

Exposure Assessment Protocol: Biological and Environmental Sampling of Per- and Polyfluoroalkyl Substances (PFAS)

FUNDED AND SPONSORED BY

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Abbreviations and Acronyms

11CI-PF3OUdS	11-chloroeicosafuoro-3-oxaundecane-1-sulfonic acid
9CI-PF3ONS	9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid
ALT	Alanine aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
DCHI	Division of Community Health Investigations
DE	Design effect
dL	Deciliter
DoD	US Department of Defense
DONA	4,8-dioxa-3H-perfluorononanoic acid
EA	Exposure Assessment
EPA	Environmental Protection Agency
EtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid
FRB	Field reagent blank
FtS 8:2	fluorotelomer sulfonic acid 8:2
FtS 6:2	fluorotelomer sulfonic acid 6:2
FtS 4:2	fluorotelomer sulfonic acid 4:2
g	Gram
GGT	Gamma-glutamyl transferase
HDL	High density lipoprotein
HFPO-DA (GenX)	hexafluoropropylene oxide dimer acid
ICC	intra-cluster correlation coefficient
L	Liter
LDL	Low density lipoprotein
LOD	Limit of detection
MeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid
Mg	Milligrams
mL	Milliliter
MRL	Minimum risk level
n-PFOA	ammonium perfluorooctanoate
n-PFOS	sodium perfluoro-1-octanesulfonate
NCEH	National Center for Environmental Health

NDAA	National Defense Authorization Act
Ng	Nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
PFAS	Per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonic acid
PFDA	perfluorodecanoic acid
PFDS	perfluorodecane sulfonic acid
PFDoA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHpS	perfluoroheptane sulfonic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFNS	perfluorononane sulfonic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFOSA	perfluorooctane sulfonamide
PFPeA	perfluoropentanoic acid
PFPeS	perfluoropentane sulfonic acid
PFTA	perfluorotetradecanoic acid
PFTTrA	perfluorotridecanoic acid
PFUnA	perfluoroundecanoic acid
Sb-PFOA	mixture of perfluoro-5-methylheptanoic acid isomers
Sm-PFOS	mixture of sodium perfluoro-5-methylheptane sulfonate isomers

This protocol has been updated to reflect changes necessary to complete the Exposure Assessments during the Covid-19 pandemic. Appropriate safety precautions, including the use of all appropriate personal protective equipment (PPE), will be implemented to keep the EA team and participants safe during the EA process. Appendix J, the PFAS EA Restart Plan, has been included that outlines the additional procedures that will be implemented during recruitment, field work and community meetings to ensure that the EAs are completed in compliance with CDC and state requirements.

<https://www.cdc.gov/coronavirus/2019-ncov/hcp/non-covid-19-client-interaction.html> for non-COVID-19

The activities that will be modified include:

- Holding virtual community meetings, including the kickoff meeting and the presentation of results. Small group (less than 10 participants) may be held as needed following applicable local, state and CDC guidelines in place at the time of the meeting.
- Ensuring that social distancing and the use of PPE are employed to comply with CDC and state guidelines during door-to-door recruitment.
- Adding information to the recruitment letter to reassure potential EA participants that all state and CDC guidelines will be in place during the EA testing.
- Asking participants about their and their family's health/COVID-19-status during their appointment reminder phone call and prior to beginning the testing process.
- Monitoring the temperature of EA team members (CDC/ATSDR and contractor staff) twice daily and taking participant's temperatures prior to entering the EA testing facility.
- Administering the exposure questionnaire over the phone instead of at the testing facility to reduce exposure time: consent form administration and collection of biological samples will occur at the testing location.

These changes are provided in modified Appendices and scripts.

Project Overview

Title

Biological and Environmental Sampling of Per- and Polyfluoroalkyl Substances (PFAS)

Protocol Summary

Under Section 8006 of the Consolidated Appropriations Act, 2018, ATSDR is required to conduct statistically based biomonitoring exposure assessments (EAs) at “no less than eight current or former domestic military installations” that have or have had documented exposures to PFAS in drinking water. This protocol describes how these EAs will be conducted.

For each site, a statistically based, community sampling design will be used to determine:

- The distribution of PFAS serum concentrations in communities with recent or past exposures to PFAS in drinking water.
- PFAS urine concentrations from a subset of participants with recent or past exposures to PFAS in drinking water.
- PFAS concentrations in indoor dust and tap water samples from a subset of homes of participants in biological sampling.

A questionnaire will be administered to all participants to gather information to characterize each individual's exposure.

Blood and urine samples from EA participants will be analyzed to determine the distribution of PFAS levels in each community. Individual and aggregated community serum and urine concentrations will be compared to reference ranges from nationally representative data. Environmental samples will be analyzed to determine PFAS exposure concentrations and, in conjunction with questionnaire data, to provide insight into environmental contributors to biological PFAS concentrations across all included sites.

Each exposure assessment will include the following goals:

- **Provide a public health service to the community:** This investigation will provide information to community members about their PFAS body burden, including an assessment of how their PFAS concentrations compare to national reference populations. The investigation will also provide information about aggregate serum concentrations and exposure in the community from which participants are selected.

Depending on the results of the investigation, ATSDR will make recommendations to further reduce exposure or conduct additional activities to better understand the impact of PFAS exposure on human health.

- **Generate information about pathways of exposures in the community:** Environmental sampling data will be combined with biological sampling results to generate information about the impact of drinking water and some non-drinking water PFAS exposure pathways on PFAS body burden in each community. For example, environmental sampling data might allow investigators to assess the relative contribution of dust to PFAS exposure, but not necessarily other exposure sources such as foods.
- **Inform future studies to evaluate the impact of PFAS exposure on human health:** The results of these EAs will inform the design and implementation of the CDC Multi-site PFAS Health Study.
 - For example, exploration of indoor dust sampling and analysis may provide valuable insight into the utility of including indoor dust sampling in future PFAS studies.
 - Similarly, collection of paired serum and urine samples will provide information on relationships between PFAS concentrations measured in these media and may generate insight into the utility of measuring PFAS in urine in future health studies.
 - Additionally, measurement of PFAS in serum and urine will generate data that could potentially be used for validation and calibration of physiologically-based pharmacokinetic modeling tools in support of historical dose reconstruction for PFAS health studies.
 - Tracking information on recruitment outcomes and response rates will allow ATSDR to improve methodology for conducting statistically representative sampling in the future.

Participating Agencies/Contractors

Institution	Role
Agency for Toxic Substances and Disease Registry	Sponsoring Agency, Funding Agency
Centers for Disease Control and Prevention	Collaborating Agency
Eastern Research Group	Contractor
Abt Associates	Contractor

Conflicts of Interest

The investigators report no conflicts of interest that would prevent them from objectively carrying out the investigation.

Introduction

Literature Review

PFAS are a large family of chemicals with several thousand members. They are used in a variety of industrial and consumer applications and products, including: fire-fighting foams; personal care and cleaning products; as well as oil, stain, grease, and water-repellent coatings on carpet, textiles, leather, and paper [1]. Perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) are the most extensively studied PFAS but there is little toxicity data available for other PFAS.

PFOS is no longer manufactured in the United States. In January 2006, the Environmental Protection Agency (EPA) initiated the 2010/15 PFOA Stewardship Program, in which the eight major companies in the PFAS industry committed to working toward eliminating emissions and product content of PFOA by 2015 [2, 3]. The goals set forth by this program were met, resulting in a significant decrease in the manufacture and use of PFOA in the United States. However, PFOA, PFOS, and other PFAS continue to be found in the environment, wildlife, and blood of the general population [4-7]. Additionally, new PFAS have been developed to replace PFOA and PFOS, many of which have been detected in environmental samples near manufacturing facilities [8-10]. Very little is known about these new PFAS, though they may have the potential to cause adverse health effects.

Due to past environmental contamination, PFAS have been detected in numerous public and private drinking water systems throughout the United States. As a result, public health agencies are concerned about the possible health risks for communities exposed to these drinking water supplies as well as other sources of PFAS contamination. In 2016 the EPA established a lifetime health advisory level of 70 parts per trillion for two PFAS (PFOA and PFOS, either separately or combined) in drinking water [11, 12]. This health advisory level offers a margin of protection for all Americans throughout their life from adverse health effects resulting from exposure to PFOA and PFOS in drinking water. These values may be revised as the science of PFAS progresses or if new values are established for other PFAS. In addition, several states have developed their own standards and guidance.

Human dietary exposure is thought to be a significant exposure route for PFAS [13-19]. Some PFAS have been shown to accumulate in fish. In the general population, fish and shellfish consumption have been associated with PFAS serum levels [20-22]. In communities near PFAS point-sources, a positive relationship between consumption of fish from PFAS-impacted waters and serum PFAS concentrations have been clearly demonstrated [23]. Additionally, several peer reviewed studies have shown a direct correlation between PFAS concentrations in soil and bioaccumulation in plants [13, 14, 24]. PFAS have been detected in potatoes and cereal seeds as well as leafy vegetables and fruits [24]. Thus, consideration of the consumption of locally-grown fruits and vegetables as well as locally-caught fish is important for exposure assessment.

Infant consumption of formula reconstituted with PFAS-contaminated water as well as consumption of breastmilk from mothers who have been exposed to PFAS are significant dietary sources of PFAS exposure for young children. Empirical data have demonstrated that infant PFOA serum concentrations

are higher than those of older individuals exposed to the same contaminated drinking water source for breastfed infants and those fed formula reconstituted with PFAS-contaminated water [25, 26]

ATSDR is currently updating its Toxicological Profile for Perfluoroalkyls. The profile will include Minimal Risk Levels (MRLs) for four PFAS (PFOA, PFOS, PFHxS, and PFNA). MRLs are estimates of daily human exposure to a hazardous substance at or below which that substance is not expected to pose a measurable risk of adverse non-cancerous (e.g., neurological, respiratory, or reproductive) effects. MRLs are used to derive screening values used by ATSDR health assessors and others to identify potentially harmful levels of contaminants at hazardous waste sites and determine whether further investigation is needed to protect communities from exposure.

Limited epidemiological studies have assessed the relationship between PFAS exposure and adverse health effects in humans. These studies have been conducted in occupationally exposed populations, residential populations exposed to PFAS through contaminated drinking water, and the general United States population.

PFOS, PFOA, PFHxS, and PFNA have been more widely studied than other PFAS. Some, but not all, studies in humans with PFAS exposure have shown that certain PFAS may:

- affect growth [27, 28], learning [29], and behavior [30-32] of infants and older children
- lower a woman's chance of getting pregnant [33, 34]
- interfere with the body's natural hormones [35, 36]
- increase cholesterol levels [37-40]
- affect the immune system [41-45]
- increase the risk of cancer [46]

Scientists are still learning about the health effects of exposures to mixtures of PFAS. More research will help scientists fully understand how PFAS may affect human health.

Although none of the results are definitive due to limitations in the study designs and relevance of the PFAS exposures in those studies to community settings and relevance of other factors that may influence health effects in affected communities, these studies have generated concerns amongst communities with identified exposures to PFAS. Though the PFAS exposure assessments described in this protocol are not designed to assess the relationship between PFAS exposure and health effects, they will measure body burden of PFAS, may result in recommendations to reduce exposure, and will inform the design and implementation of future health studies.

For instance, epidemiological studies have demonstrated a positive association between serum PFOA and elevated cholesterol levels in occupational and residential populations, though no consistent trend between serum PFOA and low density lipoprotein (LDL) and high density lipoprotein (HDL) is evident [47-50]. PFOA and PFOS have been shown to modulate expression of genes related to cholesterol metabolism and transport in men and women [51].

Evaluation of liver enzymes suggests that there is a positive association between serum PFOA and liver enzymes and an inverse association between serum PFOA and bilirubin levels [48-50, 52, 53]. This

association is evident in both occupational and residential populations. A positive relationship between liver enzyme gamma-glutamyl transferase (GGT) and PFOA serum concentration was observed in an occupational cohort [49]. A positive association between serum PFOA and serum PFOS concentrations and serum alanine aminotransferase (ALT) levels (a marker of hepatocellular damage) was observed in a large residential study [52].

There is evidence to suggest a positive association between serum PFOA and chronic kidney disease [54, 55] in the general population, although questions remain. Because of the cross-sectional nature of some studies, it is not clear if high PFOA and PFOS serum concentrations preceded the observed chronic kidney disease or vice versa [4]. Similarly, some cross-sectional studies also indicate an association between serum PFAS concentrations and early menopause, however the design of these studies makes it difficult to determine the direction of causality [4].

PFOA has been associated with kidney and testicular cancer in a survivor cohort living near a chemical plant [54]. A retrospective cohort study showed an association between length of employment at 3M Chemical Division (while PFAS production was ongoing) and prostate cancer [56]. In 2005, EPA's Science Advisory Board suggested that PFOA is "likely to be carcinogenic in humans," based on the evidence available at the time [57]. The International Agency for Research on Cancer has characterized PFOA as "possibly carcinogenic to humans [46, 58]."

Immune system responses have been observed in adults exposed to PFAS. Epidemiological studies suggest a positive association between serum PFOA and serum PFOS concentrations and suppressed antibody responses to vaccines. *In vitro* studies with human cell lines suggest that PFOA inhibits cytokines that help regulate immune responses [59]. In studies of children, evidence suggests that early development of the adaptive immune system may be vulnerable to exposure to PFOA and PFOS [60]. Studies in the Faroe Islands showed that a doubling in PFOA and PFOS exposure at seven years of age was associated with clinically-significant decreases in diphtheria antibody concentrations at 7 and 13 years of age [61]. In 2016, the National Toxicology Program concluded that both PFOA and PFOS are presumed to be an immune hazard to humans [62].

Cross-sectional studies provide some evidence for associations between exposure to PFOA and other PFAS species and asthma-related outcomes in children, though this is not yet well studied [63, 64]. There is no evidence of association between human maternal serum PFOA or PFOS and preterm birth [65], despite observed impacts on pup mortality in rodent studies [66]. A modest inverse association between maternal PFOS serum concentration and birth weight in full term infants has been observed [65].

In animals, adverse health effects have been demonstrated in response to PFOA and PFOS exposure [67, 68]. These studies identify increased liver weight as one of the primary critical effects [67, 69-73]. Other effects include changes in spleen and thymus [70, 74], as well as developmental effects [68, 75]. However, it is important to note that extrapolation from animals to humans is uncertain because of pronounced differences in elimination rates and substantial variability across species [76-78].

There is much uncertainty regarding the toxicokinetics of PFAS, especially in humans [79]. The toxicokinetic behavior of these compounds appears to be very different in humans than in laboratory

species. The rate at which PFAS are eliminated from the body is an important driver of toxicity, with longer half-lives indicating greater potential for bioaccumulation [80]. Biological half-life has been estimated in humans for several PFAS in both occupationally [81] and residentially exposed populations [80, 82, 83]. However, differences in the studied populations, including the level of exposure and the treatment of ongoing background exposures, have resulted in discrepancies across estimates [83].

PFAS excretion is an important determinant of human body burden for these compounds. In humans, PFAS are excreted primarily in the urine [78, 80, 84, 85]. Renal excretion of PFAS occurs by both active and passive mechanisms and is determined by the sum of glomerular filtration, renal tubular secretion and renal tubular absorption [77-79]. Diabetes [86] is associated with a significant lifetime risk of kidney disease [87] and diabetic kidney disease is the leading cause of end-stage renal disease in North America [87]. Diabetic kidney disease is characterized by the development of macroalbuminuria which is followed by a decline in glomerular filtration rate [88, 89]. Hepatitis C has been shown to be an important risk factor for the development of renal insufficiency and decline in glomerular filtration rate [90]. Chronic kidney disease is characterized by decreased kidney function [91]. Clinically, decreased kidney function is a diagnostic marker of chronic kidney disease and is defined by a glomerular filtration rate persistently below 60 mL/minute/1.73 m² [89, 92, 93]. Anemia is a common complication of chronic kidney disease [94]. An analysis of NHANES data demonstrated that lower estimated glomerular filtration rate was associated with higher prevalence of anemia in non-Hispanic white persons, non-Hispanic black persons, and Mexican Americans [95]. Diabetes, hepatitis C, chronic kidney disease and decreased kidney function, and anemia may have the potential to significantly impact glomerular filtration and PFAS excretion.

Dialysis treatment, often prescribed for individuals with kidney disease, may also have the potential to increase the removal of some PFAS from the body and thereby lower some PFAS serum concentrations. The primary treatment for renal failure is hemodialysis, a process by which a patient's blood is drawn out through a tube, pumped through a dialyzer to remove waste products and then returned to the patient's body. The membranes used in dialysis have been shown to provide clearance of PFAS [96]. In particular, concentrations of POFA and PFOS were reduced following dialysis with polysulfone dialysis membranes [96, 97].

Scientific evidence reported in the peer reviewed literature clearly demonstrates that PFAS accumulate in humans in protein-rich compartments including kidney, liver, and blood. This is attributed primarily to the fact that PFAS have a strong affinity for binding to serum proteins, serum albumin in particular [84]. For example, PFOA and PFNA have been reported to be 90% and 99.9% bound to human serum albumin [98]. As such, anything that causes significant blood loss will impact the excretion profile. In particular, menstrual fluid contains high levels of albumin [99]. Epidemiologic studies have shown that higher PFAS serum concentrations are measured in postmenopausal women compared with women who are still menstruating [100, 101]. Further, pharmacokinetic modeling provides additional evidence that menstrual cycles affect PFAS serum concentrations [102, 103].

Relatedly, PFAS have been demonstrated to pass through the placental barrier and into the developing fetus during gestation. PFAS have been measured in maternal serum [104-109], cord blood [27, 29, 104, 108, 110-114], placenta [76, 106, 107, 115], fetal tissue [116], and neonates [117, 118]. These studies

clearly establish gestation and birth as a significant excretion pathway for mothers and a significant exposure pathway for infants. Women who have been pregnant have a distinct excretion profile from women who have not.

Further, lactation is also a significant excretion pathway for breastfeeding women, and an exposure route for breastfed infants [119-129]. PFAS have been measured in breastmilk in many populations and the duration of breastfeeding has been demonstrated to be positively associated with serum-PFAS concentrations in children and negatively associated with PFAS-serum concentrations in mothers [130].

Justification for Exposure Assessments

Human exposure to PFAS is a growing environmental health concern. These EAs will fulfill the requirements of the 2018 Consolidated Appropriations Act, which requires CDC/ATSDR to conduct statistically-based biomonitoring EAs in “no less than eight current or former domestic military installations” that have or have had documented exposures to PFAS in drinking water.

The results of these EAs may help individual participants and their communities better understand the magnitude of their environmental exposures to PFAS. The sampling is designed to yield results that are generalizable to the entire population consuming the drinking water in each community and to allow for estimation of serum PFAS concentrations in community members who were not tested.

Limitations of the Investigation

PFAS concentrations measured in this EA cannot be used to predict the occurrence of disease for an individual and cannot explain an individual's current health problems.

Serum, urine, and environmental PFAS concentrations may improve the understanding of exposure in this community, but will not provide discrete information about all sources of exposure. Additionally, it is not possible to identify every potential confounding exposure. CDC/ATSDR will take this limitation into account when drawing conclusions. The results of this investigation may generate new hypotheses about which PFAS exposure pathways exist in this community. The results of this investigation will be applicable to all the individuals eligible for inclusion in the investigation (see 'Biological Sampling Eligibility and Recruitment' section for eligibility criteria), but cannot be generalized to groups not eligible for inclusion in the investigation.

The results will not be applicable to residents who previously lived in the community but moved away prior to this work or to residents who have moved into the community within one year of this work. While the results are generalizable to the community as a whole, they will not be able to estimate the exact concentrations for individual community members. After analysis of the data, ATSDR will include information on our website and through community meetings to provide individuals with specific information about what the data can and can not say about their exposures. The investigation is designed to estimate the mean concentration of PFOS in the population with a given level of precision. Estimates for other species or in sub-populations may not have the same level of precision in estimating means. The actual precision for other estimated means will be presented with the results.

While CDC/ATSDR will strive to obtain a representative sample at each site, the ultimate outcome of recruitment may introduce limitations in the ability to make generalizations in each community. These limitations may result in uncertainty in conclusions regarding the comparison of a community's exposure profile to nationally representative data. CDC/ATSDR will track non-response rates in order to evaluate the potential impact of the response on generalizability of conclusions.

At all sites identified for exposure assessments, mitigation actions have been implemented to reduce concentrations of PFOA and PFOS in drinking water to below 70 ppt. While there may be ongoing exposure to lower concentrations of PFAS in drinking water, the elevated exposures to PFOA and PFOS have been stopped (to the best of our knowledge). While remediation targeted PFOA and PFOS (per the EPA lifetime health advisory guidance) it is likely that the concentrations of some of the other PFAS chemicals were also reduced. In most cases, less than one biological half-life of time has passed since exposure was reduced. This means that the exposure assessments will attempt to quantify PFAS concentrations in blood and urine based on past exposure to PFAS above 70 ppt, not current exposure.

Intended/Potential Use of Exposure Assessment Findings

Individual blood and urine results will be provided to participants. Household environmental sampling results will be provided to each participant following laboratory analysis and quality assurance procedures, depending on when analytical methods become available. If a participant's blood or urine concentration level is higher than the 95th percentile reported in the National Health and Nutrition

Examination Survey (NHANES) data, or if the participant's tap water sample is higher than either the EPA lifetime health advisory or a state value, that participant will be contacted sooner in order to facilitate rapid exposure source assessment and mitigation, as needed. If PFAS concentrations in tap water samples are higher than either the EPA lifetime health advisory or a state value, we will contact local water utilities and state drinking water officials to share this information. CDC/ATSDR intends to align communications with participants regarding water sampling concentrations with EPA's guidelines for use of the lifetime health advisory. The findings from each EA will be released as a report for the general public as soon as possible and aggregate findings will be submitted for publication in the peer-reviewed scientific literature.

The findings of these EAs will inform design and implementation for the multi-site PFAS health study that will be conducted by CDC/ATSDR in consultation with the National Institute of Environmental Health Sciences (NIEHS) and the US Department of Defense (DoD).

Exposure Assessment Locations

EAs will occur at no less than eight communities associated with current or former military facilities that have or have had documented exposures to PFAS in drinking water.

ATSDR developed a multistep approach to selecting sites for the PFAS exposure assessments. The process reflects the legislative requirements and the scientific needs of the project. At each step of the process, efforts were made to collect as much information as possible.

ATSDR drew on a variety of candidate sources to assemble a list of candidate sites. Sources of information included information from other federal agencies, including DoD and EPA's UCMR3 data. ATSDR applied inclusion criteria to the candidate list to ensure that the communities were near a current or former military installation, had a completed drinking water exposure pathway for PFOA and/or PFOS above the EPA's lifetime health advisory of 70 ppt, a population of potentially exposed persons larger than the sample size calculated in the 'Sampling Strategy' section, no previous CDC/ATSDR sponsored PFAS biomonitoring at the site, and how recently PFAS exposure mitigation had been implemented.

ATSDR categorized the eligible sites based on the predominant source of drinking water to ensure the exposure assessments included communities served by both public and/or private water systems and private wells. ATSDR used information from the local water utilities to estimate the exposed population for each site. ATSDR evaluated the maximum measured concentrations of PFOA and PFOS combined in drinking water to assess the magnitude of exposure. ATSDR estimated the duration of exposure using information about initial use of AFFF at nearby military installations, service dates for drinking water wells, and information about documented releases of AFFF to surface water. Based on the information available, ATSDR chose sites that included both private well and water system sites and a geographical diversity of sites.

Listed in alphabetical order by county, the sites selected for PFAS exposure assessments are:

- Berkeley County, WV near Shepherd Field Air National Guard Base (Berkeley County)
- El Paso County, CO near Peterson Air Force Base (El Paso County)
- Fairbanks North Star Borough, AK near Eielson Air Force Base (Fairbanks North Star Borough)
- Hampden County, MA near Barnes Air National Guard Base (Hampden County)
- Lubbock County, TX near Reese Technology Center (Lubbock County)
- New Castle County, DE near New Castle Air National Guard Base (New Castle County)
- Orange County, NY near Stewart Air National Guard Base (Orange County)
- Spokane County, WA near Fairchild Air Force Base (Spokane County)

To our knowledge, all municipal systems serving the communities identified as exposure assessment sites took steps to reduce concentrations of PFOA and PFOS below 70 ppt between 2014 and 2017. Additionally, the information available for private wells within these communities indicates that either treatment systems were installed, or alternate sources of water provided between 2015 and 2018 in cases where PFOA and PFOS concentrations in private wells exceeded 70 ppt. Exposure to lower levels of a wide range of PFAS may be ongoing. When available, concentrations of PFAS over time will be used to understand the exposure history at these sites, including after mitigation, but PFAS data are not generally available prior to 2013.

Six of the selected communities (Berkeley County, El Paso County, Hampden County, New Castle County, Orange County, and Spokane County) have water systems that had PFOA + PFOS above 70 ppt. In order to draw generalizable conclusions, the sample frame in these communities will include only public water customers. The other two communities (Fairbanks North Star Borough and Lubbock County) had a large number of private wells with PFOA + PFOS above 70 ppt but no affected municipal systems. The sample frame in these communities will include only households with private wells.

Site-specific Community Engagement

ATSDR/NCEH will hold a community-wide public information meeting during the recruitment phase in all EA locations. The intended audience is prospective EA participants. A Community Event Evaluation Survey (“the Survey”) (Appendix A) will be used as a way for the EA team to receive feedback from prospective EA participants about ATSDR’s PFAS public health messaging, the ATSDR PFAS EA enrollment processes, and to gauge local feelings toward the ATSDR PFAS EA project. The survey data will help EA team members adjust and enhance public health messaging and EA project information in real time.

The goals of the Survey are:

1. To learn whether our messages and materials were understood by the audience;
2. To understand community feelings towards the project; and
3. To document whether potential participants understand how individuals are selected for potential participation in the Exposure Assessment.

The Survey questions have been adapted from the ATSDR Communications Toolkit available here: https://www.atsdr.cdc.gov/communications-toolkit/documents/16_event-evaluation-form-final-101315_508.pdf

Objectives

The overall objective of the PFAS Exposure Assessments (all sites) is to characterize exposures and biomonitoring levels in communities exposed to PFAS in drinking water, and to identify patterns in exposure amongst these communities. Each EA will attempt to answer the following questions:

- What are serum PFAS concentrations in the community? How do these concentrations compare to United States reference populations (e.g. NHANES)?
- Can PFAS be detected in urine samples from members of the community? If so, how do these concentrations compare to reference populations across the United States?
- What are environmental contributors to PFAS concentrations in blood and urine? What does environmental sampling data suggest about exposure in the community?
- What can we learn about PFAS exposures to inform future PFAS studies?

Procedures and Methods

Sampling Strategy

A one-stage cluster sample — where each household in the area receiving impacted water is a cluster and all individuals in a selected household are included in the sample — will be used to identify participants for each EA. Clusters (households) will be randomly selected from the sampling frame. This sampling design is representative of the impacted population, allowing for inferences to be made on the entire sampling frame. A step-by-step approach for the one-stage cluster sampling is described below.

1. ESTABLISH THE SAMPLING FRAME

A geographic area where PFAS exposure is known or expected will be defined and a complete list of all exposed/affected households in this area will be identified. This list comprises the sampling frame. Depending on the operations of the water system, the geographic area may be defined by the service boundaries of specific municipal water systems. The geographic area may also be defined as the area with impacted private drinking water wells.

For simple water systems expected to deliver drinking water with consistent PFAS concentrations to all end users (e.g. municipal drinking water systems with one ground water supply well, or municipal drinking water systems with a surface water source), the sampling frame will consist of all households served by the impacted water system.

The list of households served by municipal water systems can be obtained from the water company or from local municipal water supply billing information.

Sequential numbers will be assigned to each household (1, 2, 3,...N) in the sampling frame.

2. CALCULATE SAMPLE SIZE

The required sample size of independent individuals in each community for blood sampling is given by:

$$m = \left[\frac{z_{\frac{\alpha}{2}} \cdot \sigma}{E} \right]^2$$

Where,

m = sample size (individuals)

z = Z value (e.g. 1.96 for 95% confidence level)

α = level of significance

E = maximum error

σ = standard deviation of the logarithm of measured PFAS levels.

If local biomonitoring data are available to determine the standard deviation of the natural logarithm of measured PFAS levels, this data can be used. If these data are not available, national data from NHANES will be used to calculate the necessary sample size, as described below.

The geometric mean for serum PFOS was 4.72 $\mu\text{g/L}$ for the US population in 2015–2016 NHANES. The corresponding 95% confidence interval (4.40, 5.07) and the NHANES sample size of 1,993 are used to estimate the standard deviation of the ln (values). Using the upper limit of the confidence interval:

$$\hat{\sigma} = \frac{\sqrt{1993} \cdot [\ln(5.07) - \ln(4.72)]}{1.96} = 1.63$$

Then, the sample size of independent individuals to estimate the mean with precision 15% of the ln (geometric mean), and 5% level of significance is:

$$m = \left[\frac{1.96 \cdot 1.63}{0.15 \cdot \ln(4.72)} \right]^2 = 188$$

Since data are collected using a cluster design, individuals within a household are not independent. The lack of independence must be accounted for by incorporating the design effect (DE) into calculation of the required sample size. The required sample size for a cluster sample is the sample size for an independent sample multiplied by DE.

$$DE = 1 + ICC * (k - 1)$$

Where,

DE = design effect

ICC = intra-cluster correlation coefficient

k = cluster size

A pilot of representative biomonitoring for PFAS conducted by the New York State Department of Health and the Pennsylvania Department of Health resulted in retrospective calculations of the intra-cluster correlation coefficient (ICC) for PFAS in serum ranging from 0.39 to 0.54 (unpublished data from New York State Department of Health and Pennsylvania Department of Health, 2019). To be conservative, we assume an ICC of 0.54 for our calculation of the design effect.

The average household (cluster) size for the communities selected as exposure assessment sites ranges from 2.4 to 3.0 individuals per household. To be conservative, we assume a household (cluster) size of 3 for our calculation of the design effect.

Using these values, the design effect is:

$$1 + 0.54 * (3 - 1) = 2.1$$

As a conservative assumption, we use a design effect of 2.1 to calculate our required sample size.

$$sample\ size = DE$$

This results in a sample size of $2.1 * 188 = 395$ individuals accounting for intraclass correlation. To be conservative, we will use this design effect for all communities, even when a smaller average household size would result in a lower design effect.

Assuming all individuals from each selected household are included in the sample, the required number of households that should be contacted for recruitment is given by

$$n = sample\ size * \left(\frac{N}{M}\right)$$

Where,

n = required number of households
N = the total number of households in the sampling frame
M = the total number of individuals in the sampling frame

In the Hampden County community (the first exposure assessment site) $m = 395$, $N = 2,882$, $M = 7,665$ so a sample of $n = 149$ households is needed. Values for N and M taken from 2010 census data for census tract 8125 in Hampden County, MA. At the pilot sites, a within household response rate of 85% was achieved for households in which at least one person participated. Assuming this response rate applies at all sites, the total number of households that need to be contacted in order to get 395 individuals to participate is 269 households. This value is based on an 85% household participation rate ($149/0.85 = 175$) and a general response rate of 65% ($175/0.65 = 269$).

Sample size estimate will be adjusted to ensure adequate precision despite non-participating households, using an estimated household response rate of 65%. In Hampden County, $n = 149/0.65 = 229$ households will be contacted. The number of households contacted in each community will be based on the total number of households and total number of individuals in the sampling frame but will use the same sample size of individuals for all sites. The sample size of 188 independent individuals (based on the original NHANES calculation) will be used for all sites and will be modified by response rate, design effect and required household sample size.

Sampling weights for both households and children are needed to calculate prevalence estimates and make inferences about the entire population of children three years of age or older. Sampling weights can be adjusted to account for unequal probabilities of selection that may have occurred due to non-response from potential participants. Complex survey procedures in SAS/SUDAAN software or EpiInfo software will be used to account for unequal weighting, stratification and clustering in the sample (SAS Institute, Inc., Cary, NC; RTI International, Research Triangle Park, NC).

Based on the eight EAs completed in 2019 to 2020, the average response rate to the recruitment letters was 10%, and not 65%. Therefore, additional EAs that may be completed would use a response rate of 10% for the recruitment letters. A maximum of 3000 households will be sent recruitment letters.

3. SELECT HOUSEHOLDS

Each household in the sampling frame will be assigned a number (1, 2, 3,...N). A random number generator will be used to create a list of random numbers equal in size to the number of households in the sampling frame. Households will be contacted for recruitment into the EA based on estimated household size in the community and using an estimated response rate. If the response rate is lower than estimated, a reserve sample of households will be contacted for recruitment into the EA to reach the target sample size. If the reserve sample is used, households within the reserve sample will be given equal opportunity to participate as households initially invited to participate.

Biological Sampling Eligibility Criteria and Recruitment

Households identified in the sampling strategy will be contacted by mail and phone and an in-person visit, if needed. The recruitment letter and script for recruitment phone calls can be found in Appendix A. Based on response rates from national surveys (e.g., National Health and Nutrition Examination Survey, Behavior Risk Factor Surveillance System) EA staff will make up to eight attempts to reach each randomly selected household by phone. For randomly selected households we are unable to reach by phone, we will attempt one in-person visit to recruit EA participants.

In order to ensure that a sufficient number of participants are included in each EA to allow for generalizable conclusions about the exposure of the impacted community, a target for the number of participants in each EA will be set, as described in the 'Sampling Strategy' section of the protocol.

To increase the likelihood that the target sample size will be reached at each EA site, a reserve sample will be contacted for recruitment if the response rate is lower than estimated. Site-specific response rate estimates will be used to calculate the number of households to be approached for recruitment to meet the target sample size. Each randomly-selected household in this block of households will have an equal opportunity to participate, meaning that study staff will carry out the planned outreach to all households within the block, regardless of whether the target sample size was met before all households were contacted for recruitment. If, on the other hand, sample size requirements are not met after attempting recruitment from the initial sample, a reserve sample will be identified and approached using the same outreach described above. The size of the reserve sample required will vary from site to site.

To decrease the chance of participation bias – in which those that do not choose to participate are substantively different from those that do – households that choose not to participate will not be replaced. Information on recruitment, contact attempts, eligibility, enrollment, and batch number will be collected and used to assess for bias and also to weight data accordingly during the analysis phase.

EA staff will schedule biological sampling appointments for all recruited participants during the initial phone call. Recruited participants will receive a letter that confirms their participation, provides information about the assessment and includes a toll-free number for participants to call with any questions.

For sampled households that agree to participate, each individual will be screened using a series of eligibility questions when they are contacted for recruitment into the EA (Appendix A). Individuals within each selected household must meet the following inclusion criteria to participate in this investigation:

- Is three years of age or older
- Has lived in the community for at least one year
- Does not have a bleeding disorder and is not anemic

Children younger than 3 years old are excluded from the assessments because the reference values to be used for comparison for serum concentrations in this investigation are only available for children ages three and older [5, 131]. Children under three years old may still be sampled at the request of

parents or guardians; however, the child's general state of health, age, and size will be taken into consideration and EA personnel may elect not to collect a blood sample from any child identified as potentially at risk from doing so. While individual results from children under age three will be reported to parents or guardians, data from these participants will not be included in the analysis. Individuals with bleeding disorders or anemia will be excluded in order to reduce burden and risk of blood sampling.

To assess for the potential impact on PFAS excretion and resulting concentrations in blood and urine, participants with diagnosed conditions that impact kidney function (e.g., kidney disease, diabetes, hepatitis C) will be asked to self-identify via the questionnaire, but will not be excluded from the assessment. Similarly, because pregnancy can impact PFAS excretion, pregnant women will neither be targeted nor excluded, but will also be asked to self-identify via the questionnaire.

Participants will not be reimbursed or incentivized to take part in the EA and, similarly, there will be no costs to participants.

Environmental Sampling Eligibility and Recruitment

An arbitrary target of ten percent of households recruited into the EA will also be invited to participate in exploratory environmental (indoor dust and tap water) sampling. These households will be randomly selected from the list of households that have been recruited into the EA. Each household must meet the following eligibility criteria to take part in environmental sampling:

- At least one member of the household is participating in biological sampling
- Head of household reports that people who live in the home primarily drink tap water.

In order to ensure an adequate number of households are identified for environmental sampling, an estimated response rate of 65% will be used to determine how many households will be invited to participate in environmental sampling. For example, if a target of 15 households are needed for environmental sampling, then $n = 15/0.65 = 23$ will be the number of households randomly selected to receive environmental sampling invitations.

Similar to the multi-stage recruitment approach described above, if sample size goals are not met after attempting to recruit from the first batch of randomly-selected households, subsequent batches will be identified and approached. The head of household from households randomly selected for environmental sampling will be invited to participate in environmental sampling during a follow up phone call. A home visit will be scheduled for environmental sampling during the same two-week period of biological sampling.

All participating households will receive a follow-up letter confirming their participation and sampling appointments.

Figure 1 visually depicts the recruitment process.

Sample Collection Procedures

Informed Consent/Assent

Upon arrival at the centralized sample collection location, a Privacy Act Statement, PRA statement, consent, assent, and parental permission forms (Appendix B) will be provided for participants to read and sign prior to any sample collection activities. Participants will also be provided a Biological Testing Tracking Form that they will carry with them throughout the EA testing process to ensure that all appropriate forms are completed and biological samples are provided (Appendix B). Consent forms will be provided for all adults aged ≥ 18 years (Appendix B). Assent forms (Appendix B) will be provided for children aged 12–17. Parental permission forms (Appendix B) will be provided for the parents or guardians of all children participating in the investigation. Consent/assent forms include: the purpose of the assessment; procedures for sample collection; benefits and risks of participation; and contact information should participants have additional questions. All forms are written at the appropriate reading level for each group. All signed consent/assent/parental permission forms will be mailed to and securely archived at ATSDR.

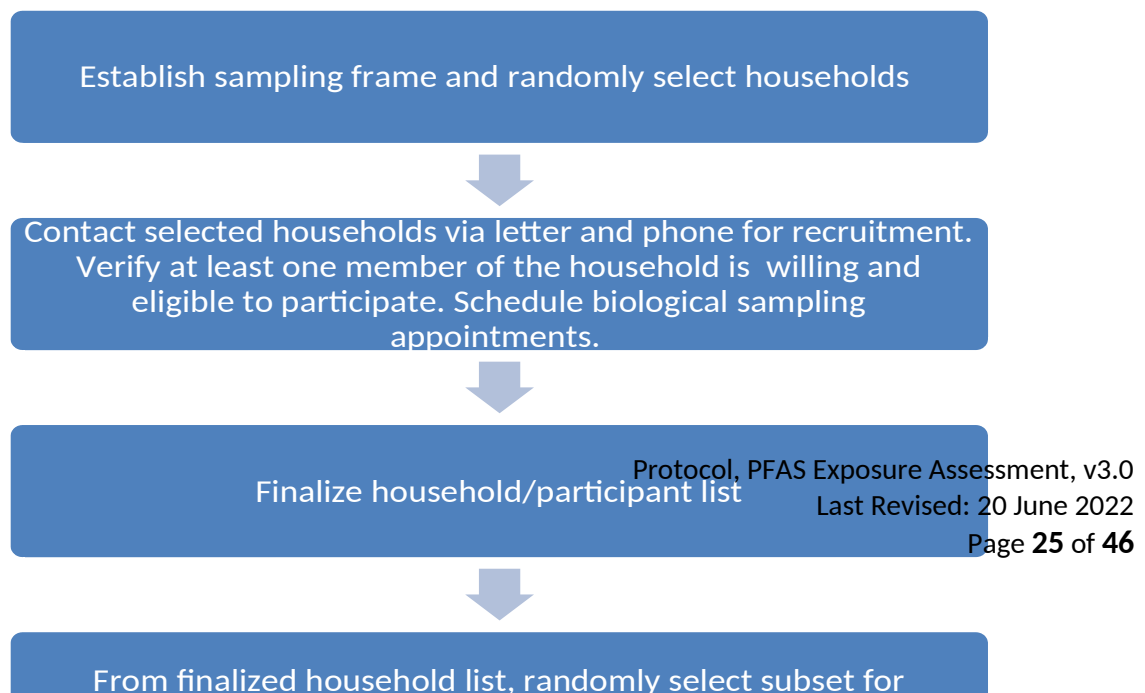
Blood Sampling

A licensed/qualified phlebotomist will collect approximately 6 mL of blood in a red top tube by venipuncture from all EA participants (adults and children). Each blood tube will be labeled with a preprinted bar-coded label associated with the participant. A collection log will also be maintained (Appendix C). Each sample tube will be placed upright in a rack; the blood will be allowed to clot for 30 minutes to 1 hour to create maximum serum yield. Following clotting, the red top tube will be centrifuged for 15 minutes at 1000 – 1300 g force.

After the contents in the tube have clotted and been centrifuged, two serum aliquots will be obtained. In the first, a minimum of 1.0 mL serum will be pipetted into a cryovial (designated as serum sample #1). The remaining serum (max 1.8 mL) will be pipetted into a second cryovial (designated as serum sample #2). Following serum aliquoting, the red top tube and its contents will be safely discarded into a biohazard multipurpose container.

To protect anonymity, the samples will be labeled with a coded identification number. The identification number on the serum sample will match the identification number on the blood sample in order to pair each individual's blood and urine samples.

FIGURE 1: PFAS EXPOSURE ASSESSMENT RECRUITMENT PROCESS



↓

Invite randomly selected households to participate in environmental sampling and schedule environmental sampling appointments.

All serum sample #1 cryovials will be placed inside storage boxes provided by the laboratory. Each box will be placed inside a plastic Saf-T-Pak™ biohazard bag along with an absorbent pad and sealed. This plastic bag will be placed inside a larger Tyvek® bag and sealed. The bagged specimen boxes will be placed inside a Styrofoam shipping container. Dry ice will be added to the shipper and serum specimens will be maintained in their frozen state. EA personnel will perform twice daily checks to ensure that samples remain frozen and add dry ice as needed. The blood samples will be shipped overnight on dry ice to the NCEH Laboratory in Atlanta, Georgia preferably on Wednesdays and Mondays during the sample collection period. Field and laboratory staff will maintain and manage proper chain of custody (Appendix D) for all serum samples.

All serum sample #2 cryovials will be packed in storage boxes and shipping containers and maintained in a frozen state on dry ice as described above. These samples will be shipped overnight on dry ice to a centralized bio-specimen repository where they will be stored. Consent forms will allow participants to choose whether or not they consent to the storage of their serum sample for future use (Appendix B).

Serum sample #1 cryovials will be received by the NCEH laboratory and analyzed for the suite of PFAS measured in the most recent NHANES cycle with publically available data. Additional analytes may be added should methods become available. Test results will be reported as nanograms of the analyte per milliliter of serum (ng/mL). All laboratory analysis will be conducted with established procedures for quality assurance and control according to NCEH methodology.

Table 1 provides the list of the PFAS currently measured in NHANES in serum and the associated limits of detection. For PFOA and PFOS, samples will be analyzed for individual PFOA and PFOS isomers, but both isomer-specific and aggregated values will be reported to allow for comparison to past NHANES that only reported aggregate measurements (up to NHANES 2011-12).

Table 1: List of PFAS proposed to be measured in serum in this investigation - abbreviation, associated chemical name, and current limit of detection (LOD)		
Abbreviation	Chemical Name	LOD (ng/mL)
MeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid	0.1
PFHxS	perfluorohexane sulfonic acid	0.1
Total PFOS	perfluorooctane sulfonic acid	--
n-PFOS	sodium perfluoro-1-octanesulfonate	0.1
Sm-PFOS	mixture of sodium perfluoro-5-methylheptane sulfonate isomers	0.1
Total PFOA	perfluorooctanoic acid	--
n-PFOA	ammonium perfluorooctanoate	0.1
Sb-PFOA	mixture of perfluoro-5-methylheptanoic acid isomers	0.1
PFNA	perfluorononanoic acid	0.1
PFDA	perfluorodecanoic acid	0.1
PFUnA	perfluoroundecanoic acid	0.1
PFDoA	perfluorododecanoic acid	0.1

* ng/mL - nanograms per milliliter

Urine Sampling

All participants will be mailed a labeled urine collection cup, urine collection instructions (Appendix E), insulated cooler, and ice pack, which they'll be instructed to store in their freezer. The morning of their blood sampling appointment, participants will collect a first-morning urine sample (filling at least one quarter of the cup, if possible), cap the container, seal the container in a plastic bag, and place in a refrigerator until they travel to the blood sampling location. Participants will transport their sample to EA staff in an insulated cooler with the frozen ice pack.

Urine samples will be placed inside storage boxes provided by the laboratory. Each box will be placed inside a plastic Saf-T-Pak™ biohazard bag along with an absorbent pad and sealed. This plastic bag will be placed inside a larger Tyvek® bag and sealed. The bagged specimen boxes will be placed inside a Styrofoam shipping container. Dry ice will be added to the shipper and urine specimens will be maintained in their frozen state. EA personnel will perform twice daily checks to ensure that samples remain frozen and will add dry ice as needed. All urine samples will be shipped overnight on dry ice to a centralized bio-specimen repository on Wednesdays and Mondays during the sample collection period. Field and bio-specimen repository staff will maintain and manage proper chain of custody (Appendix D) for all urine samples.

In order to evaluate whether the available method for measuring PFAS in urine is able to detect PFAS in urine samples, an initial subset of ten percent of urine samples will be analyzed for PFAS. If possible, the initial subset of urine samples will be selected randomly from participants with the highest drinking water exposures. If it is not possible to stratify participants based on drinking water exposure, ten percent will be randomly selected from the entire sampling frame. For each sample selected for laboratory analysis, biorepository personnel will pipette 1.8 mL of urine into a cryovial and ship samples overnight on dry ice from the bio-specimen repository to the NCEH laboratory in Atlanta, Georgia. Biorepository staff and NCEH/ATSDR personnel will maintain and manage proper chain of custody (Appendix D) for all urine samples.

If the geometric mean PFAS urine concentrations in a community are higher than the NHANES 95th percentile, all urine samples for the community will be shipped from the repository to the NCEH laboratory and analyzed. The geometric mean will be used in this instance because it minimizes the effect of very high or very low values.

To protect anonymity, the samples will be labeled with a coded identification number. The identification number on the urine sample will match the identification number on the serum sample in order to pair each individual's blood and urine samples.

Urine samples selected for laboratory analysis will be analyzed for PFAS and creatinine. Additional analytes may be added should methods become available. If the geometric mean PFAS concentrations in this initial subset are elevated compared to the U.S. national reference population, as defined by the 2013-2014 NHANES 95th percentile, all other urine samples from the site will be analyzed.

PFAS test results will be reported as nanograms per milliliter of urine (ng/mL). Creatinine results will be reported as milligrams per deciliter of urine (mg/dL). Laboratory processing, analysis methods, quality assurance and quality control measures will be conducted in accordance with NCEH laboratory methods.

Table 2 provides the list of PFAS to be measured in urine in this investigation and associated limits of detection. For PFOA and PFOS, samples will be analyzed for individual PFOA and PFOS isomers, but both isomer-specific and aggregated values will be reported.

Abbreviation	Chemical Name	LOD (ng/mL)
PFBS	perfluorobutane sulfonic acid	0.1
PFHpS	perfluoroheptane sulfonic acid	0.1
PFHxS	perfluorohexane sulfonic acid	0.1
Total PFOS	perfluorooctane sulfonic acid	--
n-PFOS	sodium perfluoro-1-octanesulfonate	0.1
Sm-PFOS	mixture of sodium perfluoro-5-methylheptane sulfonate isomers	0.1
PFBA	perfluorobutanoic acid	0.1
PFPeA	perfluoropentanoic acid	0.1
PFHxA	perfluorohexanoic acid	0.1
PFHpA	perfluoroheptanoic acid	0.1
Total PFOA	perfluorooctanoic acid	--
n-PFOA	ammonium perfluorooctanoate	0.1
Sb-PFOA	mixture of perfluoro-5-methylheptanoic acid isomers	0.1
PFNA	perfluorononanoic acid	0.1
PFDA	perfluorodecanoic acid	0.1
PFUnA	perfluoroundecanoic acid	0.1
HFPO-DA (GenX)	hexafluoropropylene oxide dimer acid	0.1
DONA	4,8-dioxa-3H-perfluorononanoic acid	0.1
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	0.1

* ng/mL - nanograms per milliliter

Questionnaire

An EA team member will administer a questionnaire (Appendix F) to each participant to gather information on risk factors for exposure to PFAS through food pathways, contact with contaminated soil, and water consumption. This information will be used to interpret biomonitoring data and to help understand any unexpected or unusual results. An EA team member will administer the questionnaire and record each participant's responses using the Epi Info suite of software tools either in person or over the phone. An ATSDR staff person will train EA teams on how to ask each question, which questions

should be asked of which participants, and how to accurately and consistently record responses. All questionnaire records will be provided to ATSDR.

Participants will be asked their current address, how long they have lived there, and to identify their primary source of drinking water. Participants will be asked to characterize how much water they drink on a daily basis, and to identify where their drinking water comes from (bottled water, tap water, filtered water, etc...). Participants will also be asked for information about their residential history prior to living in their current home. This information will help characterize the extent and duration of their potential exposure to PFAS-contaminated drinking water in the community.

PFAS are excreted primarily in the urine and are highly bound in the blood. Participants will be asked about the frequency with which they donate blood (participants over the age of 17 only) and if they have been told they have kidney disease in order to characterize excretion. PFAS are also excreted during pregnancy and lactation. Adult women will be asked about their history of pregnancy and breastfeeding. This information will be used to help interpret unusual or unexpected biomonitoring data.

In the event that unexpected serum PFAS concentrations are measured in an individual, ATSDR may use this information to provide context to the result. For example, if an individual reports that they have kidney disease and has PFAS serum concentrations that are significantly higher than others in the household, ATSDR may inform the individual that kidney disease may reduce the rate at which PFAS are cleared from the body, which could have contributed to the observed PFAS serum concentration. Similarly, if a woman who reports multiple pregnancies and significant breastfeeding duration and has lower PFAS serum concentrations than others in her household, ATSDR may inform the individual that pregnancy and breastfeeding are excretion pathways that may have resulted in lower PFAS serum concentrations in her body. In all cases, ATSDR will explain that an individual's PFAS body burden is the product of many complex factors, including but not limited to drinking water exposure, non-drinking water exposure, as well as active and passive excretion.

ATSDR will not use this information to assess impact at the aggregate level or to inform comparisons to the NHANES cohort.

Children (ages 12 - 17) participating in the study will not be asked questions that are inappropriate for their age (pregnancy, blood donation) and will be allowed to obtain parental assistance in answering questions they have difficulty answering.

In order to characterize the potential for additional exposures, participants will also be asked about their occupational history; use of certain consumer products; and the frequency with which they work with the soil, consume locally grown vegetables, and eat locally caught fish. In an effort to understand recent behavioral changes that may impact PFAS exposure, participants will be asked to identify any recent changes related to drinking water, consumption of locally caught fish and locally grown vegetables, or other changes that may impact their exposure to PFAS.

Answers to questions on the questionnaire will not be used to disqualify participants from the study.

Environmental Sample Collection Teams

Sample collection teams comprised of at least two EA staff people, at least one of whom is an ATSDR staff person, will travel together to each household selected to participate in environmental sampling.

Drinking Water Sampling

A subset of 10% of participating households will be selected for environmental sampling, including drinking water sampling. Identification and recruitment of these households is described in more detail in the 'Environmental Sampling Eligibility and Recruitment' section of this protocol.

A drinking water sample will be collected from the primary drinking water location (e.g. kitchen tap) at each household. If point of use filtration is in place, every attempt will be made to collect a sample prior to filtration and after filtration. Samples will be collected in accordance with EPA Method 537.1. Each sample will be collected in a 250 mL polypropylene bottle with a polypropylene screw cap. A preservation reagent (Trizma) will be added as a solid to each sample bottle prior to shipment to the field (or prior to sample collection).

Sample handlers will wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. The tap will be opened and allowed to flush until the water temperature has stabilized (approximately 3 to 5 min). Samples will be collected from the flowing system, taking care not to flush out the sample preservation reagent. Bottles should be filled to near capacity, but samples do not need to be collected headspace free. After collecting the sample, the sample handler will cap the bottle and agitate by hand until preservative is dissolved. The sample will be kept sealed from time of collection until extraction at the laboratory.

A field reagent blank (FRB) will be prepared along with each household tap water sample, and field duplicates collected when indicated per EPA Method 537.1. Prior to the investigation, laboratory staff will prepare two bottles for each household, one empty (preservative free) bottle, and another filled with reagent water and preservatives, which will be sealed. These bottles will be shipped to the sampling site along with the sample bottles. At the sampling site, the sampler will open the bottle containing the preserved reagent water and pour it into the empty sample bottle, after which the sampler will seal and label this bottle as the FRB. The FRB will be shipped back to the laboratory along with the tap water sample and analyzed to ensure that PFAS were not introduced into the sample during sample collection/handling.

Samples placed in insulated shipping containers with ice packs and cooled to less than 10 degrees Celsius (but not frozen). Tap water samples will be maintained in their cooled state with EA personnel performing twice daily checks of cooler temperature and adding ice packs as needed. Water samples will be shipped overnight to a laboratory accredited to perform EPA Method 537.1. Staff will maintain and manage proper chain of custody (Appendix D) for all water samples.

Samples will be analyzed for PFAS according to EPA method 537.1 by an EPA-approved laboratory. Test results will be reported as ng/L. All laboratory analysis will be conducted with established procedures for quality assurance and control. Table 3 provides the list of PFAS that will be measured in drinking water in this EA.

Table 3. List of PFAS to be measured in drinking water in this investigation – Abbreviation, Chemical Name, and Lowest Concentration Minimum Reporting Level (LCMRL)		
Abbreviation	Chemical Name	LCMRL (ng/L)
HFPO-DA	hexafluoropropylene oxide dimer acid	4.3
EtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid	4.8
MeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid	4.3
PFBS	perfluorobutane sulfonic acid	6.3
PFDA	perfluorodecanoic acid	3.3
PFDoA	perfluorododecanoic acid	1.3
PFHpA	perfluoroheptanoic acid	0.63
PFHxS	perfluorohexane sulfonic acid	2.4
PFHxA	perfluorohexanoic acid	1.7
PFNA	perfluorononanoic acid	0.83
PFOS	perfluorooctane sulfonic acid	2.7
PFOA	perfluorooctanoic acid	0.82
PFTA	perfluorotetradecanoic acid	1.2
PFTrA	perfluorotridecanoic acid	0.53
PFUnA	perfluoroundecanoic acid	5.2
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	1.5
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	1.8
DONA	4,8-dioxa-3H-perfluorononanoic acid	0.55
ng/L – nanograms per liter		

Indoor Dust Sampling

Measurements of household dust may act (to some extent) as a proxy for the presence of the analytes in products used in the household and as a descriptor of direct exposures (dust ingestion). Dust collection is intended to generate additional information about the contribution of non-drinking water exposures to overall PFAS exposure. Dust collection is exploratory and will yield information about how levels of PFAS in indoor dust samples correlated with PFAS serum concentrations. A subset of 10% of participating households will be selected for environmental sampling, including indoor dust sampling. Identification and recruitment of these households is described in more detail in the ‘Recruitment’ section of this protocol.

A composite dust sample will be collected from the floor of three locations inside each selected home – the primary living space as identified by the home owner (e.g., living room, family room, television room), the kitchen, and the bedroom in which EA participants spend the most time, as identified by the homeowner. Vacuum sampling will be used to collect dust samples. Unless otherwise indicated, the method described in the EPA technical standard operating procedure for High Volume Indoor Dust Sampling at Residences for Risk-Based Exposure to Metals (available here:

https://www.epa.gov/sites/production/files/documents/r8-src_src-dust-01.pdf), will be used to collect samples. This protocol is suitable for the collection of interior dust samples from either hard or smooth and highly textured surfaces, including brickwork and rough concrete as well as carpeting.

Dust will be removed from the designated surface area by means of a flowing air stream passing through a sampling nozzle at a specific velocity and flow rate. Dust will be separated from the air mechanically by a cyclone and collected in a catch bottle attached to the bottom of the cyclone. After collection, the bottle will be tightly capped, labeled, and placed upright in a storage container. All sampling equipment will be decontaminated and inspected for cleanliness prior to collection of each sample. Quality control samples will be collected to identify any potential cross-contamination between samples. Samples will be kept at an ambient temperature and shipped to an EPA approved and DOD accredited laboratory for analysis.

Indoor dust samples will be analyzed for 24 PFAS and PFAS precursors by an EPA-approved laboratory. An EPA-validated isotope dilution method (such as SWA 846) will be used to measure PFAS in indoor dust samples when it is available and approved for use. Samples will be held at the identified laboratory until the analytical method is available for analysis.

Table 4 identifies the species to be measured in indoor dust samples.

Table 4. List of PFAS and PFAS precursors to be measured in indoor dust samples in this investigation - Abbreviation and Chemical Name	
Abbreviation	Chemical Name
PFTA	perfluorotetradecanoic acid
PFTrA	perfluorotridecanoic acid
PFDoA	perfluorododecanoic acid
PFUnA	perfluoroundecanoic acid
PFDA	perfluorodecanoic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFPeA	perfluoropentanoic acid
PFBA	perfluorobutanoic acid
PFDS	perfluorodecane sulfonic acid
PFNS	perfluorononane sulfonic acid
PFOS	perfluorooctane sulfonic acid
PFHpS	perfluoroheptane sulfonic acid
PFHxS	perfluorohexane sulfonic acid
PFPeS	perfluoropentanes ulfonic acid
PFBS	perfluorobutane sulfonic acid
PFOSA	perfluorooctanesulfonamide
FtS 8:2	fluorotelomer sulfonic acid 8:2
FtS 6:2	fluorotelomer sulfonic acid 6:2
FtS 4:2	fluorotelomer sulfonic acid 4:2
EtFOSAA	N-ethyl perfluorooctanesulfonamidoacetic acid
MeFOSAA	N-methyl perfluorooctanesulfonamidoacetic acid

Anticipated Risks and Benefits

Participants may experience some discomfort and bruising in the area where the blood sample was collected. Blood samples will be collected by licensed and trained phlebotomists. Risk of harm from participation in this investigation is considered minimal.

All participants in this EA will be informed of the concentration of PFAS in their serum and how their serum concentrations compare to national reference populations. The subset of participants whose urine was randomly selected for analysis will be informed of the concentration of PFAS in their urine; as detailed in the methods above, if the geometric mean for a subset exceeds the NHANES 95th percentile, all urine samples from that site will be analyzed and results shared with participants. The subset of

participants whose households were randomly selected for dust and tap water PFAS analysis will receive these results.

Participants will be informed that their participation in this EA will help advance the understanding of PFAS exposure and will inform future PFAS health studies. Upon completion of the EA, participants will be provided an interpretation of their results by a public health professional.

The total time it will take participants to read through and sign consent/assent/parental permission forms, respond to the questionnaire, and provide a blood and urine specimen is estimated to be less than one hour. The total time it will take participants to have environmental samples collected is estimated to be about 20 minutes.

All sample collection and analysis is provided at no cost to participants.

Privacy Protections

Personal privacy will be protected to the fullest extent possible by applicable federal and state laws and regulations. For the data sets collected at each site, the HIPAA Safe Harbor de-identification method will be applied to extract specific personally identifiable information (PII) and store them separately from other information. When the data sets from all sites are reconciled at CDC/ATSDR, statistical re-identification risk assessment principles and methods will be used to fully de-identify the data set. All documents with personally identifiable information (i.e., consent forms, assent forms, collection logs, etc.) will be kept in locked cabinets and all electronic data will be stored on a password-protected network servers behind firewalls, accessible only to those staff working directly with raw data. Coded biospecimens and environmental samples will be sent to the laboratories—no personally identifiable information will be included. Any reports produced from this information will not identify specific individuals.

Records will be retained and disposed of in accordance with the ATSDR Records Control Schedule. Physical copies of assessment materials and reports will be maintained at ATSDR until no longer needed by program officials and will be kept in accordance with the corresponding retention schedules. Computer documents will be disposed of when no longer needed by program officials. Personal identifiers will be deleted from records when no longer needed and will be retained no longer than five years. Disposal methods will include erasing computer files, shredding paper materials, or transferring records to the Federal Records Center when no longer needed for evaluation and analysis. Records are retained for 20 years.

In compliance with federal and state privacy protection laws and regulations, the limited data set may be shared with other federal, state and/or local public health and environmental agencies via data use agreements for research purposes to advance the scientific understanding of human exposures to PFAS. These agencies must also protect this private information. Each state health department will act in compliance with their respective Sunshine Laws, which may impact the potential for information sharing.

Data Handling and Analysis

Data Handling

A detailed data management plan for all data collected in the exposure assessment can be found in Appendix I.

For all serum and urine samples, sample analysis, data quality assurance and quality control will be performed by the NCEH laboratory according to CDC/ATSDR protocols. Data that meets the required quality assurance and quality control specifications will be used for analysis.

For tap water data, data quality assurance and quality control will be performed by the identified laboratory as described in EPA Method 537.1 and only valid data will be used for analysis.

For indoor dust data, data quality assurance and data quality control will be performed by the identified laboratory according to the EPA-approved method (when available) or in accordance with another identified method capable of quantifying PFAS in a solid matrix should no EPA method be available in time to satisfy the needs of this project.

Questionnaire data will be collected using the Epi Info software tool and will be kept in a secure and encrypted electronic database.

All data will be transmitted via secure connections and methods to ATSDR or ATSDR contractor for incorporation into a centralized data management repository protected by CDC/ATSDR network firewall and additional security access controls. All results will be electronically transmitted in spreadsheet format using a secured and password-protected network.

Statistical Analysis of Serum and Urine Data from Individual Participants

National values for 12 PFAS in serum are available from the 2013–2014 NHANES and are considered to be national measures of exposure for the general US population. The NHANES geometric mean and 95th percentile for each PFAS serum concentration will be used as a comparison value for each EA participant.

National values for 18 PFAS in urine are being developed from the 2013–2014 NHANES. These will be considered national measures of exposure in the US population. The NHANES geometric mean and 95th percentile for each PFAS urine concentration will be used as a comparison values for each EA participant, when available. Only 10% of collected urine samples will be analyzed initially. If the geometric mean PFAS urine concentrations in a community are higher than the NHANES 95th percentile, all urine samples for the community will be shipped from the repository to the NCEH laboratory and analyzed as described above.

Questionnaire data will be used to aid in the interpretation of anomalous biomonitoring results.

Statistical Analysis of Aggregate Data

Individual participant data will be analyzed in aggregate to estimate community-level PFAS exposure. Aggregate urine data analysis will only be performed if all samples are analyzed at a site. Based on prior studies showing a log-normal distribution of PFAS concentrations in humans, and to allow for direct

comparison to NHANES data, the geometric mean with associated 95th percentile confidence interval, 90th percentile, and 95th percentile will be calculated for total PFAS and for each species, provided the proportion of censored data (data below the limit of detection [132]) does not equal or exceed 40%. For datasets in which the rate of censoring is below 40%, the limit of detection divided by square root of 2 (LOD/ $\sqrt{2}$) will be substituted for non-detect values [133]. For datasets in which censoring is equal to or greater than 40%, only high sample percentiles will be reported (e.g., 90th percentile, 95th percentile) [132]. Given the limitations in using substitution when calculating summary statistics for censored data, a sensitivity analysis of aggregated PFAS data will be performed using other statistical methods to account for censoring. For datasets in which <50% data are censored, Kaplan-Meier method [132] will be used to estimate the geometric mean with associated 95th percent confidence interval, 90th percentile, and 95th percentile. For datasets in which 50–80% of data are censored, maximum likelihood estimation will be used, and for datasets with >80% censoring, only high sample percentiles will be reported. Given that no nationally representative comparison values using these methods are available, results of this sensitivity analysis will be used only as a comparison to results obtained using simple substitution of censored values.

Where possible, summary statistics will be stratified based on participants characteristics (male/female, age categories, drinking water source, etc...) as reported in the questionnaires.

Environmental Data

Concentrations of PFAS in tap water samples will be compared to federal and/or state drinking water guidelines/advisories for PFAS as they are available.

Prior to any statistical evaluation, one participant will be identified from each household that is expected to be most exposed to the conditions in that household. For example, an adult who does not work outside of the home and who reports drinking primarily unfiltered tap water would be preferable over an adult who works outside of the home or who frequently drinks bottled water.

Following an exploratory analysis to confirm the distribution of the environmental data we will apply statistical tests as appropriate. If appropriate, Pearson's correlation test will be applied to evaluate the strength of the association between PFAS concentrations in drinking water and indoor dust, and the serum and urine concentrations measured in the sentinel participant. Data distributions will be assessed and transformed as necessary to meet statistical assumptions such as normality. Statistical significance of correlation will be evaluated using a two-sided Student's t-test based on a 95% confidence level. Correlation coefficients and statistical significance will only be determined when the rate of detection is greater than 60%.

Other statistical analyses (e.g., regression modeling) may be performed as appropriate and will be described in detail in the final reports.

Anticipated Products

Individual test results with a written explanation of meaning will be provided by mail to the participants (Appendix G). Biological sampling results for individuals will be provided separately from environmental sampling results. Following dissemination of individual results, an EA team member will be available to discuss individual questions by phone or email.

At the conclusion of each exposure assessment, a report will summarize the overall aggregate findings and conclusions of the assessment, but will not reveal personal identifiers. If warranted, recommendations for additional actions such as continued monitoring, educational activities, or interventions to reduce exposure will be made. Aggregate findings from all exposure assessments will be summarized in a final report and in manuscript(s) submitted for publication in the peer-reviewed scientific literature.

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