0.09	1.68	0.20	1.42	< 0.01	1.32	0.08	1.25	0.17	1.51	0.16
<b>Fast Food Consumption</b>												
Low Frequency	1.19	0.31	0.81	0.65	1.02	0.87	1.01	0.96	1.07	0.71	0.96	0.92
<b>Handwashing</b>												
Low Frequency	1.78	< 0.05	0.72	0.41	1.28	< 0.05	1.39	< 0.05	1.23	0.09	1.69	0.06
<b>Microwaveable Meals/Popcorn Intake</b>												
Low Frequency	1.00	1.00	0.66	0.38	0.75	0.06	0.95	0.78	0.73	0.09	0.75	0.42
<b>Vacuuming Frequency</b>												
Low Frequency	0.98	0.94	0.87	0.77	2.04	0.06	1.08	0.68	1.33	0.12	0.95	0.89
<b>Water Filtration Use</b>												
Yes	0.85	0.53	2.22	< 0.05	0.93	0.60	0.81	0.18	0.72	< 0.05	0.73	0.27
<b>Stain Repellent Clothing Use</b>												
Low Frequency	1.46	0.17	0.72	0.45	0.96	0.79	1.06	0.75	1.13	0.48	1.10	0.27

Exponentiated beta coefficients represent the multiplicative change in the serum concentration relative to the reference group for categorical variables. Reference groups are the opposite frequency category (e.g. high frequency).

### *PFASs in Hand Wipes*

Four different PFASs were measured in the hand wipe samples with detection frequencies > 50% (6:2 FTOH, 6:2 diPAP, 8:2 diPAP, and PFHxA). The detection frequencies of these four compounds were 97.4%, 100%, 100%, and 63.2%, respectively. Geometric means for the four PFASs present in the wipes ranged from 0.05-19.32 ng (Table 2). PFHxA was present in hand wipes in the lowest levels, with concentrations ranging from 0.01-0.33 ng, with a geometric mean of 0.05 ng. In contrast, 6:2 FTOH was present in hand wipes in the highest levels, ranging from 0.20-618.83 ng. In general, the fluorotelomer alcohols were present in larger quantities on hand wipes (and thus hands) than the polyfluoroalkyl phosphates. The perfluoroalkyl carboxylic acids were present in very low concentrations, and detected infrequently.

### *PFASs in Wristbands*

Five PFASs (6:2 FTOH, 8:2 FTOH, 6:2 diPAP, 8:2 diPAP, and PFNA) were measured in the silicone wristbands (n=25) worn by participants over a period of 5 days. Detection frequencies were 100%, 100%, 100%, 64% and 64%, respectively. Geometric means for the five PFASs in the bands ranged from 0.25-190 ng (Table 2). Of the compounds, PFNA was present in the lowest amounts, with concentrations ranging from 0.08-1.61 ng and a geometric mean of 0.25 ng. 6:2 FTOH was present in wristbands in the highest concentrations, with a range of 25.03-996.25 ng. In general, the wristbands contained more fluorotelomer alcohols than polyfluoroalkyl phosphates across the cohort.

### *PFASs in Dust*

Four compounds (6:2 FTOH, 6:2 diPAP, 6:2/8:2 diPAP, and PFHxA) were measured in the dust samples with adequate detection frequencies (> 50%). PFHxA and 6:2 diPAP were found in all samples, and 6:2 FTOH and 6:2/8:2 diPAP were found in 91.4% and 94.3% of samples, respectively. Geometric means of the dust compounds ranged from 7.24-312 ng/g dust (Table 2). PFHxA was present in the lowest amounts, with concentrations ranging from 1.19-73.9 ng/g dust while 6:2 FTOH had a large range in concentration levels (10.5-48,171 ng/g). In general, the 6:2 FTOH and 6:2 diPAPs were more abundant in the dust samples than the 6:2/8:2 diPAP compounds, but all three compounds were higher than the PFCAs.

### *Dust Questionnaire Results*

Dust samples were also analyzed for significant differences between various survey responses. Most survey responses were not associated with levels measured in dust; however, the possession and use of waterproof clothing proved to be suggestive for higher 6:2 FTOH dust levels ( $p < 0.1$ ). Age of furniture was significantly associated with dust PFAS levels, with 6:2 FTOH levels ( $p < 0.05$ ) significantly higher in individuals that had older furniture.

### *Correlations of PFASs within Sample Matrices*

Correlation analyses were conducted among the individual PFASs within each matrix (see Table 8). In the serum, several statistically significant correlations were observed between the analytes (Table 8). The two 6 chain PFASs, PFHxA and PFHxS, were significantly correlated ( $r_s = 0.38$ ,  $p < 0.05$ ) in the serum. Spearman correlations between serum PFOA, PFNA, PFOS, and PFDA were also significant, with  $r_s$  values ranging from 0.55-0.75 ( $p < 0.001$ ). In the hand wipes, there were no significant correlations observed, but intra-correlations ranged between -0.21 and 0.23. The range of negative and positive values for intra-correlations among the hand wipes indicates that the hand wipe data is extremely variable in the cohort.

Within the wristbands, there were four significant correlations detected, with coefficients ranging from 0.48 ( $p < 0.05$ ) to 0.62 ( $p < 0.001$ ) (Table 8). These correlations indicate strong co-exposures between 6:2 FTOH, 6:2 diPAP, 8:2 FTOH, and 8:2 diPAP. Additionally, there was one suggestive correlation between 6:2 FTOH and 8:2 FTOH, with a  $r_s = 0.36$  ( $p = 0.08$ ) indicating a trend of association among the two analytes reflective of co-exposure or degradation. Significant correlations between the fluorotelomer alcohols and diPAPs were also seen in the wristbands, with  $r_s$  values ranging from 0.48 to 0.62 ( $p < 0.05$ ). Within household dust, there was one significant correlation between 6:2 diPAP and 6:2/8:2 diPAP, with a correlation coefficient of 0.40 ( $p < 0.05$ ).

**Table 8.** Spearman correlation matrix for PFCs in paired serum, hand wipe, wristband, and dust samples.

	Serum						Hand Wipes				Wrist Bands					Dust			
	PFHxA	PFHxS	PFOA	PFNA	PFOS	PFDA	PFHpA	6:2 FTOH	6:2 diPAP	8:2 diPAP	PFNA	6:2 FTOH	8:2 FTOH	6:2 diPAP	8:2 diPAP	PFHxA	6:2 FTOH	6:2 diPAP	6:2/8:2 diPAP
<b>Serum</b>	PFHxA	1.00																	
	PFHxS	0.38*	1.00																
	PFOA	-0.01	0.16	1.00															
	PFNA	-0.03	0.04	0.75***	1.00														
	PFOS	0.20	0.23	0.55***	0.60***	1.00													
	PFDA	-0.14	0.10	0.58***	0.70***	0.72***	1.00												
<b>Hand Wipes</b>	PFHpA	-0.01	0.12	0.15	0.02	-0.17	-0.08	1.00											
	6:2 FTOH	-0.11	0.18	0.12	-0.12	0.16	0.18	0.20	1.00										
	6:2 diPAP	-0.43**	-0.39*	-0.18	-0.12	0.03	-0.04	-0.07	0.22	1.00									
	8:2 diPAP	0.03	-0.06	0.10	0.01	0.02	-0.08	-0.21	0.23	0.12	1.00								
<b>Wrist Bands</b>	PFNA	-0.36^	0.23	-0.13	-0.02	-0.02	0.04	0.03	0.16	0.10	-0.17	1.00							
	6:2 FTOH	-0.13	-0.00	0.24	-0.02	-0.02	0.05	0.46*	0.61**	0.45*	0.15	-0.19	1.00						
	8:2 FTOH	-0.15	-0.17	-0.39^	-0.33	-0.35^	-0.24	-0.13	0.15	0.49*	0.10	-0.08	0.36^	1.00					
	6:2 diPAP	-0.17	-0.16	0.04	0.00	0.05	0.07	0.23	0.17	0.80***	-0.09	-0.04	0.53**	0.58**	1.00				
	8:2 diPAP	-0.07	-0.14	0.09	0.00	0.14	0.14	-0.22	0.04	0.43*	0.44*	-0.18	0.28	0.48*	0.62***	1.00			
<b>Dust</b>	PFHxA	0.12	0.37*	0.22	0.23	0.21	0.31^	0.16	0.11	-0.38*	-0.19	-0.24	-0.19	-0.25	-0.26	-0.02	1.00		
	6:2 FTOH	0.15	-0.09	0.09	-0.08	0.17	0.17	0.03	0.33^	-0.06	-0.04	-0.11	0.35	-0.11	-0.01	-0.06	-0.02	1.00	
	6:2 diPAP	0.11	-0.23	-0.04	0.09	0.05	-0.05	-0.19	-0.20	0.22	0.07	-0.73***	0.28	0.24	0.09	0.08	0.06	0.27	1.00
	6:2/8:2 diPAP	-0.13	-0.55**	-0.20	-0.06	-0.33^	-0.26	-0.21	-0.40*	0.17	0.40*	-0.31	-0.10	0.39^	0.21	0.57**	0.03	-0.16	0.40*

Analyses were conducted using analytes in which the detection frequency was > 50%. Shaded correlations indicate relationships between the same PFC measured in two different sample matrices. ^ < 0.10. \* < 0.05. \*\* < 0.01. \*\*\* < 0.001.

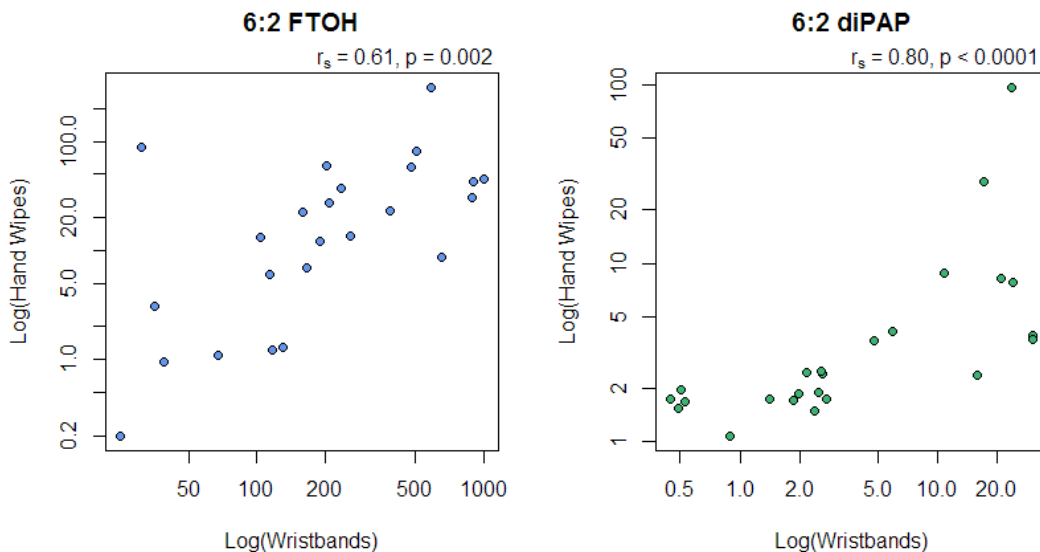
### *Correlations of PFASs between Sample Matrices*

Spearman correlations between sample matrices were also calculated to assess important exposure routes. Between hand wipes and serum, two significant correlations were observed. PFHxA in the serum was negatively correlated to 6:2 diPAP in the hand wipes, with a  $r_s = -0.43$  ( $p < 0.01$ ) (Table 8). As the amount of 6:2 diPAP in the hand wipes increased, the amount of perfluorohexanoic acid in the serum of the cohort decreased. Additionally, another negative correlation between serum PFHxS and hand wipe 6:2 diPAP was observed, with a  $r_s = -0.39$  ( $p < 0.05$ ). These negative correlation trends indicated that high amounts of PFAS precursors on hand wipes were generally associated with lower serum levels inside the body (Table 8). No significant correlations were observed between wristband and serum PFAS levels. Three correlations were marginally significant ( $r_s = -0.39, -0.36, -0.35$ , all  $p < 0.1$ ), however, these again reflected a negative association with serum PFAS levels.

Between dust and serum PFAS levels, two significant correlations were found, one negative ( $r_s = -0.55$ ,  $p < 0.01$ ) and one positive ( $r_s = 0.37$ ,  $p < 0.05$ ) (Table 8). PFHxS in serum was negatively correlated with dust 6:2/8:2 diPAP levels, while PFHxS was positively correlated with PFHxA in household dust. Two additional correlations between serum and dust PFASs were marginally significant. Serum PFOS and dust 6:2/8:2 diPAP had a  $r_s$  value of  $-0.33$  ( $p = 0.07$ ) while serum PFDA and dust PFHxA had a Spearman's rho of  $0.31$  ( $p = 0.09$ ). In general, dust PFAS levels were positively correlated with serum PFASs when the dust analyte was a perfluoroalkyl acid, but negatively correlated when the dust compound was a polyfluoroalkyl phosphate.

Spearman correlations were also run across the other sample matrices, between hand wipes and wristbands, hand wipes and dust, and wristbands and dust. When comparing hand wipe and wristband PFASs, there were several significant positive correlations between the two matrices, implying that exposure through hands or near hands was similar in the cohort (Figure 5). Seven significant correlations were detected with values ranging from 0.4-0.8, indicating a very strong relationship between the exposure in the hand wipes and wristbands (Table 8). The dust was also compared to both hand wipe and wristband PFAS concentrations, and several significant inter-correlations were found. Interestingly, significant correlations between dust and hand wipe PFASs ranged from  $-0.4$  to  $0.4$  for the polyfluoroalkyl phosphates ( $p < 0.05$ ) (Table 8). In addition, there was one suggestive correlation for 6:2 FTOH from both dust and hand

wipes, with a rho of 0.33 ( $p = 0.06$ ). Between dust and wristbands, correlations were generally inconsistent. PFNA in wristbands was negatively correlated with 6:2 diPAP in the dust ( $r_s = -0.73$ ,  $p < 0.001$ ), while 8:2 diPAP in wristbands was positively correlated with 6:2/8:2 diPAP in the dust ( $r_s = 0.57$ ,  $p < 0.01$ ).



**Figure 5.** Correlation plots between the same PFAS in hand wipes and wristbands.

## Discussion

Overall, our results indicate that exposures to PFASs are common but variable in our adult population. We discovered detectable levels of PFASs in all exposure matrices. Even-chain PFAS acids were generally detected more frequently in serum than odd-chain compounds, reflective of the heavier usage of even-chain chemicals in commerce as well as degradation pathways of these compounds (Kotthoff et al., 2015). While we did not examine the presence of PFAS precursor molecules in the serum, we expect that our internal serum levels are adequately representative of exposure due to the short half-lives of precursors in the body (Butt et al., 2014). Levels of these PFASs in the serum were generally similar to those in the U.S. population, but our cohort tended to have lower blood concentrations of PFOS, PFNA, and PFOA (Olsen et al., 2012; Calafat et al., 2007). Conversely, our cohort's serum concentrations of PFHxS were elevated compared to the U.S. population, and PFHxA was more frequently detected in the Duke group than in previous studies (Olsen et al., 2012; Calafat et al., 2007; Health Canada, 2013).

Decreases in the serum PFOS, PFNA, and PFOA concentrations and increases in PFHxA and PFHxS are potentially reflective of phase-outs of long-chain PFASs and replacements with shorter-chain compounds. The high PFHxS levels seen in this cohort could also be due to laboratory bias since the measured levels of this compound in our Standard Reference Material #1957 used to validate the serum method appeared to be higher than previously reported values (Keller et al., 2010). Additionally, PFHxS levels are known to be extremely variable in this SRM, and previous inter-laboratory relative standard deviations for PFHxS have ranged between 24% and 53% (Keller et al., 2010). For PFHxA, the high detection frequency of this PFAS may be due to the fact that the lab where these samples were analyzed was recently established, resulting in very little blank contamination and low detection limits.

Several factors were determined to have a significant impact on the levels of PFASs present in the blood of our study participants. Sex was a major predictor of serum levels in multiple regressions, and males had 1.15-2.13 times as much as females in our cohort. This is similar to previously published data, whereby males consistently had higher blood PFAS levels than females (Calafat et al., 2007; Health Canada, 2013). Increased exposure in males could be due to a variety of reasons, including fewer excretion pathways compared to females, longer half lives in males, and behavioral differences that lead to increased exposure (Fromme et al., 2009). Females are hypothesized to have lower serum half-lives of persistent PFAS compounds due to gender-specific elimination pathways, including placental transfer of precursors and metabolites during pregnancy and breastfeeding as well as depuration during menstruation (Yang et al., 2016; Wong et al., 2014). Support for this theory comes from research indicating that women with hysterectomies have higher PFAS serum levels than those that do not, suggesting that menstruation and pregnancy are important routes of elimination for females (Knox et al., 2011). This study also found that postmenopausal females tended to have higher serum PFAS levels than those still menstruating, which also supports this theory (Knox et al., 2011). To our knowledge, the females in the Duke cohort were nulliparous so offloading during pregnancy and breastfeeding are not expected depuration mechanisms. However, the elimination of PFASs through menstruation could explain some of the sex differences in serum levels in this group.

Additionally, sex-related differences in personal behaviors may also be driving higher serum levels of PFASs in males. The results from the self-administered questionnaire helped to provide more insight on how different behaviors may contribute to exposure, which has not been

well explored previously in the literature. To our knowledge, this is the first investigation that has studied the associations between personal behaviors and serum PFAS levels. Our results indicated that there were multiple behaviors associated with either increases or decreases in serum concentrations, including deep cleaning carpets, eating microwaveable meals or microwave popcorn, dusting, and water filtration use. There were also several behaviors that were not statistically significant, but showed suggestive trends in terms of increases or decreases in specific PFAS levels, such as smoking status or vacuuming. Most significant or near-significant behaviors were associated with unidirectional changes in PFAS serum concentrations. However, water filtration appeared to be associated with higher PFHxA and lower PFOA levels in the serum. Exploring the mechanism behind this could be a potential subject for future study. In addition, behavioral differences between males and females indicated variations in exposure, particularly with behaviors that males engaged in less frequently. Specifically, males were less likely to indicate that they washed their more frequently compared to females. Males were also less likely to use water filtration of some kind, which may be associated with higher serum levels of PFOA in males versus females. As such, these results may indicate that personal behaviors—in addition to differences in physiology—play a role as to why males have consistently higher PFAS serum levels compared to females.

In the hand wipes, wristbands, and dust, precursor compounds (FTOHs and diPAPs) were present in much higher quantities than the acid end products. The larger geometric means of 6:2 FTOH over 8:2 FTOH as well as 6:2 diPAP over 8:2 diPAP in the hand wipes, wristbands, and dust reflect shifts in manufacturing to increase the preferential production of short chain compounds in consumer products (Houtz et al., 2016). In general, these PFAS levels indicate that dust ingestion, dermal absorption, and hand to mouth transfer activity are likely to be indirect sources of exposure, with precursor compounds making up the majority of the exposure through these routes. In the dust specifically, ranges of the carboxylate and sulfonate acids were much lower than previously published data, and the detection frequencies of these compounds in our study were lower than those in other studies (Strynar and Lindstrom, 2008; Fraser et al., 2013; Kato et al., 2009). PFHxA was one exception, as this compound was detected more frequently in the Duke cohort dust samples than in previously published literature.

For the precursor compounds, 6:2 FTOH was detected more frequently and had higher maximum levels in the dust of this cohort compared to other studies, while 8:2 FTOH had a

lower detection frequency and similar maximum values in this study compared to published research (Strynar and Lindstrom, 2008; Fraser et al., 2013). Interestingly, the dust levels of the polyfluoroalkyl phosphates were all lower in this study compared to previous studies, with smaller means and ranges of 6:2, 6:2/8:2, and 8:2 diPAPs (De Silva et al, 2012). The 8:2 diPAPs were detected much less frequently in this study than in De Silva's, which could be indicative of decreased use in consumer products in the eight years since sampling occurred in that study. Additionally, there appears to be a wide range of average levels of diPAPs in dust across countries, so it is possible these differences between our study and De Silva's could be attributable to differing levels of PFASs in U.S. and Canadian households (Eriksson and Kärrman, 2015). Ultimately, the totality of the dust data in this cohort is suggestive that precursor levels are responding to shifts in PFAS production stimulated by regulatory phase-outs of long chain compounds.

### *Limitations*

This study has several limitations that need to be taken into consideration when analyzing the results. The cohort size was fairly small (n=40), so our ability to draw meaningful conclusions may be limited by statistical power. Lack of clear correlative trends between sample matrices could be due to a variety of reasons. First, paired hand wipe, dust, wristband, and serum samples were collected only once throughout the study, which may not adequately capture the day to day variability of precursor exposure on the hands. Second, many participants had recently moved into new apartments or housing situations less than 3 months before dust sampling occurred, so poor correlations with serum levels may be expected within this cohort given the long half-lives of the compounds. Other researchers have looked at correlations between house, office, and vehicular dust with serum PFAS levels and similarly reported a lack of correlative trends between the two exposure matrices (Fraser et al., 2013). Third, while the hand wipe and wristband data clearly demonstrate that exposure via inhalation and dermal absorption are probable, the lack of a correlation with serum PFAS concentrations may indicate that these snapshots of exposure are not reflective of long-term integrated exposures. That is, a single day's estimation of precursor exposure is not likely to be heavily predictive of an internal concentration. This relationship between precursor exposure in the dust, wristbands, and hand wipes could be explored in future studies by intentionally allowing several years to pass before

measuring serum PFAS levels in the precursor-exposed population. Fourth, this study did not measure the major hypothesized exposure pathways (diet and water), so these results are missing important variables that could have explained a large amount of the variability in our serum measurements.

Other limitations in this study include the unequal ratio of females to males, which might cause us to draw spurious conclusions about the ways that gender is associated with PFAS exposure and influences behavior. Additionally, the convenience sample cohort was relatively homogenous, with a preponderance of white participants with similar education status and little variation among behavioral traits. Lack of behavioral variability in the questionnaire limited our ability to pick predictive variables in the multivariate regressions, so pertinent exposure factors like diet were not adequately analyzed in this cohort. The size and characteristics of our cohort may hinder our ability to generalize these results to the greater U.S. population, but this does not invalidate our results.

### *Future Research*

Addressing the lack of variation among certain personal behaviors could be a focus of future research. Examining the role of specific dietary preferences--such as vegetarianism or veganism--in relation to PFAS serum levels would be particularly relevant, especially when considering the fact that diet is commonly believed to be the primary contribution to PFAS exposure. Similarly, all except one of our participants lived within the same city limits, where the water comes from two intermingled drinking water plants. The single individual who lived outside of the town of Durham did not have significantly different serum levels in relation to the rest of the cohort. However, future research could focus on whether or not county of residence and water consumption patterns--including water filtration use--could be influencing differences in PFAS serum levels. In addition, many of our participants only had older furniture or pre-owned furniture of indeterminate age, with very few individuals with any new furniture. Exploring the exposure of people with newer furniture would be another topic for potential future study, especially in light of the relatively recent shifts in manufacturing to shorter chain compounds.

## Conclusions

To our knowledge, this study is the first of its kind to assess background exposure to perfluoroalkyl substances in a cohort of the adult U.S. population using hand wipes and silicone wristbands. Our results indicate that exposure to PFAS precursors is occurring in household dust, in hand wipes, and in wristbands, and that short chain compounds dominate these matrices. These findings, in addition to increases in 6-chain compounds in the serum, suggest that the phase out of long chain PFASs are influencing perfluoroalkyl exposure in this cohort. Decreased exposure also appears to be associated with particular cleaning behaviors, and frequent hand washing may be an effective way to minimize one's contact with these endocrine-active compounds.

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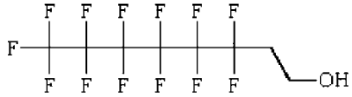
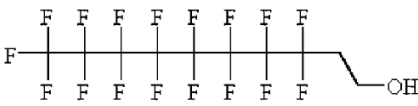
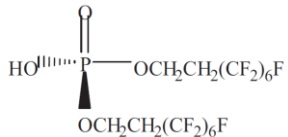
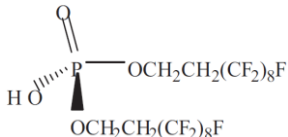
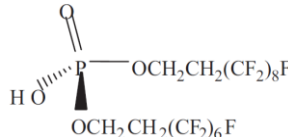


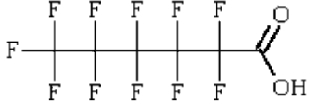
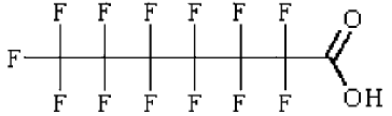
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## Appendix A

**Table A1.** Names and structures of target analytes in this study (Adapted from Ding et al., 2012; Strynar and Lindstrom, 2008).

Common Name	Acronym	Structure
2-(perfluorohexyl) ethanol	6:2 FTOH	
2-(perfluorooctyl) ethanol	8:2 FTOH	
6:2 di-substituted polyfluoroalkyl phosphate	6:2 diPAP	
8:2 di-substituted polyfluoroalkyl phosphate	8:2 diPAP	
6:2/8:2 di-substituted polyfluoroalkyl phosphate	6:2/8:2 diPAP	
Perfluorobutanoic acid	PFBA	
Perfluoropentanoic acid	PFPeA	
Perfluorohexanoic acid	PFHxA	
Perfluoroheptanoic acid	PFHpA	

Perfluorooctanoic acid	PFOA	
Perfluorononanoic acid	PFNA	
Perfluorodecanoic acid	PFDA	
Perfluorobutane sulfonate	PFBS	
Perfluorohexane sulfonate	PFHxS	
Perfluorooctane sulfonate	PFOS	

## Appendix B

**Table B1.** LC-MS/MS target analyte acronym, full name, MRM, and internal standard

<b>Acronym</b>	<b>Full Name</b>	<b>MRM</b>	<b>ISD</b>
<i>Perfluorinated Sulfonates (PFSAs)</i>			
PFBS	perfluorobutane sulfonate	299.1>99.2	<sup>18</sup> O <sub>2</sub> -MPFH <sub>x</sub> S
PFH <sub>x</sub> S	perfluorohexane sulfonate	399.1>99.1	<sup>18</sup> O <sub>2</sub> -MPFH <sub>x</sub> S
PFOS	perfluorooctane sulfonate	499.1>99.1	<sup>13</sup> C <sub>4</sub> -MPFOS
<i>Perfluorinated Carboxylates (PFCAs)</i>			
PFBA	perfluorobutanoate	213.1>169.1	<sup>13</sup> C <sub>4</sub> -MPFBA
PFPeA	perfluoropentanoate	263.2>219.1	<sup>13</sup> C <sub>2</sub> -MPFH <sub>x</sub> A
PFH <sub>x</sub> A	perfluorohexanoate	313.1>269	<sup>13</sup> C <sub>2</sub> -MPFH <sub>x</sub> A
PFHpA	perfluoroheptanoate	363.1>319	<sup>13</sup> C <sub>4</sub> -MPFOA
PFOA	perfluorooctanoate	413.1>369	<sup>13</sup> C <sub>4</sub> -MPFOA
PFNA	perfluorononanoate	463.2>419.1	<sup>13</sup> C <sub>5</sub> -MPFNA
PFDA	perfluorodecanoate	513.1>469.1	<sup>13</sup> C <sub>2</sub> -MPFDA
<i>Polyfluoroalkyl Phosphates (diPAPs)</i>			
6:2 diPAPs	6:2 fluorotelomer phosphate diester	988.9>543	<sup>13</sup> C <sub>4</sub> -M6:2 diPAP
6:2/8:2 diPAPs	6:2/8:2 fluorotelomer phosphate diester	888.9>443	<sup>13</sup> C <sub>4</sub> -M6:2 diPAP
8:2 diPAPs	8:2 fluorotelomer phosphate diester	788.9>443	<sup>13</sup> C <sub>4</sub> -M8:2 diPAP

**Table B2.** GC-MS target analyte acronym, full name, SIM ions (quantification ion is underlined), and internal standard.

<b>Acronym</b>	<b>Full Name</b>	<b>SIM</b>	<b>ISD</b>
6:2 FTOH	6:2 fluorotelomer alcohol	<u>365</u> , 327	<sup>2</sup> H <sub>2</sub> -, <sup>13</sup> C <sub>2</sub> -6:2 FTOH
8:2 FTOH	8:2 fluorotelomer alcohol	<u>465</u> , 427	<sup>2</sup> H <sub>2</sub> -, <sup>13</sup> C <sub>2</sub> -8:2 FTOH

**Appendix C**

**Questionnaire**

Please answer all questions as honestly as possible.

All of your responses will be kept strictly confidential and this paper version will be destroyed at the conclusion of the study.

**Date:** \_\_\_\_\_

**Study ID Number:** \_\_\_\_\_

**Collected by:** \_\_\_\_\_

**DEMOGRAPHICS:**

Participant's Name: \_\_\_\_\_  
\_\_\_\_\_ F

Sex: \_\_\_\_\_ M

Participant's Height (inches): \_\_\_\_\_

Weight (lbs): \_\_\_\_\_

Street Address: \_\_\_\_\_

City: \_\_\_\_\_ State: \_\_\_\_\_ Zip: \_\_\_\_\_

Email Address: \_\_\_\_\_

Age (years): \_\_\_\_\_

Marital Status:

\_\_\_\_ Married \_\_\_\_ Single \_\_\_\_ Widowed \_\_\_\_ Divorced \_\_\_\_ Partners living together

Ethnicity: Hispanic or Latino

Non-Hispanic or Non-Latino

Race: (may answer Yes to more than one)

American Indian/Alaskan Native:	No	Yes
Asian	No	Yes
Native Hawaiian or other Pacific Islander	No	Yes
Black or African American	No	Yes
White	No	Yes

Education (check all that apply):

\_\_\_\_ Primary School  
\_\_\_\_ Some High School  
\_\_\_\_ High School  
\_\_\_\_ Some College  
\_\_\_\_ Associate's Degree  
\_\_\_\_ Bachelor's Degree  
\_\_\_\_ Graduate Deg. (e.g. Master's, Ph.D., M.D.)

**PERSONAL BEHAVIORS**

1. On average, how many times a day do you wash your hands with soap and water?  
 0-5 times daily  
 5-10 times daily  
 10-20 times daily
  
2. On average, how many times a week do you take a shower?  
 Never  
 1-2 times  
 3-4 times  
 5-6 times  
 7 times  
 Over 7 times
  
3. What percentage of the time do you wash your hands with soap and water before you eat?  
 Never  
 Less than half of the time  
 More than half the time  
 All of the time
  
4. On average, how many times a day do you use hand sanitizer?  
 Never  
 0-2 times daily  
 3-5 times daily  
 6-8 times daily  
 9-11 times daily  
 >11 times daily
  
5. Do you smoke cigarettes?  
 Yes, currently  
     If so, for how many years? \_\_\_\_\_  
     How many per day? \_\_\_\_\_  
 No, but past smoker  
     If so, how many years ago did you quit? \_\_\_\_\_  
 Never smoked
  
6. Do you own any waterproof or stain repellent clothing?  
 Yes  
 No  
     If yes, please list items:  
     \_\_\_\_\_  
     How frequently do you wear these clothes?  
          Never  
          Once a month  
          Once a week  
          More than once a week  
          Daily
  
7. Are you employed?  
 Yes  
 No

If you work more than 10 hours per week, please list your occupation:

---

#### PERSONAL CARE PRODUCTS

8. How often do you use the following products per month?

a. Makeup:

Never

Once a week

Weekdays

\_ \_ Every day

b. Do you have makeup on now?

Yes

No

Please list the types of makeup that you wear at least five times per week:

Example: eyeliner, lipstick, liquid foundation

---

---

c. Lotion:

Never

Once a week

Weekdays

Every day

d. Do you have lotion on now?

Yes

No

e. Does your lotion have sunscreen in it that you know of?

Yes

No

Not sure

f. How often do you apply sunscreen (non-lotion products applied specifically to protect against the sun, such as Banana Boat, Coppertone, etc.):

Never

Once a week

Weekdays

Every day

g. Do you have sunscreen on now?

Yes

No

#### ENVIRONMENT

9. On average, how many hours a day do you spend inside?

0-2 hours

3-5 hours

6-8 hours

9-12 hours

13-18 hours

19-23 hours

24 hours

10. On average, how many hours a day do you sleep?  
 \_\_\_ Hours
11. Of the hours a day spent inside, please rank the following rooms as 1 (spend the most time in) to 5 (spend the least time in):  
 \_\_\_ Bedroom  
 \_\_\_ Kitchen  
 \_\_\_ Family room/sitting room/office  
 \_\_\_ Computer room or lab  
 \_\_\_ Garage  
 \_\_\_ Other: Please specify: \_\_\_\_\_
12. How many hours a day do you spend in a car?  
 \_\_\_\_\_
13. How many hours a day do you spend on public transportation (e.g. a bus)?  
 \_\_\_\_\_
14. On average, how many hours a day do you spend in public buildings?  
 \_\_\_ 0-2 hours  
 \_\_\_ 3-5 hours  
 \_\_\_ 6-8 hours  
 \_\_\_ 9-12 hours  
 \_\_\_ 13-15 hours  
 \_\_\_ 15+ hours
15. On average, how many hours a day do you spend at home?  
 \_\_\_ 0-2 hours  
 \_\_\_ 3-5 hours  
 \_\_\_ 6-8 hours  
 \_\_\_ 9-12 hours  
 \_\_\_ 13-15 hours  
 \_\_\_ 15+ hours
16. How old is the building that you live in, approximately?  
 \_\_\_ 0-5 years old  
 \_\_\_ 5-10 years old  
 \_\_\_ 11-20 years old  
 \_\_\_ 21-30 years old  
 \_\_\_ 31+ years old
17. If there is carpeting in your residence, have you had it cleaned using a deep cleaning machine?  
 \_\_\_ Yes  
 \_\_\_ No  
 \_\_\_ N/A (no carpet present)
- If yes, from where did you get the carpet cleaner?  
 \_\_\_ Own the carpet cleaner  
 \_\_\_ Rented the carpet cleaner  
 \_\_\_ Used a professional carpet cleaning service

18. Does your furniture have any stain repellent properties (beads up water, labeled as 'stain resistant')?

Yes

If yes, list what types of furniture:

\_\_\_\_\_

No

Not sure

19. How old is your furniture? List each furniture piece with its associated age.

Example: \_\_\_\_\_ bed, dresser, nightstand 2-5 years old

\_\_\_\_\_ 0-1 years old

\_\_\_\_\_ 2-5 years old

\_\_\_\_\_ 6-10 years old

\_\_\_\_\_ 11-15 years old

\_\_\_\_\_ 16-20 years old

\_\_\_\_\_ 21+ years old

\_\_\_\_\_ Not sure

20. If there is carpeting in your residence, does it have stain repellent properties (beads up water, labeled as 'stain resistant')?

Yes

No

Not sure

N/A (no carpet present)

21. If there is carpeting in your residence, how old is it?

0-1 years old

2-5 years old

6-10 years old

11-15 years old

16-20 years old

21+ years old

Not sure

N/A (no carpet present)

22. How many times a month do you vacuum your home?

Never

Once a month

Once a week

More than once a week

23. Approximately how long ago was the last time you vacuumed? \_\_\_\_\_

24. How many times a month do you dust your home?

Never

Once a month

Once a week

More than once a week

25. Approximately how long ago was the last time you dusted? \_\_\_\_\_

26. Do you dust the dashboard of your car more than once every three months?

Yes

No

#### DIET AND WATER INTAKE

27. Do you have specific dietary requirements?

None

Vegan

Vegetarian

Gluten-free

Paleo

Other

If other, please specify: \_\_\_\_\_

28. How often do you eat fast food?

Never

Once a month

Once a week

More than once a week

Daily

29. How often do you eat microwave popcorn or microwave meals?

Never

Once a month

Once a week

More than once a week

Daily

30. How much water do you drink per day?

None

0-2 cups

2-5 cups

5-8 cups

9+ cups

31. What is your main drinking water source?

Tap water

Bottled water

32. Do you use any water filtration devices for your tap water (Brita, PUR, whole house filtration systems)?

Yes

No

33. Do you drink from a reusable water container?

Yes

No

If yes, please indicate how much water you drink out of it per day:

- 0-2 cups
- 2-5 cups
- 5-8 cups
- 9+ cups

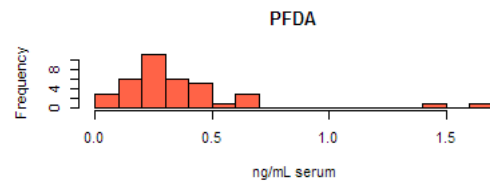
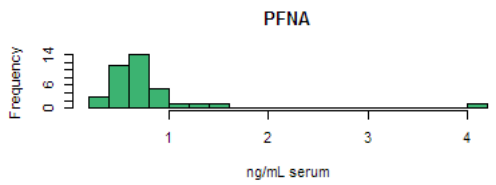
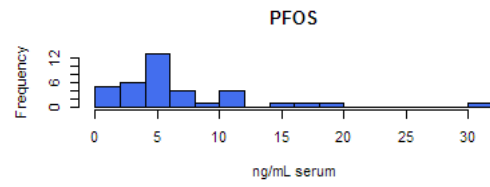
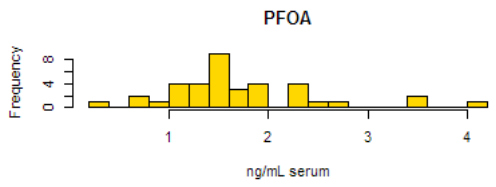
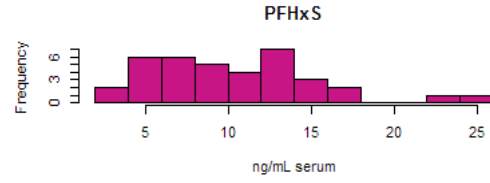
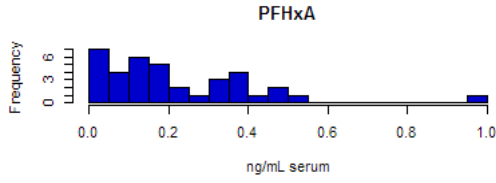
34. How frequently do you drink out of disposable plastic-coated or wax-coated cups (disposable coffee or soda cups)?

- Never
- Once a month
- Once a week
- More than once a week
- Daily

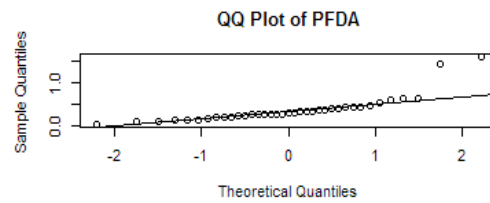
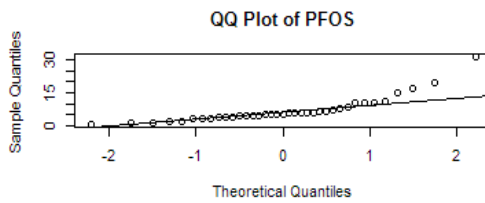
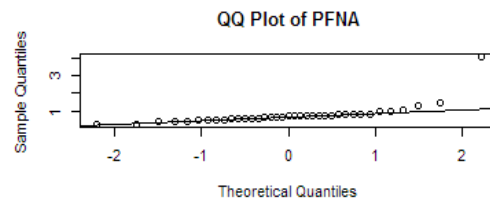
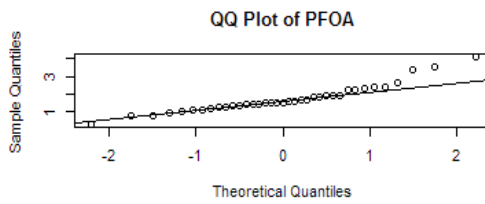
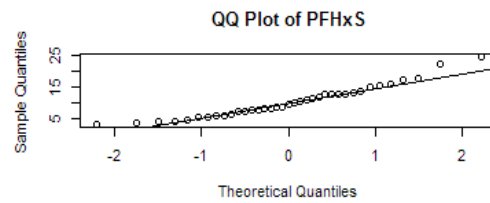
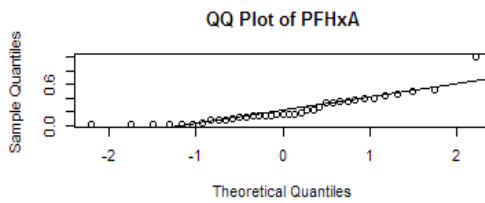
## Appendix D

### Serum Statistics-Duke Cohort

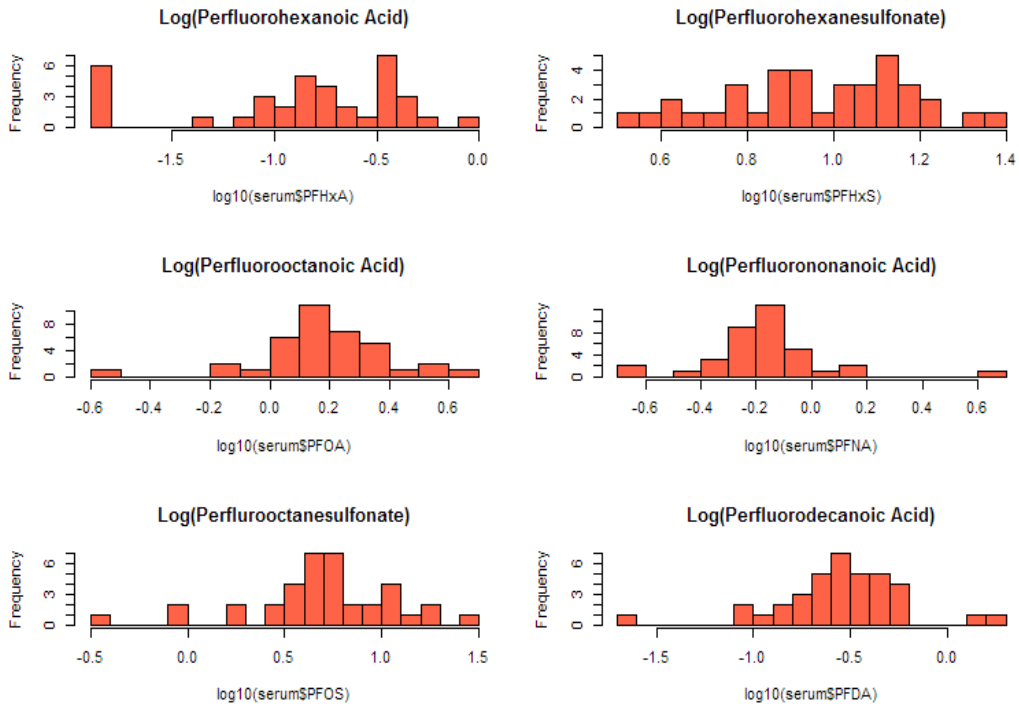
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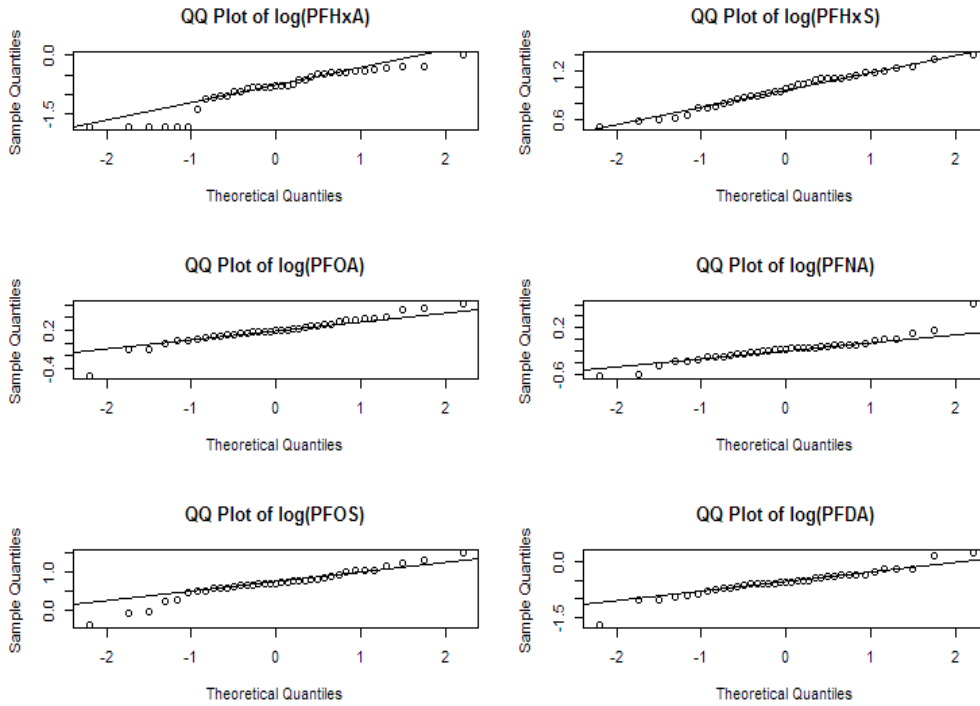
QQ Plots:



Log-transformed Histograms:

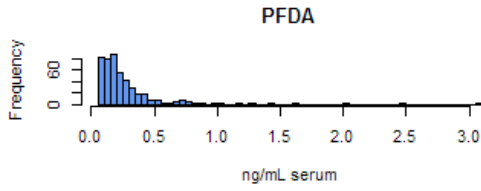
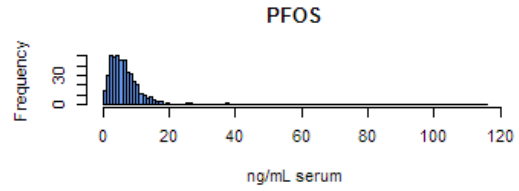
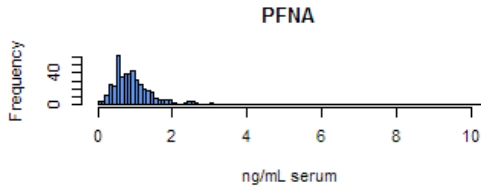
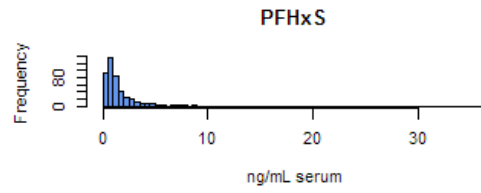
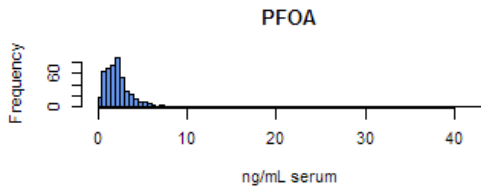


Log-transformed QQ Plots:

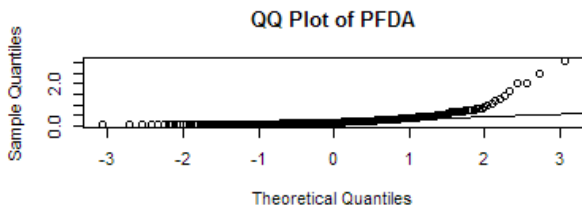
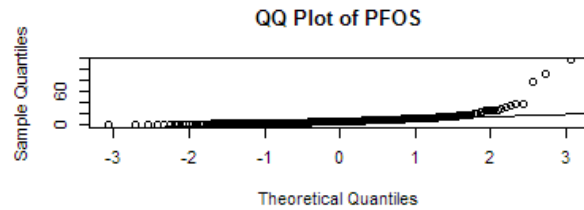
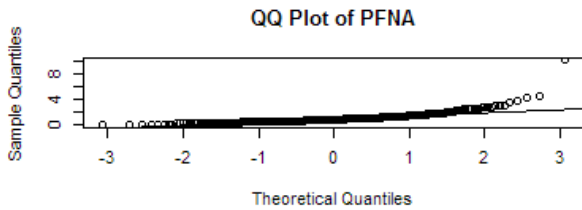
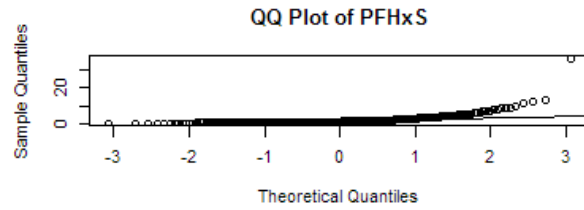
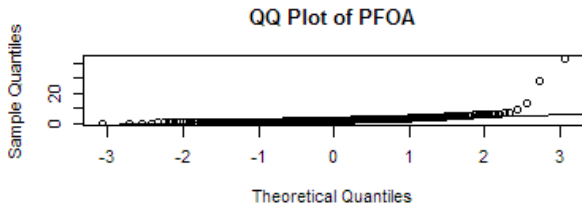


*Serum Statistics-NHANES Cohort*

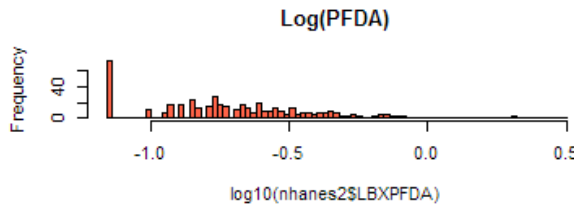
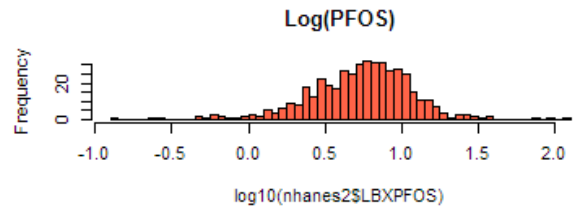
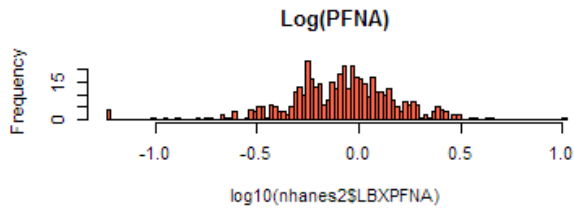
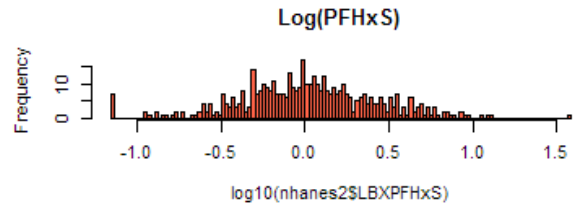
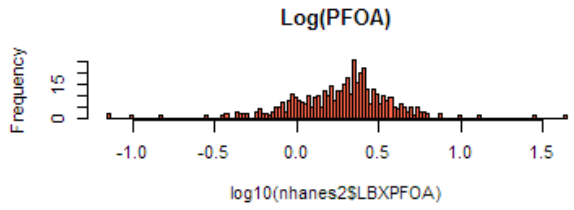
Histograms:



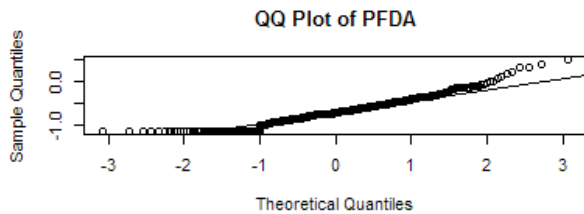
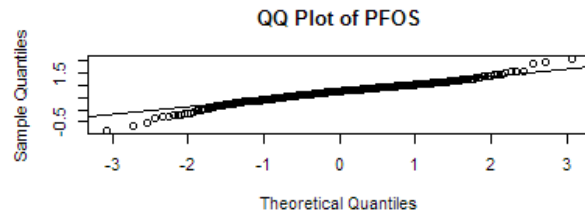
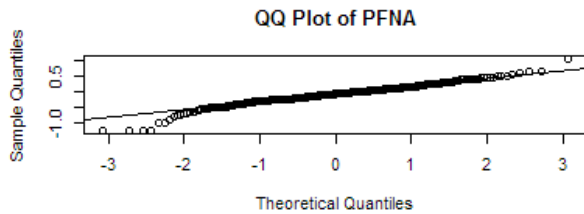
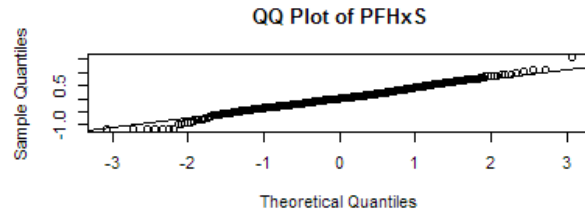
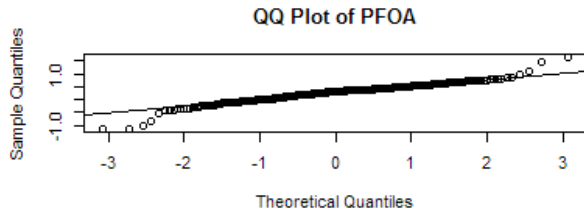
QQ Plots:



Log-Transformed Histograms:



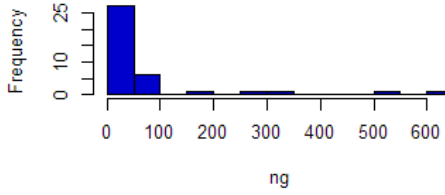
Log-Transformed QQ Plots:



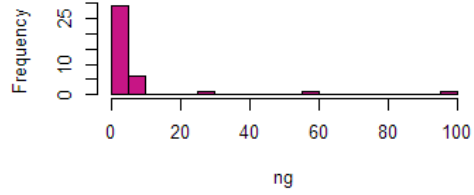
*Hand Wipe Statistics*

Histograms:

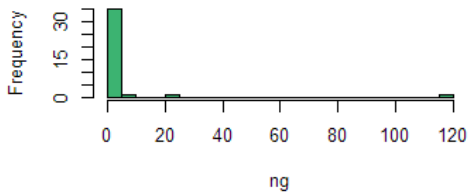
**6:2 FTOH in Handwipes**



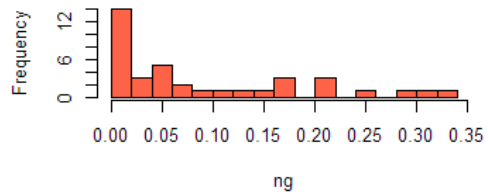
**6:2 diPAPs in Handwipes**



**8:2 diPAPs in Handwipes**

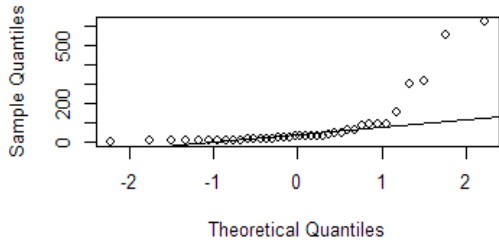


**PFHpA in Handwipes**

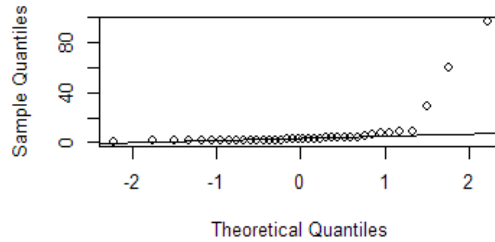


QQ Plots:

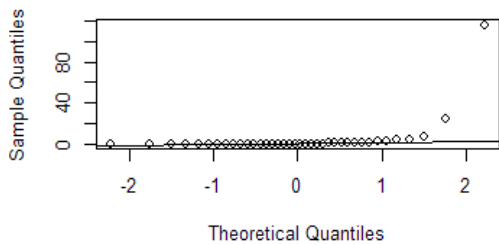
**QQ Plot of 6:2 FTOH in Handwipes**



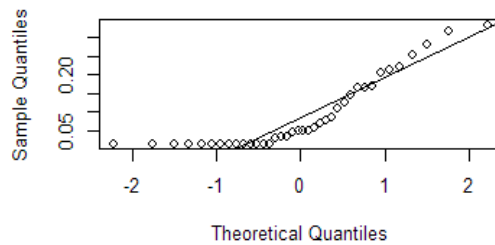
**QQ Plot of 6:2 diPAPs in Handwipes**



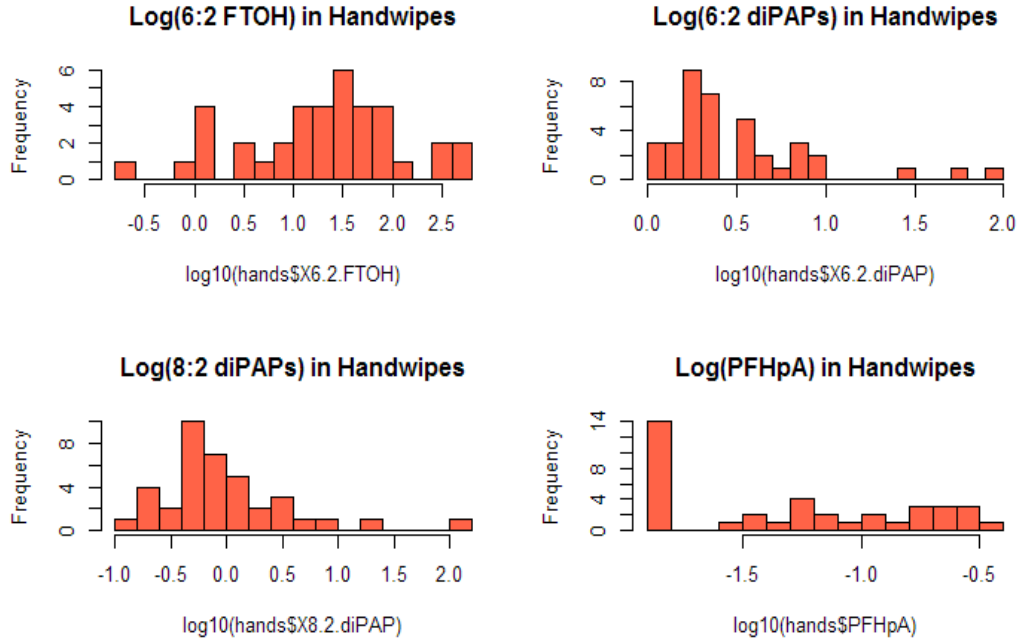
**QQ Plot of 8:2 diPAPs in Handwipes**



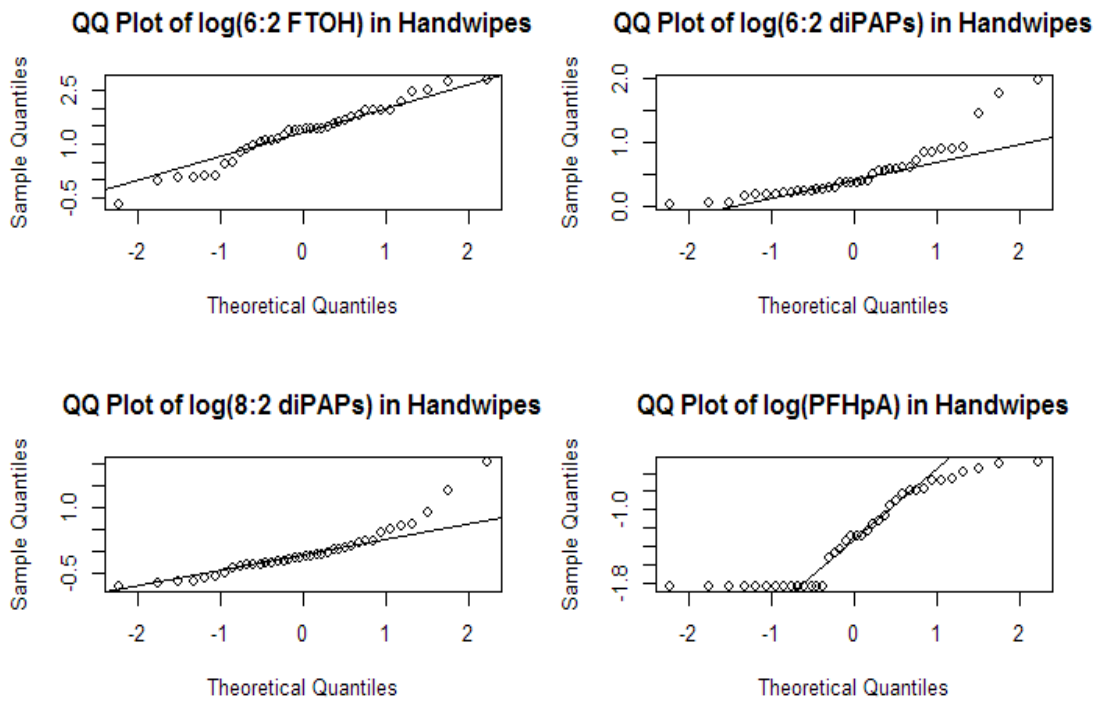
**QQ Plot of PFHpA in Handwipes**



Log-transformed Histograms:

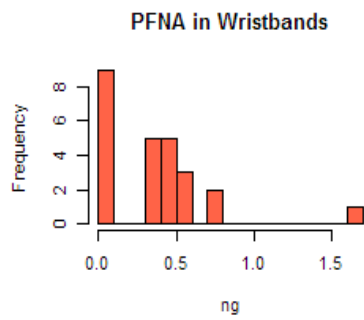
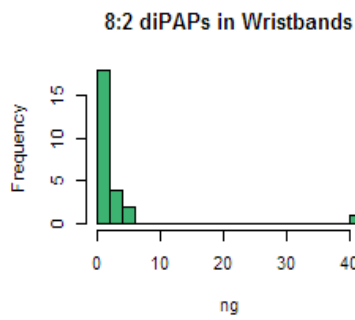
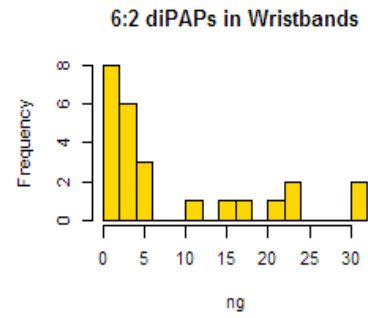
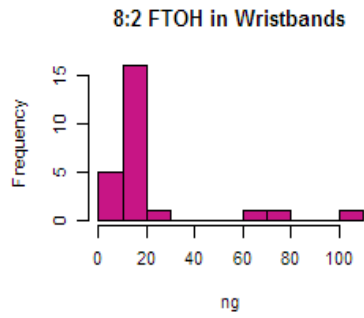
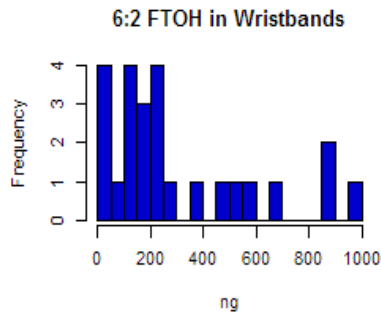


Log-transformed QQ Plots:

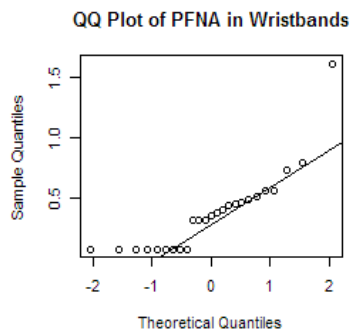
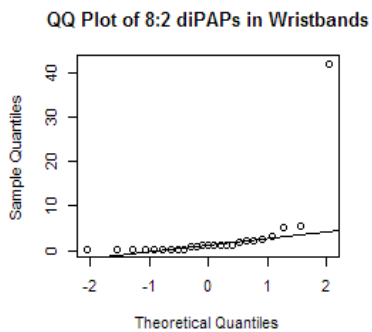
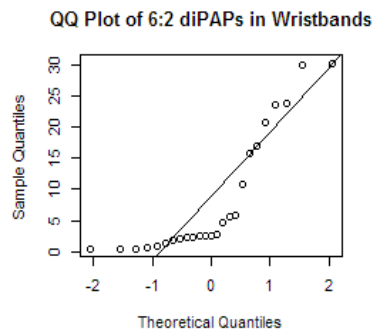
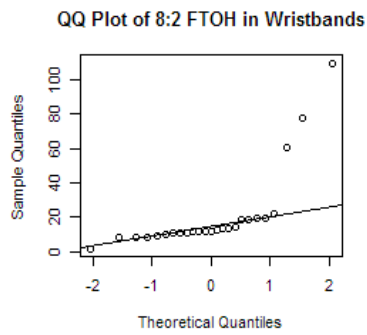
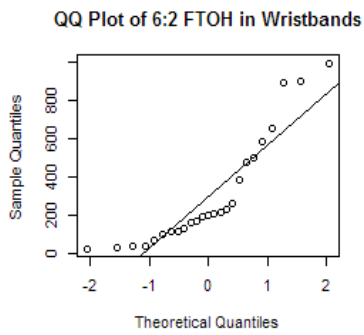


## Wristband Statistics

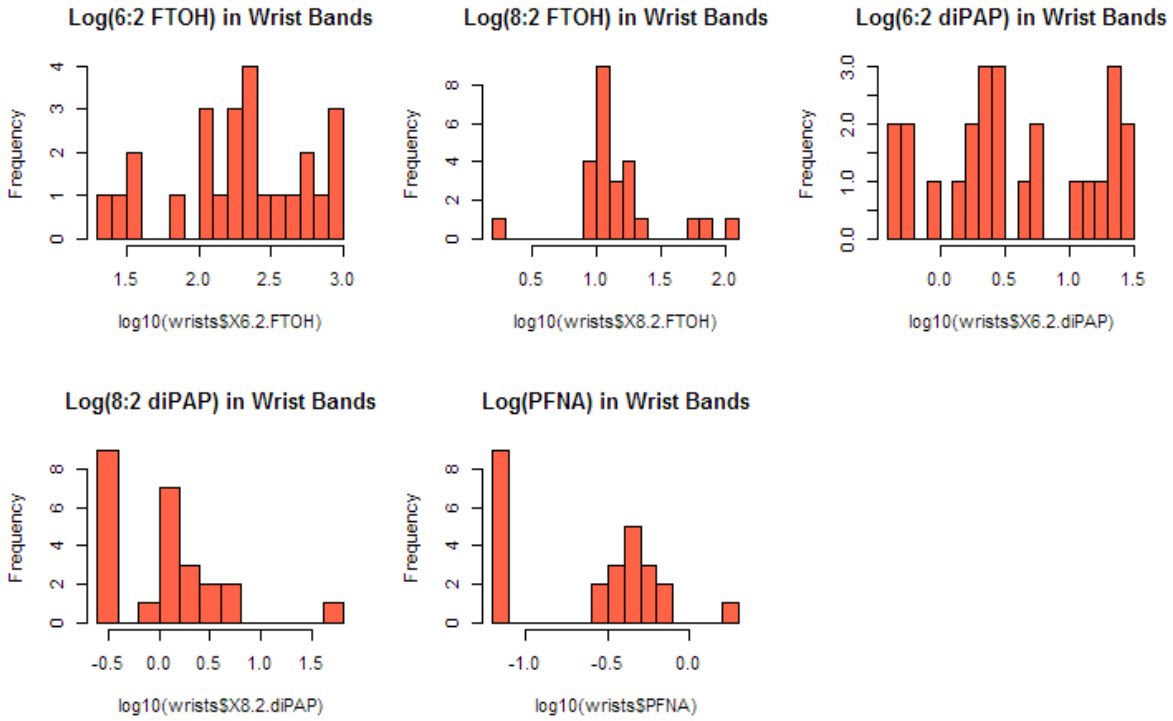
Histograms:



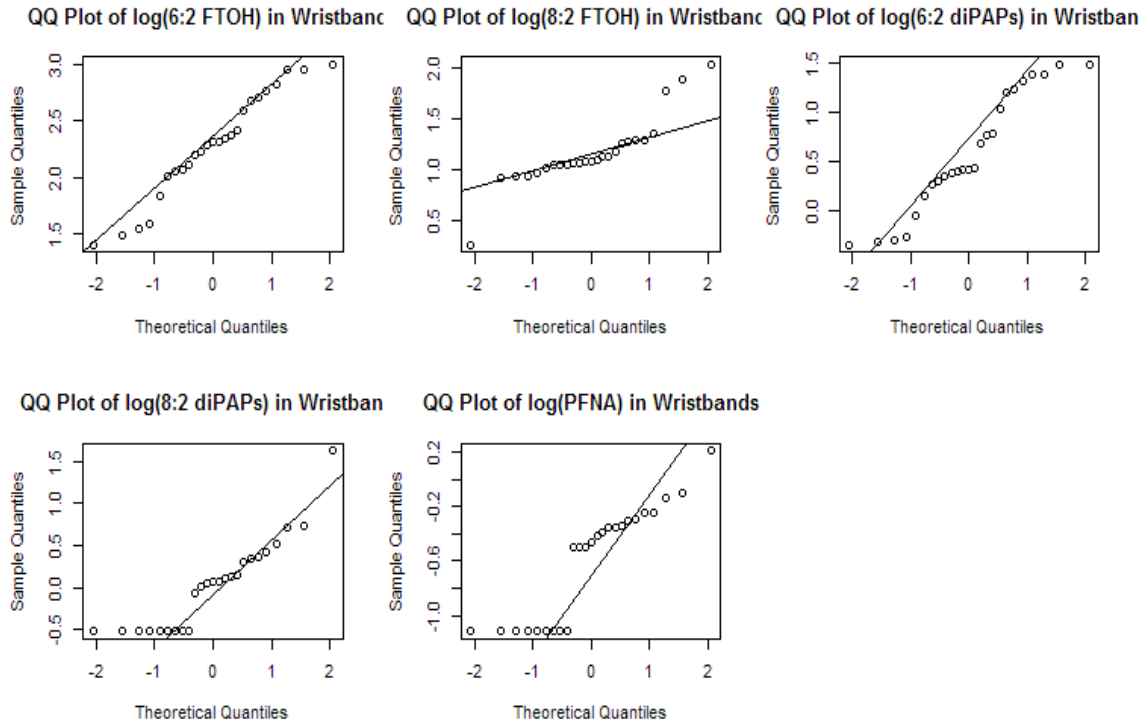
QQ Plots:



Log-transformed Histograms:

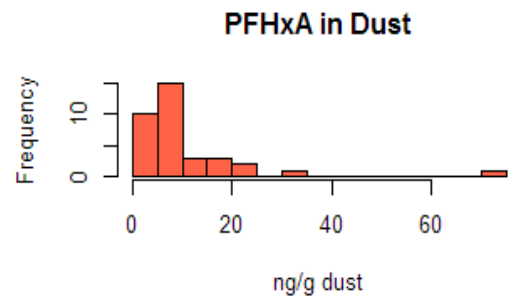
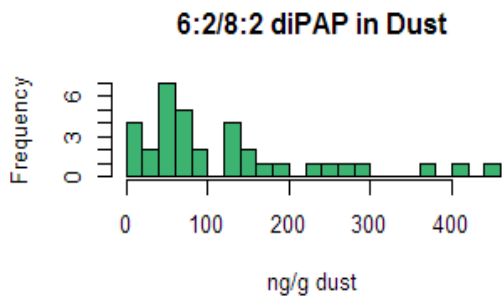
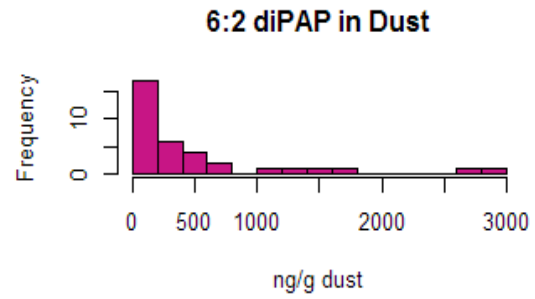
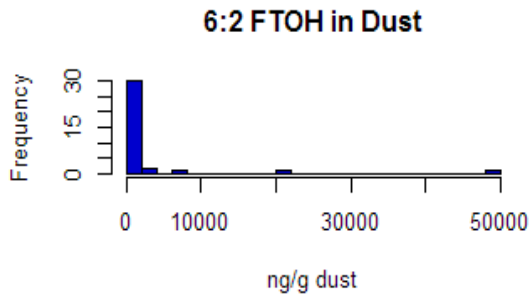


Log-transformed QQ Plots:

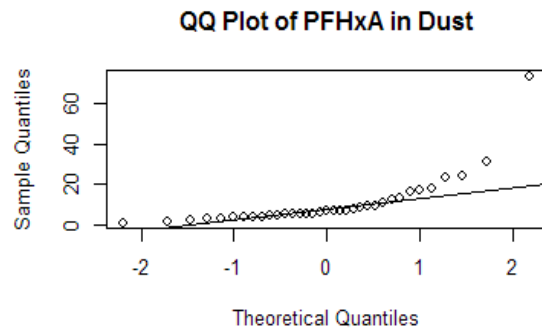
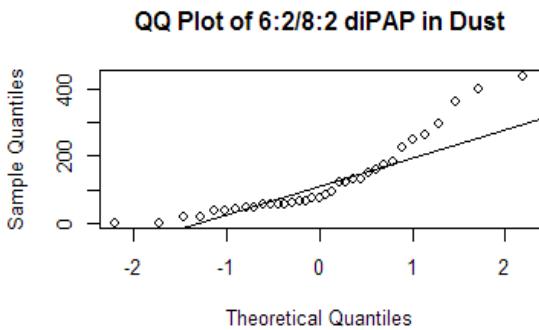
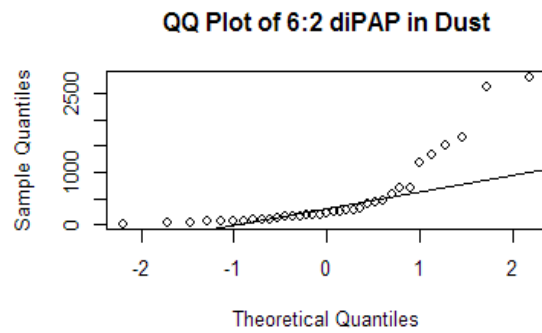
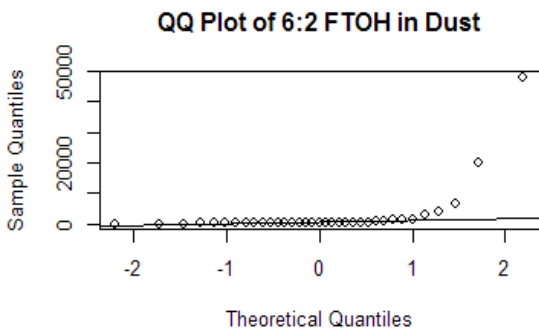


Dust Statistics

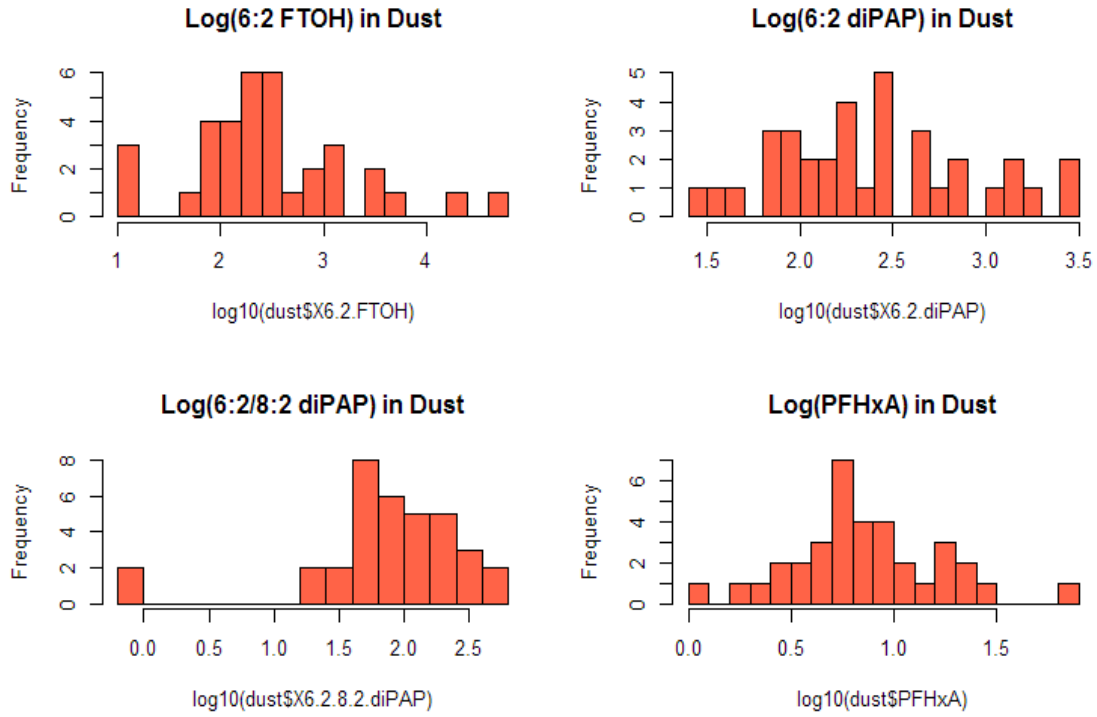
Histograms:



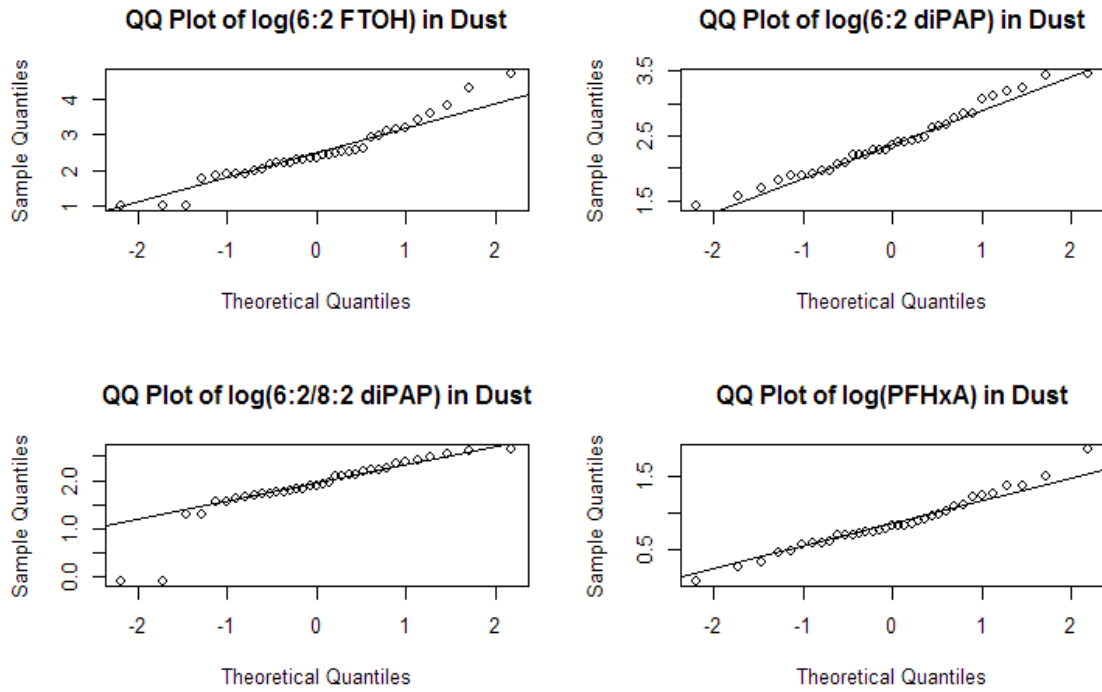
QQ Plots:



Log-transformed Histograms:

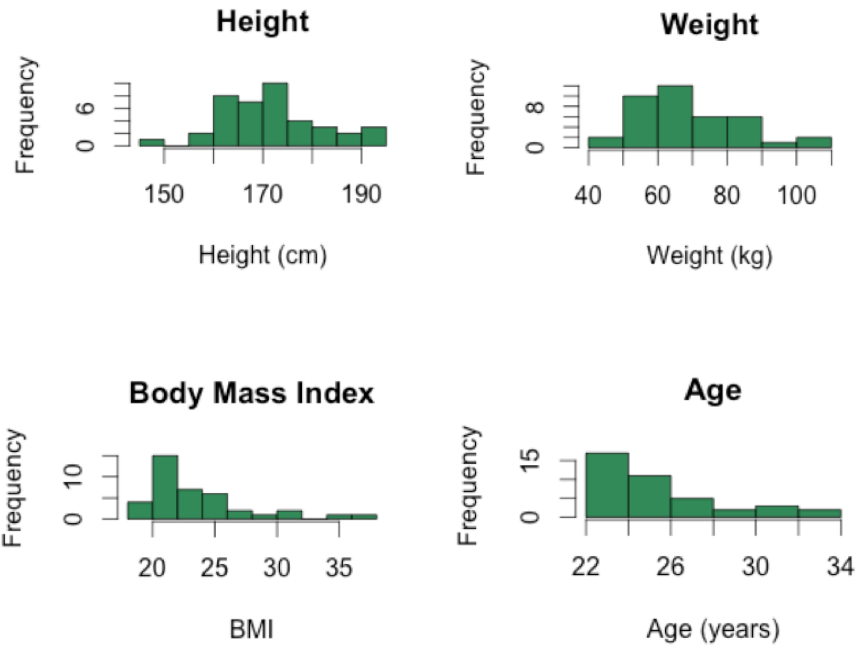


Log-transformed QQ Plots:



Survey Statistics

Histograms:



Spearman Correlation Matrix:

