

# Pease Study

# Proof of Concept Protocol

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**Human Health Effects of Drinking Water Exposures to Per- and Polyfluoroalkyl Substances (PFAS) at Pease International Tradeport**

Portsmouth, New Hampshire

November 14, 2024

Prepared by the

United States Department of Human and Health Services (HHS)  
Agency for Toxic Substances and Disease Registry (ATSDR)  
Office of Community Health Hazard Assessment (OCHHA)  
Atlanta, Georgia 30341



**This protocol was updated to reflect changes necessary to restart the Pease Study during the COVID-19 pandemic. Appropriate safety precautions, including the use of all appropriate additional personal protective equipment (PPE), were implemented to keep the Pease Study team and participants safe during the study data and sample collection. The Pease Study Restart Plan (Appendix G in the Manual of Procedures - Attachment 14) was developed and outlines the additional procedures that will be implemented during recruitment, field work and community meetings to ensure that the Pease Study continued in compliance with CDC, state, and local requirements.**

**The activities that were modified included:**

- **Holding virtual Community Assistance Panel (CAP) meetings. Small group sessions (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 and consent documents to reassure potential Pease Study participants that all state and CDC guidelines will be followed.**
- **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 Pease Study team members (CDC/ATSDR and contractor staff) twice daily and taking participant's temperatures prior to entering the Pease Study Office.**
- **Offering participants to administer the questionnaire over the phone instead of at the study office to reduce exposure time: consent form administration and collection of biological samples will still have to occur at the study office.**

**These changes are provided in modified Pease Study protocol Attachments including study recruitment materials (Att6-7), study fact sheet (Att9c), screening scripts (Att6c-7c), appointment reminder script (Att10) and card (Att9a), consent package (Att9b), appointment tracking form (Att9c), and study Manual of Procedures (Att14, Appendix G).**

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## **1. PROJECT OVERVIEW**

### **1.1 Summary**

#### ***1.1.1 Literature Review***

Per- and polyfluoroalkyl substances (PFAS) are a family of chemicals used in industrial applications and consumer products. A number of PFAS chemicals including perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexane sulfonate (PFHxS) persist in the environment and have long serum half-lives in humans (Wang 2017).

A detailed review of epidemiological studies published to date was included in the ATSDR feasibility study (ATSDR 2017a; released Nov 2017). Health effects of PFAS exposure in children were also recently reviewed by Rappazzo (2017). Most studies of the health effects from PFAS exposures have focused on PFOA and PFOS, but have only evaluated PFHxS and perfluorononanoic acid (PFNA) and other PFAS chemicals sparingly (ATSDR 2017a). These include studies that evaluated data from the National Health and Nutrition Examination Survey (NHANES), occupational studies, studies of West Virginia and Ohio residents and workers exposed to PFOA from a chemical plant (the “C8” studies), and national surveys conducted in other countries where exposures to PFAS were found mostly from consumption of food and beverages in PFAS-contaminated packaging.

The scientific evidence linking PFAS exposures with adverse health effects is rapidly growing but is inconsistent for a variety of reasons, including differences concerning exposure levels, methods of ascertaining diseases and the exposure and effect biomarkers measured. For some health endpoints, only one or a few studies currently exist. Nevertheless, studies have found associations with changes in lipids (Steenland 2009; Zeng 2015, Mora 2018), levels of uric acid (Steenland 2010), thyroid and sex hormones (Wen 2013; Lopez-Espinosa 2016, Preston 2018), liver (Darrow 2016, Mora 2018), and immune function (Grandjean 2012, 2017), as well as reduced birth weight (Bach 2015, Verner 2015), reproductive effects and some cancers (Lopez-Espinosa 2011; Barry 2013). While C8 studies provided extensive and high quality information on PFOA studying a large cohort of highly exposed residents (60,000+) and workers living in the vicinity of the production facility; it didn’t address the number of other PFAS compounds and exposures routes. Because of this research gap, there is a need for more epidemiological research on the health effects of PFAS exposures. Except for the C8 studies, there is scant information on the health effects of exposures to PFAS-contaminated drinking water.

### **1.1.2 Pease Site History**

PFOS, PFOA, PFHxS and other PFAS are constituents in aqueous film-forming foam (AFFF), used to extinguish flammable liquid fires. Since the 1970s, military bases in the U.S. have used AFFF with PFAS constituents for firefighting training as well as to extinguish fires. At some military bases, such as the Pease Air Force Base in Portsmouth, NH, AFFF use has resulted in the migration of PFAS chemicals through soils to ground water and/or surface water sources of drinking water for the base and/or surrounding communities (ATSDR 2017a). After the base closed in 1991, the three on-base supply wells served the Pease International Tradeport (“Pease”), a business and aviation industrial park, with water in one supply well contaminated with PFAS at concentrations measured in 2014 as high as 2.4 µg/L for PFOS and 0.35 µg/L for PFOA, which were above the EPA advisory levels (NH DHHS 2016). In 2009, the US EPA established provisional drinking water health advisory levels for PFOS and PFOA of 0.2 µg/L and 0.4 µg/L, respectively, and in 2016, EPA established a new lifetime health advisory for the total PFOA and PFOS combined of 0.07 µg/L (US EPA 2009, 2016).

In 2015, the New Hampshire Department of Health and Human Services (NH DHHS) initiated the Pease biomonitoring program in response to the affected community members’ request for clinical testing following their consumption of PFAS-contaminated water. This program established a design framework which was adopted by ATSDR for this current study (NH DHHS 2016).

### **1.1.3 Health Study Feasibility Assessment**

In 2017, ATSDR published a feasibility assessment of possible future drinking water epidemiological studies at Pease (ATSDR 2017a). As part of this feasibility assessment, ATSDR reviewed the published epidemiological studies that evaluated the health effects of PFAS exposures and the available information. Most of the epidemiological studies were cross-sectional in design because cross-sectional studies are especially suitable for evaluating effect biomarker tests such as liver, kidney, immune and thyroid function (Checkoway 2004). ATSDR concluded that cross-sectional epidemiological studies of children and adults at one site (e.g. at Pease) were feasible for some health endpoints (e.g., lipids, kidney function), but the size of populations would be insufficient for other important health endpoints (e.g., thyroid, liver and immune function, autoimmune diseases). Therefore, the feasibility assessment concluded that a multi-site PFAS study was necessary.

For the multi-site study, the studies at different sites will be cross-sectional and involve separate evaluations of children (ages 4-17) and adults (ages ≥18). The children and adult studies will evaluate



multiple communities impacted by PFAS-contaminated public drinking water supply wells and/or private wells. The criteria for selecting study sites include:

1. Documented past or present PFAS drinking water concentrations at the tap above the current EPA Lifetime Health Advisory for PFOS + PFOA (i.e., 70 µg/L or 70 parts per trillion [ppt]), or PFAS serum biomonitoring results indicating levels above NHANES serum levels for PFOA, PFOS and/or PFHxS,
2. Size of the population exposed,
3. Amount of information available on the contaminated drinking water system or private wells, and
4. If biomonitoring for PFAS has previously occurred at the site.

The overall goal will be to recruit at least 2,000 children and 6,000 adults for the multi-site study from the sites exposed to PFAS-contaminated drinking water. The participants will be categorized based on the measured serum concentration of PFAS compounds or on modeled estimated historical serum levels (e.g., referent or low, medium, high). At sites with preceding biomonitoring such as at Pease (NH DHHS 2016), the child and adult studies will evaluate changes in PFAS concentration over time. The studies will reconstruct historic serum PFAS concentrations by estimating half-lives and elimination rates as well as water contamination modeling to inform the pharmacokinetic (PK) or physiologically based pharmacokinetic (PBPK) modeling. Historical serum PFAS reconstruction will enable the evaluation of exposure lags and vulnerable periods as well as statistical analyses that can control for reverse causations (Dhingra 2017).

#### ***1.1.4 Summary of Pease Study Goals***

The research study, titled the “Human health effects of drinking water exposures to per- and polyfluoroalkyl substances (PFAS) at Pease International Tradeport, Portsmouth, NH” (hereafter, the “Pease Study”), will be a proof of concept study for the multi-site health study. It will be a cross-sectional study and will recruit from the convenience sample of children and adults who participated in the 2015-7 Pease biomonitoring program (NH DHHS, 2016; Daly 2018). The goal is to enroll at least 350 children (ages 4-17) and 1,000 adults aged ≥18 years. To meet sample size requirements, those that met biomonitoring eligibility criteria but were not enrolled in the 2015-7 Pease biomonitoring program may be recruited. Eligible exposed participants had to work at, live on, or attend childcare at the Pease Tradeport or Pease Air Force Base, or live in a home near the Pease facilities that were served by a PFAS-contaminated private well between 2004 and May 2014. In order to restrict this study to drinking water exposures, adults

occupationally exposed to PFAS will not be eligible for the study (e.g., ever firefighters). Likewise, children whose birth mothers were occupationally exposed will not be eligible. Eligible females who are pregnant may enroll. The federal regulations do not allow people who are prisoners or under house arrest to take part in this type of study.

The main goals of the study are to 1) evaluate the procedures and test the study protocols in order to identify any issues that need to be addressed before embarking on a multi-site study; and 2) examine associations between measured and historically reconstructed serum levels of PFAS including PFOA, PFOS, and PFHxS (see **Section 3.10**), and selected health outcomes as described below and detailed in study hypotheses (see **Section 2.5.2**).

The adult and children studies will also include smaller referent groups of children (n=175) and adults (n=100) from other areas of Portsmouth, NH, who were never exposed to PFAS-contaminated drinking water. Birth mothers of referent children likewise must never have had exposure to contaminated drinking water from Pease. Referent results will be used to compare serum concentration in exposed versus 'unexposed' and evaluate the need for the referent groups in the multi-site study. Categorization based on measured serum PFAS concentrations in communities with exposure may provide enough participants at background levels without a need to sample external comparisons. As most Americans have measurable concentrations of at least some PFAS (Calafat 2007, Ye 2017), participants will be categorized based on the measured concentration of PFAS compounds or on modeled estimated historical levels (e.g., referent or low, medium, high).

Based on our literature review of epidemiological studies of PFAS, we propose to examine association between PFAS compounds and lipids, renal function and kidney disease, thyroid hormones and disease, liver function and disease, glycemic parameters and diabetes, as well as immune response and function in both children and adults. In addition, we plan to investigate PFAS differences in sex hormones and sexual maturation, vaccine response, and neurobehavioral outcomes in children. In adults, additional outcomes of interest include cardiovascular disease, osteoarthritis and osteoporosis, endometriosis, and autoimmune disease.

The underlying justifications for the selected research hypotheses and health outcomes were: (1) to follow-up a suggestive positive result (e.g., OR or RR >1.2) for a disease or effect biomarker in one or more PFAS epidemiological studies of children aged 4 or older and/or adults; or (2) to follow-up a finding for a disease or biomarker in the C8 studies and/or PFAS studies utilizing NHANES data; and/or 3) to study a biomarker or disease for which a multi-site study of individuals aged 4 years or older would have sufficient

statistical power but for which there is a lack of data on the effect of PFHxS exposure on the disease or biomarker. With the proposed sample sizes for the overall multi-site study (n=6,000 for adults and n=2,000 for children) we should have sufficient power to detect mean differences and odds ratios in the ranges of those observed in other well designed epidemiologic studies.

The adult and children studies will obtain blood specimens from participants to measure PFAS serum levels and several effect biomarkers in addition to completing a questionnaire. The child and adult studies will evaluate changes in serum PFAS concentration over time using the existing biomonitoring and new PFAS results and will contribute to half-lives and elimination rate estimations for PBPK modeling. Urine specimens will be collected and stored for future analyses.

The results from the Pease Study will contribute to the body of scientific research examining health outcomes related to community PFAS exposures from contaminated drinking water. They will inform the direction and design of the multi-site and other future PFAS studies.

## **1.2 Study Investigators and Roles**

The health study team at ATSDR is responsible for the development of protocols for the Pease Study and the future PFAS multi-site study. A table describing the roles of investigators, oversight steering committee, external experts, as well as other collaborators and consultants is found in **Attachment 1**. The study investigators declare no conflicts of interest, financial or otherwise, that may prevent them from exercising objectivity in carrying out the study.

Serum specimens for PFAS analyses will be submitted to the CDC NCEH DLS, Atlanta, GA. Core clinical and research effect biomarkers will be analyzed by commercial laboratories as specified in the protocol. Urine specimens will be collected and stored for future analysis and study.

The NH DHHS will provide a recruitment frame and provide support for ATSDR to recruit from its existing list of participants in its Pease biomonitoring program. ATSDR will work with local officials to recruit additional exposed and referent participants to meet sample size goals. ATSDR will work with local officials and school districts to abstract pertinent information from children's school records. Similarly, ATSDR will also reach out to local medical societies to facilitate medical records verification among local practitioners.

The protocol has undergone external peer review. In addition, the Pease Community Assistance Panel (CAP) will have an opportunity to provide input on the protocol and will assist in recruitment outreach. The study protocol has been reviewed and approved by the CDC Institutional Review Board (IRB, CDC

protocol number 7061) under CDC's Federal wide Assurance (FWA) No. 00001413 and by the Office of Management and Budget (OMB; OMB control number 0923-0061).

## **2. INTRODUCTION**

### **2.1 Authority**

ATSDR is authorized to conduct the Pease Study under Section 8006 of the Consolidated Appropriations Act, 2018, and research in general, under the 1980 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), as amended by the 1986 Superfund Amendments and Reauthorization Act (SARA) (42 U.S.C. 9601, 9604).

### **2.2 Background**

#### ***2.2.1 Characterization of Pease Drinking Water PFAS Contamination***

From approximately the early 1970s, aqueous film-forming foam (AFFF) for firefighting training and extinguishing flammable liquid fires was used in a number of military and non-military sites around the country. Several PFAS are components of AFFF, including perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS). PFAS chemicals in the AFFF likely leached into the soil and groundwater at and around the installation using AFFF.

The requirements for the site characterization and documentation of drinking water system(s) contamination by PFAS from AFFF include information on the following parameters:

1. Enumeration of supply wells that provided drinking water to the site. Range of years in which the site received water from each of those wells.
2. By year (or month if available), the proportion of the total water supply provided by each supply well. How did water supply change after the contamination was detected?
3. Characteristics of the drinking water distribution system including Information on whether mixing from the supply well(s) occurred at the treatment plant before entering distribution system or if each supply well served a specific area in the system. Also, information on water purchased from other systems and areas of the distribution system served by purchased water.
4. If the water system is supplied by surface water, characteristics of this supply.

5. Description of when and how samples from monitoring or supply wells (or surface water) were obtained when the contamination was detected. Maximum and average sample concentrations for each of supply wells.
6. Which PFAS were detected, when, and at what levels of concentration; how they relate to EPA's lifetime advisory limits.
7. Any historical samples or measurements available?
8. Any information on the historical use of AFFF (training exercises, fire incidents, spills, etc.) at the site (e.g., military base airstrip) which was the source of the drinking water contamination.
9. Any previous human biomonitoring program conducted? If yes, what were the results in regard to concentration and descriptive/predictive factors of those concentrations (i.e., volume of water consumed, length of residence at site, differences in age, race, or other population characteristics)?

Much of the above information is already available in considerable detail for the Pease site as described below. The Pease Tradeport, Portsmouth, NH, opened in 1993 on land formerly occupied by the Pease Air Force Base, which closed in 1991. From approximately 1970 until 1991, the base used aqueous film-forming foam (AFFF) for firefighting training and extinguishing flammable liquid fires. PFAS chemicals in the AFFF likely leached into the soil and groundwater at the base sometime during the 1970s.

Three major supply wells provided drinking water to the base: the Haven, Smith, and Harrison wells. Before 1981, the wells fed directly into the distribution system so that a particular area of base would primarily receive water from the nearest well. After 1981, the water from the three supply wells mixed at the treatment plant before entering the distribution system. These same three supply wells provided drinking water to the Pease Tradeport after it opened.

In 2013, sampling of monitoring wells near the former fire training areas at the base detected PFOS and PFOA as high as 95 µg/L and 56 µg/L. Sampling of the three supply wells serving the Pease Tradeport for PFAS contamination occurred in April and May 2014. In the April sampling, the Haven well had PFOS, PFOA, and PFHxS levels of 2.5 µg/L, 0.35 µg/L, and 0.83 µg/L, respectively. In the May sampling, the Haven well had PFOS, PFOA, and PFHxS levels of 2.4 µg/L, 0.32 µg/L, and 0.96 µg/L. Other PFAS were also detected in the Haven well. The Harrison well had much lower levels of these contaminants with maximum PFOS, PFOA, and PFHxS levels of 0.048 µg/L, 0.009 µg/L, and 0.036 µg/L, respectively. The Smith well had maximum levels of PFOS and PFHxS of 0.018 µg/L and 0.013 µg/L, respectively, with an estimated level of PFOA of about 0.004 µg/L.

From the opening of the Pease Tradeport through 1999, the Haven well on average provided about 56% of the total water supply at Pease, with the Smith well providing 44% and the Harrison well out of service. In 2000-2001, the Haven well supplied 88% of the supply and the Smith well supplied 12%. From 2003 until removal from service in May 2014, the Haven well on average supplied about half the water supply. By 2006, the Harrison well was back in service and the Smith and Harrison wells together supplied on average about half of the water supply at the Pease Tradeport. After May 2014, the Smith and Harrison wells supplied 56% of the Pease Tradeport water supply and the City of Portsmouth provided the other 44%.

No water samples of the Pease Tradeport distribution system for PFAS are available from the period when the Haven well was in operation. However, it is possible to estimate the concentrations of PFOS, PFOA and PFHxS in the distribution system, by using a simple mixing model and assuming that contamination concentrations are approximately uniform throughout the system. The model takes into account the pumping rates for each of the three wells, the total water demand, and the concentrations of PFAS in the wells during the April and May 2014 sampling. Using this simple approach, the estimated levels of PFOS, PFOA, and PFHxS in the Pease Tradeport distribution system in April 2014 would be approximately 1.4 µg/L, 0.2 µg/L, and 0.5 µg/L, respectively. For comparison, the EPA's lifetime health advisory level for the combined concentrations of PFOA and PFOS is 0.07 µg/L. No drinking water health advisory level currently exists for PFHxS or other PFAS chemicals.

### ***2.2.2 2015 Pease Biomonitoring Program***

In 2015, NH DHHS established the Pease biomonitoring program for PFAS. Those participants had to work at, live on, or attend childcare at the Pease Tradeport or Pease Air Force Base (AFB), or live in a home in or before 2014 that was served by a private well with Pease-related PFAS contamination (for any period of time). Ever firefighters (persons who were ever employed as firefighters) were included in the biomonitoring program.

This was an exposure-based convenience (or volunteer) sample, not a statistically based sample. Nevertheless, the testing program provided important information on the extent and magnitude of exposures to the PFAS-contaminated drinking water at the Pease Tradeport. The Pease biomonitoring program obtained blood specimens for PFAS analyses from 1,578 persons (NH DHHS 2016; the report was published in 2016 but some biomonitoring activities were ongoing throughout 2017 and we refer to those efforts as occurring in 2015-17 with n=1,836).

The results from the blood-testing program indicated that the exposed population had serum levels of PFOS and PFHxS that were about two to three times higher than the U.S. population based on data from NHANES 2013-4 and from other epidemiological studies in the U.S. (**Attachment 2**). In analyses conducted by NH DHHS (2016), geometric mean PFHxS serum levels were higher for persons who drank  $\geq 4$  cups of water per day compared to those who drank  $< 4$  cups per day (4.76  $\mu\text{g/L}$  versus 3.77  $\mu\text{g/L}$ ). NH DHHS measured 8 to 14 PFAS congeners at 3 analytical laboratories. Among PFOA, PFOS, PFHxS and PFNA concentrations, water consumption had the strongest effect on PFHxS serum levels. In particular, water consumption had the highest effect on PFHxS serum levels among persons aged  $\leq 19$  years ( $\beta = 0.31$ , SE = 0.15, marginal effect = 36.4%). Geometric mean PFOS and PFOA serum levels were also higher among persons who drank  $\geq 4$  cups of water per day compared with those who drank  $< 4$  cups per day (NH DHHS 2016). Linear trends were observed for geometric mean serum levels of PFOS, PFOA, and PFHxS and increasing time spent at the Pease Tradeport. The trend was strongest for PFOS and PFHxS (NH DHHS 2016). Findings from the report have been recently published (Daly 2018).

### **2.3 General Approach for Pease Study Recruitment**

In considering possible study designs, ATSDR focused on the methods used in previous epidemiological research of PFAS exposures (ATSDR 2017a). Adopting study design methods consistent with previous research facilitates the interpretation and synthesis of findings across studies. Most of the epidemiological studies of PFAS exposures were cross-sectional and evaluated serum PFAS measurements. Some studies also evaluated cumulative PFAS serum levels estimated from historical reconstruction models. ATSDR concluded that epidemiological studies of populations at Pease should be cross-sectional and evaluate measured serum PFAS measurements as well as historically reconstructed estimates of cumulative PFAS serum levels. ATSDR also concluded that methods used to evaluate health-related endpoints in the studies should be consistent with methods used in previous epidemiological research of PFAS exposures, given adequate sample size and power.

NH DHHS and the Pease CAP will assist ATSDR to conduct outreach to encourage participation. At Pease, ATSDR will recruit from the existing convenience sample of children (aged 4-17), their parents, and other adults (aged  $\geq 18$ ) who previously participated in the Pease biomonitoring program. Parent, parents, and parental in this study protocol refers to children biological or adopted parents or their legal guardians. If sample size goals are not met, those that met biomonitoring eligibility criteria but were not enrolled in 2015-7 will be recruited. ATSDR will also recruit a small referent group of children unexposed to PFAS-contaminated drinking water through outreach to Portsmouth schools and day care centers (for those 4–

5-year-old). Recruitment of a small group of adult referents will include outreach to current adult education students among the five colleges at Pease (i.e., students who first attended these schools after closure of the Haven Well), Portsmouth government employees (AFSCME Local #1386), Portsmouth community organizations, and/or specific Portsmouth neighborhoods.

## **2.4 Study Objectives**

The main goals of the studies of children and adults at Pease are to (1) evaluate the methods and procedures in the study protocols to identify any issues that need to be addressed before embarking on a national multi-site study; and (2) evaluate the associations between specific health effects and serum PFAS concentrations among those exposed to PFAS-contaminated drinking water at Pease.

## **2.5 Study Questions**

### ***2.5.1 Literature Review***

A literature review was conducted for the Pease feasibility assessment and can be accessed in the final feasibility report (ATSDR 2017a). The literature review from the Pease feasibility assessment concluded that most information on potential health effects concerned exposures to PFOA. In particular, numerous studies have been conducted of West Virginia and Ohio residents and workers exposed to PFOA from a chemical plant (the “C8” studies) (Frisbee 2009). Studies of other workforces also focused primarily on PFOA exposures. The literature review found that less information was available about the potential health effects of PFOS exposures, and little information was available on the potential health effects of exposures to PFHxS. Because the primary contaminants in the drinking water at the Pease Tradeport were PFOS and PFHxS, epidemiological studies of the Pease populations have the potential to fill key knowledge gaps and address the community’s concerns.

The literature review identified many health-related endpoints evaluated in previous epidemiological studies of PFAS exposures. These included cancers, changes in lipids, effects on thyroid and immune function, and developmental delays. They also included effects on kidney and liver function and sex hormones, and diseases such as endometriosis, ulcerative colitis and osteoporosis (ATSDR 2017a).

The literature review found that most of the epidemiological studies of PFAS exposures were cross-sectional and evaluated serum PFAS measurements. Some studies also evaluated cumulative PFAS serum levels estimated from modeling methods. ATSDR concluded that any study of populations exposed to the PFAS-contaminated drinking water at the Pease Tradeport should be cross-sectional and should evaluate



measured serum PFAS measurements as well as estimated cumulative PFAS serum levels. ATSDR also concluded that methods used to evaluate health-related endpoints in the Pease Tradeport populations should be consistent with methods used in previous epidemiological research of PFAS exposures.

#### 2.5.1.1 Health Effects in Children

There is some evidence that PFAS exposures are associated with decreased birth weight, small fetus size for gestational age, measures of intrauterine growth retardation, and preterm birth. In particular, two meta-analyses have found an overall decrease in birthweight associated with PFOA and PFOS (Johnson 2014, Negri 2017, Verner 2015; Bach 2015). However, the findings across studies are inconsistent for adverse birth outcomes, and few studies have evaluated PFHxS. Several studies of infants have found that prenatal PFAS exposures affect thyroid function, but only two studies have evaluated thyroid function in older children (Lopez-Espinosa 2012; Lin 2013, Preston 2018).

A few studies of children have found elevated uric acid with PFAS exposures, but the possibility of reverse causation exists (Geigere 2013; Kataria 2015; Qin 2016). Positive findings occurred in some of the four studies of PFAS exposures and testosterone and other sex hormones, but the findings were not consistent across studies and further research is necessary (Maisonet 2015; Lopez Espinosa 2016, Zhou 2016). Growing evidence suggests that exposure to per- and polyfluoroalkyl substances (PFASs) may disrupt lipid homeostasis and liver function, but data in children are limited. Indicators of adiposity and glucose metabolism were also linked with PFAS in a large follow up study of children and adolescents (Domazet 2016). Recent study (Mora, 2018) suggests that prenatal and mid-childhood PFAS exposure may be associated with modest, but somewhat conflicting changes in the lipid profile and ALT levels in children.

There is some evidence from four studies that PFAS exposures might be associated with attention deficit hyperactivity disorder (ADHD), but findings have not been consistent across studies (Stein 2011; Liew 2015; Ode 2014; Hoffman 2010). In the Stein (2011) study, the ORs for ADHD and PFOS and PFHxS were 1.27 (95% CI 0.99-1.64) and 1.6 (95% CI 1.21-2.08), so there was some evidence of an increased risk, although not strong. A study using NHANES data obtained an OR of 1.5 (95% CI 1.02-1.08) for 1µg/L increase in serum PFOS and ADHD (Hoffman 2010). Other studies have found conduct and coordination problems associated with PFOS (Fei 2011) and executive function deficits with PFOS and PFHxS (Vuong 2016). Evaluating the evidence for PFAS exposures and neurobehavioral outcomes is difficult for several reasons: 1) the studies used different methods to measure the outcomes, 2) studies are inconsistent in the outcomes evaluated, and 3) too few studies exist. For example, there is little evidence that PFAS affects IQ, primarily because only two studies evaluated it; one in Taiwan, which observed deficits (Lien

2016), and one at C8 which did not (Stein 2011). We believe it is worth evaluating whether the PFAS mixture at Pease (and other sites with contamination due to AFFF use) is associated with IQ deficits or other neurobehavioral outcomes.

A few studies have found associations between PFAS exposures and a decline in antibody response to specific vaccines (Grandjean 2012, 2017), but only two studies evaluated the same vaccine (i.e., rubella; Granum 2013, Stein 2016).

In summary, there are considerable data gaps concerning the health effects in children of PFAS exposures. This is because of the small number of studies conducted, inconsistencies in methods and findings across studies, and limited sample sizes in some studies. As for other adverse outcomes, few studies have evaluated the effects on children of PFHxS exposures.

A systematic review of PFAS studies of children concluded that there was "...generally consistent evidence for PFAS' association with dyslipidemia, immunity including vaccine response and asthma, renal function, and age at menarche" (Rappazzo 2017). The review noted the limited number of studies for any one particular health outcome, the variability in outcome measurement, and the need for longitudinal studies.

#### 2.5.1.2 Health Effects in Adults

Based on its detailed assessment of the epidemiological literature, ATSDR concluded that there was limited information concerning associations with PFAS exposures and most cancers and other adult diseases (ATSDR 2017a). In particular, few studies have evaluated PFHxS exposures and the risk of cancers and other adult diseases.

Epidemiologic studies of subjects exposed to PFOA and PFOS at background levels and at occupational settings have reported positive associations with number of health outcomes and conditions. Lipid and cholesterol concentrations were associated with increased PFOA or PFOS (Frisbee et al., 2010; Nelson et al., 2010; Winqvist 2014b; Steenland et al., 2015), as were increased uric acid levels (Costa et al., 2009; Steenland et al., 2010; Shankar et al., 2011; Geiger et al., 2013; Gleason et al., 2015), concentrations of thyroid and sex hormones (Olsen and Zobel, 2007; Knox et al., 2011; Jain, 2013; Wen et al., 2013; Winqvist 2014b), immune parameters (Dalsager et al., 2016), and reproductive effects (Joensen et al., 2013; Kristensen et al., 2013; Crawford et al., 2017).

Associations with elevated liver enzymes were found with PFAS in most cross-sectional studies (Olsen et al., 2000; Sakr et al., 2007a; Lin et al., 2010; Gallo et al., 2012; Gleason et al., 2015) but were weaker or

found no association in the cohort studies of liver function (Sakr 2007b, Darrow 2016). Structural protein cytokeratin 18 (CK-18) and its components have been used as a new non-invasive serum biomarker for non-alcoholic fatty liver disease and suspected steatohepatitis for adults and children (Fieldstein 2013, Shen 2012, Vos 2008). Prevalent coronary heart disease was positively associated with PFAS exposure in a cross-sectional examination of NHANES (Shankar 2012) but not in cohort designs (Winquist 2014b; Mattsson 2015).

Two studies of osteoarthritis show association with PFOA in cross sectional analyses (Innes 2011, Uhl 2013) but no association in longitudinal analyses (Innes 2011). Another cross-sectional NHANES study (Khalil 2016) found an association with osteoporosis among women for PFHxS. Two NHANES studies (Lin 2014, Khalil 2016) also found associations with bone mineral density. Although, these studies are cross-sectional, they provide important evidence for a link between PFAS exposures and osteoarthritis and osteoporosis.

In evaluation of kidney function, data from Watkins et al. (2013) and Dhingra et al. (2017) showed that while measured PFOA showed positive association, modeled PFOA concentrations had no relation to eGFR illustrating example of potential reverse causality.

There is increasing evidence showing associations between PFAS and markers of glucose homeostasis and insulin resistance, and associations with adult type 2 diabetes risk in men and women (Cardenas 2017; He 2018; Sun 2018).

Roles of inflammatory cytokines and adipokines have been explored in several studies of liver disease such as non-alcoholic fatty liver disease/steatohepatitis and in atherosclerosis (Hennig 2007, Wahlang 2016, and Clair 2018). Proinflammatory responses, alterations in leptin signaling, and increases in TNF-alpha and IL-2 were reported in mechanistic studies with various persistent organohalogen pollutants in relation to diabetes and metabolic syndrome (Ferrante 2014; Wieser 2013). These associations have not yet been explored specifically with PFAS compounds.

Some positive associations have also been found for cancer outcomes; with C8 studies finding associations between PFOA and kidney and testicular cancer (Alexander and Olsen, 2007; Barry et al., 2013; Bonefeld-Jorgensen et al., 2014; Hardell et al., 2014; Steenland et al., 2015).

Some studies have observed no association between PFAS exposure and health effects such as specific cancers (Alexander and Olsen, 2007; Lundin 2009), lipids or metabolic function (Fisher, 2013). Effects of confounding, bias, and chance on observed associations with PFAS compounds were explored in several

reviews of immune and cancer outcomes (Chang 2014, Chang 2015) and in studies of PFAS and menopause and endometriosis (Ruark 2017, Ngueta 2017).

In summary, most epidemiological studies of PFAS have evaluated PFOA and PFOS, but the epidemiological evidence is still limited for these two chemicals. The epidemiological evidence for other PFAS, e.g., PFHxS and PFNA, is considerably more limited. Therefore, additional research on the effects of PFAS, including PFHxS, PFNA, PFOS, and PFOA, is necessary to determine whether exposures increase the risk of adult diseases.

The proposed scope of the funding and sample size estimated for this health study would be too small and insufficient to evaluate cancer health outcomes.

### **2.5.2 Hypotheses**

For children (aged 4-17 years), the Pease Study will evaluate the following main hypotheses, following the outline of the biochemical analytical plan (**Attachment 3**):

Higher serum levels of PFOA, PFOS, PFHxS, or other PFAS are associated with:

1. Lipids (higher total cholesterol, low-density lipoprotein, and triglycerides, and higher prevalence of hypercholesterolemia; obesity).
2. Impaired renal function (a higher level of uric acid, a higher prevalence of hyperuricemia, and a lower estimated glomerular filtration rate (eGFR)).
3. Liver function/damage biomarkers (alanine transaminase (ALT),  $\gamma$ -glutamyltransferase (GGT), direct bilirubin, cytokeratin-18 (CK-18)), and non-alcoholic fatty liver disease/steatohepatitis (determined by CK-18 levels).
4. Glycemic parameters (glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide, pro-insulin) and diabetes (type 1 and 2).
5. Measures of thyroid function (differences in thyroid stimulating hormone - TSH, total thyroxin - TT4, and TT3; higher prevalence of hypothyroidism).
6. Differences in sex hormones, growth and sexual maturation (testosterone, estradiol, and sex hormone-binding globulin (SHBG); insulin-like growth factor - 1 (IGF-1), age at menarche, delayed puberty).

7. Immune response including prevalence of hypersensitivity-related outcomes (e.g., asthma, atopic dermatitis; higher levels of immunoglobulins (IgG, IgA and IgM) and lower antibody responses to rubella, mumps, and diphtheria vaccines).
8. Neurodevelopmental outcomes (lower intelligence quotient (full scale IQ), attention-deficit and hyperactivity disorder (ADHD)).

For adults (aged  $\geq 18$  years), the Pease Study will evaluate the following main hypotheses.

Higher serum levels of PFOA, PFOS, PFHxS, or other PFAS are associated with:

1. Lipids (higher total cholesterol, low-density lipoprotein and triglycerides) and a higher prevalence of hypercholesterolemia).
2. Higher prevalence of coronary artery disease and hypertension (including pregnancy induced hypertension).
3. Renal function (higher level of uric acid and a higher prevalence of hyperuricemia, lower estimated glomerular filtration rate (eGFR)) and higher prevalence of kidney disease.
4. Glycemic parameters (glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide, pro-insulin) and diabetes (type 1 and 2).
5. Differences in thyroid hormones (thyroid stimulating hormone (TSH), TT4, and TT3), and higher prevalence of hypothyroidism.
6. Liver function/damage biomarkers (alanine transaminase (ALT),  $\gamma$ -glutamyltransferase (GGT), direct bilirubin, cytokeratin-18 (CK-18)) and liver disease.
7. Higher prevalence of osteoarthritis
8. Higher prevalence of osteoporosis.
9. Higher prevalence of endometriosis.
10. Measures of immune response and inflammation (serum levels of IgA, IgE, IgG, IgM, C - reactive protein (CRP), antinuclear antibodies (ANA), inflammatory cytokines and adipokines (interleukin 1- $\beta$  (IL-1 $\beta$ ), interleukin 6 (IL-6), interleukin 8 (IL-8), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), leptin, adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1).

11. Higher prevalence of autoimmune diseases such as ulcerative colitis, rheumatoid arthritis, lupus, and multiple sclerosis.

## **2.6 Intended Use of Study Findings**

Given that epidemiological research on the health effects of PFAS is at a relatively early stage, these health studies should make an important contribution to the scientific literature as the first epidemiological child and adult studies in the U.S. to evaluate potential health effects occurring in populations exposed to PFAS-contaminated drinking water caused by the use of AFFF.

The studies will provide the PFAS serum level and the results of the effect biomarker tests to each study participant. The participant can use this information for medical decision-making.

The health study will provide summaries of the findings to the participating affected communities.

Finally, any issues identified with the procedures, methods and approaches will be addressed in the protocol for the multi-site study.

## **3. METHODS**

### **3.1 Study Design**

The child and adult studies will be cross-sectional with separate evaluation of children (ages 4 – 17 years) and adults (aged  $\geq 18$  years). The participants will be recruited from existing convenience samples from the Pease biomonitoring program, additional exposure groups, and from the referent groups.

The general components of the data collection are as follows:

- ATSDR will obtain adult consent, and parental permission and child assent (ages 7 years and older), to participate in this research study.
- ATSDR will administer adult and child questionnaires and seek medical records verification of self-reported diseases and medical histories.
- ATSDR will administer neurobehavioral test batteries to the children and their parents and seek to abstract children's school records, in particular, special education records.
- ATSDR will obtain blood samples from each participant for analyses of PFAS and a number of effect biomarkers.

- As part of the current protocol, both children and adults will be asked to provide a urine sample for future analyses. This may include PFAS and/or relevant effect biomarkers.
- ATSDR will seek consent to store residual blood and urine samples for future analyses.

### 3.2 Study Populations and Eligibility

The target area for the adult and child studies is the Pease Tradeport in Portsmouth, NH, served by PFAS-contaminated drinking water caused by the use of AFFF at the former Pease Air Force Base (ATSDR 2017a, NH DHHS 2016). The target population consists of those exposed to PFAS contaminated drinking water at Pease. Those eligible include those who worked, resided, or attended childcare at the Pease Tradeport or Pease Air Force Base prior to the closing of the Haven supply well in May 2014, were exposed in utero or during breastfeeding, or lived in a home near Pease that was served by a PFAS-contaminated private well. Therefore, ATSDR is recruiting adults and children who had PFAS-contaminated drinking water exposure from 2004 to May 2014.

Persons eligible to take part in this research study will focus on the 2015-2017 Pease biomonitoring program participants (NH DHHS, 2016). Ever firefighters (a person who was ever employed as firefighter) were included in the Pease biomonitoring program, but firefighters and others with occupational PFAS exposure from sources other than the drinking water will not be included in the Pease Study. In addition, children whose birth mothers had occupational exposures to PFAS from sources other than drinking water will be excluded. The goal is to enroll at least 350 children (ages 4-17) and 1,000 adults aged  $\geq 18$  years with community exposure to PFAS. In the event that recruitment does not reach the desired sample sizes, ATSDR will recruit additional persons who were eligible for but did not participate in the Pease biomonitoring program. Again, the exclusion for non-drinking water occupational exposures will apply. Pregnant females will not be excluded. In addition, Section 46.306(b) of the regulations, 45 CFR 46, does not allow prisoners, including persons on house arrest, to take part in this type of research.

ATSDR will recruit approximately 175 referent children (ages 4 – 17 years) and 100 adults (aged  $\geq 18$  years) from the Portsmouth area who were not exposed to PFAS contaminated drinking water at Pease, and do not have a history of occupational exposure to PFAS. For referent children, their birth mothers also must not have exposure to drinking water at Pease. From an exposure point of view, a smaller number of referents need to be enrolled because a sizeable proportion (e.g. lower tertile) of 'exposed' Pease participants are expected to be in range of the general population concentrations for a number of PFAS compounds (**Attachment 2**) (NH DHHS 2016).

### **3.2.1 Children**

The eligibility criteria for children is as follows:

1. Aged 4 – 17 years at the start of the study,
2. Resided or attended day care in areas served by PFAS-contaminated drinking water (i.e. during the period when the combined concentrations of PFOS and PFOA in the drinking water serving the area exceeded 70 parts per trillion) caused by AFFF use, or were exposed in utero or during breastfeeding when the mother consumed the contaminated drinking water,
3. Drinking water exposure from Pease occurred between 2004 and May 2014.
4. ATSDR will exclude children whose birth mothers were ever employed as a firefighter, ever participated in fire training exercises using AFFF foam, or were ever employed at industrial facilities that used PFAS chemicals in the manufacturing process.
5. Females who are pregnant may enroll.
6. Youth in prison, including those under house arrest, are not eligible.

The requirement that the child's last exposure to PFAS from drinking water at Pease be from 2004 to May 2014 takes into account the half-lives of about 3 years for PFOA and PFOS, and about 5 years for PFHxS, observed in a recent study of drinking water exposures caused by AFFF use at a military facility in Sweden (Li 2017). Based on these half-lives, those last exposed more than 15 years ago will have greatly diminished current serum levels of these PFAS chemicals, making the use of these serum measurements to predict past exposures more problematic.

At Pease, about 370 children who participated in the 2015 biomonitoring program at Pease and who were aged 1–13 years at the time of blood draw would be aged 4–17 years in 2018 and would be eligible for the child study. Most of these children attended daycare at Pease. We do not know exactly how many children attended daycare at the Pease Tradeport before June 2014 but the records search of two daycare centers was undertaken (see below). In addition, those children who attended daycare but didn't participate in the 2015 biomonitoring would also be eligible in order to reach sample size goals.

The Discovery Child Enrichment Center began operation in 1994. Its yearly enrollment was approximately 149 children ages 6 weeks to 5 years. A records search by the director of the Center identified 695 children who attended the daycare during 1996–2015 and who would be aged of 6–17 years in 2018. The number of children who attended this day care prior to June 2014 and would be between the ages of 4 and 17 years in 2018 could be within the range of 250 – 450 individuals. The Great Bay Kids' Company is also



located at the Pease Tradeport and began operation in 2010. Its annual enrollment is approximately 270 children aged  $\leq 12$  years. Assuming that most of the children enrolled would be  $\leq 5$  years of age, and that most of the children attend daycare for 4 years, about 300 children might have attended this daycare during the period of interest and would be aged 4–17 years in 2018.

Assuming that a minimum of about 500 children attended the two day-care centers at Pease before June 2014 and would be aged 4–17 years in 2018, we would require a participation rate of about 70% to recruit 350 Pease children into the study. Such a participation rate is possible given the high visibility of the study, strong interest in the community, and the commitment of the Pease CAP and associated organizations to conduct outreach for the study. It would also be feasible to recruit at least 175 children in the same age range from the schools in Portsmouth, NH, who were unexposed to the PFAS-contaminated drinking water at the Pease Tradeport and whose parents did not work at the Pease Tradeport or have occupational exposures to PFAS.

The age range for the child study (4-17 years) was determined by taking into account the age ranges in previous PFAS studies and the age range appropriate for the candidate endpoints. The study will limit inclusion to those  $\geq 4$  years of age because those children exposed in utero on or before the closing of the Haven Well (May 2014) would be at least 4 years of age at the start of the study. In addition, most of the neurobehavioral tests that will be used in the study are appropriate for children aged  $\geq 4$  years of age.

The study will recruit a comparison population consisting of children aged 4 – 17 years from those attending school in Portsmouth, NH, who were never exposed to PFAS-contaminated drinking water during their lifetime, including in utero and during breastfeeding. Therefore, the children's birth mothers also must never have exposure to drinking water from the former Pease Air Force Base or the Pease International Tradeport. Recruitment of the comparison population will match the age distribution of children who participated in the Pease biomonitoring program.

### **3.2.2 Adults**

The population of interest at Pease is adults aged  $\geq 18$  years who worked at the Pease Tradeport prior to the closing of the Haven supply well in May 2014, resided or worked at Pease Air Force Base, or lived in a home near Pease that was served by a PFAS-contaminated private well; and who consumed PFAS-contaminated drinking water at Pease any time during their lifetime. The eligibility criteria for adults is as follows:

1. Aged  $\geq 18$  years at the start of the study.

2. Resided or worked in areas served by PFAS-contaminated drinking water (i.e. during the period when the combined concentrations of PFOS and PFOA in the drinking water serving the area exceeded 70 parts per trillion) caused by AFFF use.
3. Drinking water exposure from Pease occurred between 2004 and May 2014.
4. ATSDR will exclude persons ever employed as a firefighter, ever participated in fire training exercises using AFFF foam, or ever employed at industrial facilities that used PFAS chemicals in the manufacturing process.
5. Females who are pregnant may enroll.
6. Adults in prison, including those under house arrest, are not eligible.

Apart from the occupational exposure exclusion, the study will recruit adults who participated in the Pease biomonitoring program. Adults who did not participate in biomonitoring program but meet eligibility criteria could also enroll in the current study in order to meet sample size requirements. About 1,430 adults have participated in this program. A participation rate of 70% would result in a sample size of about 1,000. In addition, for some of the outcomes of interest, e.g., serum level of total cholesterol, hyperuricemia, and cardiovascular disease, a sample size of 1,000 would be sufficient for PFAS serum levels categorized into quartiles.

The study will recruit a comparison population of 100 adults, unexposed to the contaminated drinking water at Pease, consisting of adults aged  $\geq 18$  years, taking into account the age distribution of the adults who participated in the Pease biomonitoring program.

### **3.3 Sample Size Considerations**

The Pease feasibility assessment included sample size calculations for a wide range of health-related outcomes (ATSDR 2017a). Sample size calculations selected a type 1 (“ $\alpha$  error”) of .05 and type 2 error (“ $\beta$  error”) of .20. The tables present sample sizes per stratum for specific outcomes for children (Table 1) and for adults (Table 2). To determine effect sizes that are reasonable to detect, we selected epidemiological studies considered most representative of the U.S. population exposed to PFAS-contaminated drinking water caused by AFFF use at military facilities. Studies conducted using NHANES data had PFOA and PFHxS serum levels similar to or lower than those observed at number of potential study sites. In some of the more recent NHANES studies, the PFOS serum levels were only moderately higher than at Pease or other sites. Therefore, the PFOS, PFOA and PFHxS results in the NHANES studies were used in many of the sample size calculations. For those outcomes not included in NHANES studies, the C8 studies were used. The C8 results were considered more representative of U.S. populations (e.g.,

in background disease rates and prevalence of non-PFAS risk factors) than studies conducted in other countries, although the PFOS, and especially the PFOA, serum levels in the C8 studies were higher than at Pease. The total sample sizes for children and adults should allow for the categorization of PFAS serum levels (or cumulative PFAS serum levels) into e.g. quartiles of exposure: reference level, low, medium and high.

**Attachment 4** includes additional information and assumptions pertinent to selected health outcomes to be studied.

### **3.3.1 Children**

For children, **Table 1** (and **Attachment 4a**) provide the sample size calculations for several health outcomes of interest assuming a type 1 (“ $\alpha$  error”) of .05 and type 2 error (“ $\beta$  error”) of .20. It was considered important that a study have a total sample size so that exposures could be categorized into tertiles (i.e., reference, medium, and high) or preferably into quartiles (i.e., reference, low, medium and high). Per stratum estimates of needed sample size have been calculated based on different prevalence of outcomes and detected odds ratios or mean difference.

The proposed sample size (350 exposed and 175 referents) is large enough to effectively evaluate some of the health outcomes identified in the literature review and the recent systematic review (Rappazzo 2017) as potentially associated with PFAS in children (i.e. difference in lipids or renal function). A larger multi-site study ( $\geq 2,000$  children) is needed to effectively evaluate other health outcomes of interest (ATSDR 2017a).

The health outcomes and biomarkers studied would include mean difference in total cholesterol (ranging from 156 to 637 per stratum), uric acid levels (556 per stratum), estimated glomerular filtration rate (eGFR; 275 per stratum), testosterone (about 400 per stratum) and insulin growth factor-1 (IGF-1; 146 per stratum). Based on our estimations, we would also be able to detect mean differences for TT4 (1,080 per stratum), obesity, atopic dermatitis, and in full scale IQ that are similar to or smaller than those reported in some studies of children exposed to PFAS compounds (Wang 2011, Karlsen 2017, Lin 2013; Stein 2014).

An NHANES study of estimated glomerular filtration rate observed statistically significant findings with a total sample size of just under 2,000 children (Kataria 2015). For thyroid function, estradiol, delayed puberty, and asthma, a total sample sizes of 2,000 children may be sufficient, although larger sample sizes would be optimal (Lopez-Espinosa 2011, 2012; Stein 2016).

We should note that several well designed epidemiological studies of children exposed to PFAS that reported robust associations with these health outcomes had similar or smaller sample sizes than the one proposed for Pease Study, e.g., studies conducted in Taiwan (Zeng 2015, Lin 2013), Avon, UK (Maisonet 2015a, b), and C8 studies of IQ (Stein 2013) and ADHD behaviors (Stein 2014). Valuable findings on immune function including vaccine response that we would also like to study were also reported from studies of similar sample sizes (Grandjean 2012, 2017; Granum 2013, Stein 2016).

In summary, a total sample size of  $\geq 2,000$  would be sufficient to evaluate a wide range of biomarkers and outcomes including lipids (and hypercholesterolemia), uric acid (and hyperuricemia), estimated glomerular filtration rate, testosterone, IGF-1, neurobehavioral measures (executive function, attention, IQ) and ADHD, rhinitis, and obesity. The child study at Pease would provide an important contribution towards that goal and would be able to address at least some of the more prevalent outcomes as a stand-alone or “pilot” study.

**Table 1.** Child Study (ages 4-17 years)

Health-related Endpoint	Relevant Study	Observed Effect Size	Assumptions	Sample Size/Stratum $\alpha$ error = .05 $\beta$ error = .20
Total Cholesterol (mg/dL)	Frisbee 2010, C8 Study 1,971 boys <12 yrs 2,773 boys 12-18 yrs 1,886 girls <12 yrs 2,520 girls 12-18 yrs	PFOS: 5 <sup>th</sup> vs 1 <sup>st</sup> quintile Age: <12 yrs 12-18 Boys: +6.2 +9.3 Girls: +4.6 +9.4	Mean PFOS serum levels were about 20 $\mu$ g/L. SD for total cholesterol=29.3 mg/dL	+4.6: 637/stratum +9.3: 156/stratum
High cholesterol		OR = 1.6	Prevalence=34.2%	300/stratum
Thyroid function TT <sub>4</sub>	Lopez-Espinosa 2012, C8 1,078 1-5 yrs 3,132 6-10 yrs 6,447 >10 – 17 yrs	PFOS, 4 <sup>th</sup> vs 1 quartile: 2.3% change (mean difference = 0.17 $\mu$ g/dL)	Mean PFOS serum levels were about 20 $\mu$ g/L. SD for TT <sub>4</sub> as estimated at 1.4. Percent change in TT <sub>4</sub> was converted to mean difference assuming the median TT <sub>4</sub> was ref. level.	1,080/stratum
Thyroid disease		PFOA: OR=1.44 (PFOS: OR < 1.0)	Prevalence=0.6% (used PFOA results)	>16,000/stratum
Uric Acid	Kataria 2015, NHANES 1,960; 12-18 yrs	PFOS: 4 <sup>th</sup> vs 1 <sup>st</sup> quartile = +0.19 mg/dL	Mean PFOS serum level = 12.8 $\mu$ g/L. SD = 1.19.	556/stratum
Hyperuricemia	Geiger 2013, NHANES 1,772; 12-18 years	PFOS: 4 <sup>th</sup> vs 1 <sup>st</sup> quartile, OR=1.65	Mean PFOS serum level =16.6. Prevalence=16%	400/stratum

eGFR	Kataria 2015 1,960; 12-18 yrs	PFOA mean serum level =3.5 µg/L. mean difference= -6.6	Standard deviation=27.6	275/stratum
Testosterone	Lopez-Espinosa 2016, C8 1,169 boys; 6-9 yrs 1,123 girls; 6-9 yrs	PFOS (IQR): -5.8% boys (diff=1.9) -6.6% girls (diff=2.45)	Percent change was converted to mean difference assuming median testosterone level was ref. level. SD estimated at 11.85 for girls and 9.63 for boys.	Boys: 404/stratum Girls: 368/stratum
IGF-1 (Insulin- like growth factor – 1)	Lopez-Espinosa 2016, C8	PFHxS (IQR): Boys: -2.5% (diff=17.3) Girls: -2.1%	Percent change was converted to mean difference assuming median IGF-1 in boys as ref. level. SD estimated as 52.6	146/stratum
Delayed Puberty	Lopez-Espinosa 2011. C8 3,072 boys, 8-18 yrs 2,903 girls, 8-18 yrs	PFOS: mean serum level was about 19 µg/L.	OR for delayed puberty and the number of days delayed puberty had narrow CIs	Insufficient information to calculate sample size, but sample sizes in this study were enough for sufficient precision.
ADHD	Stein 2011, C8 10,546; aged 5-18 yrs.	PFHxS mean serum level was 5.2 µg/L. 4 <sup>th</sup> vs 1 <sup>st</sup> quartile, OR=1.5	Prevalence: ADHD Dx: 12.4%	764/stratum
Asthma	Stein 2016, NHANES 640; 12-19 yrs	PFOA mean serum level = 3.6 µg/L. OR=1.2	Prevalence = 11%	2,400/stratum
Atopic dermatitis	Wang 2011 (Taiwan) 244; infants, 2 yrs	PFOS mean serum level=5.5 µg/L., 4 <sup>th</sup> quartile OR=2.19	Prevalence=10.7%	220/stratum
Obesity	Karlsen 2017 (Faroës)	PFOA mean serum level=2.22 µg/L. OR=1.88	Prevalence=17%	250/stratum

Note: Observed effect sizes focused on the results for serum levels of PFOS and/or PFHxS.

Abbreviations: eGFR –estimated glomerular filtration rate, TT4 – total thyroxine; IGF-1 – insulin-like growth factor 1; ADHD – attention-deficit and hyperactivity disorder.

### 3.3.2 Adults

For adults, **Table 2** (and **Attachment 4b**) provides the sample size calculations for several health outcomes of interest assuming a type 1 (“ $\alpha$  error”) of .05 and type 2 error (“ $\beta$  error”) of .20. In this exposure based study, we assume an appropriate coverage of range of exposures that will enable stratification/categorization to tertiles or quartiles of exposure. Per stratum estimates of needed sample size (e.g. first vs. fourth quartile) have been calculated based on different measures of association such as odds ratios or detected mean difference.

We propose to enroll at least 1,000 in the Pease adult study which would have enough power to effectively evaluate some health outcomes of interest described below. To effectively evaluate broader spectrum of PFAS exposure related health outcomes of interest, a sample size of  $\geq 6,000$  participants would be needed for a multi-site study.

For more prevalent outcomes like elevated lipids levels (cholesterol) or uric acid, the range of 229 to 660 participants per stratum (i.e. quartile) or 200 to 550 per stratum, respectively, given observed differences would be needed. That would translate to overall sample size of about 800 to 2,600 participants being sufficient to detect differences at the specified level of precision and power (Steenland, 2009, 2010; Fisher 2013; Shankar 2011). Similar sample sizes would also be required to compare other common health outcomes such as cardiovascular disease (Shankar 2012). Larger samples sizes would be needed for liver function or osteoarthritis, with a total sample in the range of 3,000 to 4,000 subjects (Uhl 2013; Gallo 2012; Steenland 2010).

For thyroid disease and thyroid function, a total sample size of 6,000 may be sufficient although probably not optimal. However, NHANES studies of thyroid function and thyroid disease obtained statistically significant findings with total sample sizes considerably less than 6,000 (Melzer 2010; Wen 2013). NHANES studies of liver function also obtained statistically significant findings with total sample sizes considerably less than 6,000 (Gleason 2015;  $n=4333$ ). For biomarkers of immune function (e.g., immunoglobulins, C-reactive protein and cytokines) and fatty liver disease, there was insufficient information to calculate sample sizes. However, a total sample size of 6,000 should be sufficient to evaluate these biomarkers as we assumed similar endpoint differences of those outcomes.

For ulcerative colitis, a sample size of 6,000 might be sufficient if the effect size in the C8 study (i.e.,  $OR=3.05$ ) was consistent for PFOA serum levels considerably lower than those in the C8 study. For more modest effect sizes (e.g.,  $ORs < 2.75$ ), a total sample size of 6,000 would not be adequate to evaluate associations with ulcerative colitis.

In addition, several epidemiological studies of adults exposed to PFAS that reported robust statistical associations with these health outcomes had similar or smaller sample sizes than the one proposed for Pease Study, e.g., NHANES studies (Nelson 2010, Wen 2013), a C8 longitudinal study (Fitz-Simon 2013), a C8 immune study (Looker 2014), and studies in China (Fu 2014) and Korea (Ji 2012).

In summary, a total sample size of  $\geq 6,000$  in multi-site study should be sufficient to evaluate a broad range of biomarkers and outcomes such as lipids (and hypercholesterolemia), uric acid (and hyperuricemia),

cardiovascular disease, osteoarthritis, immune biomarkers and biomarkers for fatty liver disease. It also may be sufficient to evaluate thyroid disease, thyroid function, and liver function. The adult study at Pease would provide an important contribution towards that goal and would be able to address at least some of the more prevalent outcomes as a stand-alone or “pilot” study.

**Table 2.** Adult Study.

Health-related Endpoint	Relevant Study	Observed Effect Size	Assumptions	Sample Size/Stratum $\alpha$ error = .05 $\beta$ error = .20
Total Cholesterol (mg/dL)	Steenland 2009, C8 46,294 aged $\geq$ 18 yrs	PFOA, mean serum level = 19.6 $\mu$ g/L, 10 <sup>th</sup> vs 1 <sup>st</sup> decile: +11 mg/dL	SD=41.9	228/stratum
High cholesterol		4 <sup>th</sup> vs 1 <sup>st</sup> quartile, OR=1.51	Prevalence=15%	660/stratum
High Cholesterol	Fisher 2013, Canada	PFHxS, mean serum level = 2.2 $\mu$ g/L, 4 <sup>th</sup> vs 1 <sup>st</sup> quartile, OR=1.57	Prevalence=44%	290/stratum
Cardiovascular disease	Shankar 2012, NHANES 1,216 aged $\geq$ 40 years	PFOA mean serum level = 4.2 $\mu$ g/L, 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: OR=2.01	Prevalence = 13%	250/stratum
Uric Acid	Steenland 2010, C8 53,458 aged $\geq$ 20 yrs	PFOA mean serum level = 20.2 $\mu$ g/L, 10 <sup>th</sup> vs 1 <sup>st</sup> decile: +0.22 mg/dL	SD=1.55	780/stratum
		Hyperuricemia, 5 <sup>th</sup> vs 1 <sup>st</sup> quintile: OR=1.26	Prevalence:24%	1,525/stratum
Uric Acid	Shankar 2011, NHANES 3,883 aged $\geq$ 20 yrs	PFOA mean serum level = 3.5 $\mu$ g/L, 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: +0.44 mg/dL	SD = 2.5	507/stratum
		Hyperuricemia, 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: OR=1.97	Prevalence: 19.2%	200/stratum
		PFOA mean serum level = 17.9 $\mu$ g/L Hyperuricemia, 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: OR=1.5		550/stratum
Liver function Elevated ALT	Gallo 2012, C8 46,452 aged $\geq$ 18 yrs	PFOA and PFOS mean serum levels were 28 $\mu$ g/L and 20.3 $\mu$ g/L, respectively. PFOA: OR=1.54 PFOS: OR=1.25	Prevalence = 11.2%	725/stratum 2,917/stratum
Liver function ALT ( $\mu$ IU/mL)	Gallo 2012, C8 46,452 aged $\geq$ 18 yrs	The top quintile of serum PFOS in the Pease population was 15	SD=1.47	1,958/stratum

		$\mu\text{g/L}$ . This would approximately correspond to a mean difference in ALT of +1.8 $\mu\text{IU/mL}$		
Liver function Elevated ALT	Gleason 2015, NHANES 4,333 aged $\geq 12$ yrs	PFHxS mean serum level = 1.8 $\mu\text{g/L}$ . 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: OR=1.37	Assumed similar prevalence as in the C8 study	1,570/stratum
Thyroid disease	Melzer 2010, NHANES 1,900 men, aged $\geq 20$ yrs 2,066 women, aged $\geq 20$ yrs	PFOA, mean serum level=3.5 $\mu\text{g/L}$ , 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: Thyroid disease ever: Women, OR=1.64 Men, OR=1.58 Thyroid disease with current meds Women, OR=1.86 Men, OR=1.89	Prevalences: 16.18% 3.06%  9.89% 1.88%	410/stratum 2,035/stratum  365/stratum 1,575/stratum
Subclinical hypothyroidism	Wen 2013, NHANES 672 males aged $\geq 20$ yrs 509 females aged $\geq 20$ yrs	PFHxS mean serum level averaged about 2 $\mu\text{g/L}$ . Unit increase in $\text{Ln}(\text{PFHxS})$ : Women, OR=3.10 Men, OR=1.57	Prevalences: 1.6% 2.2%	475/stratum 2,918/stratum
Osteoarthritis	Innes 2011, C8 49,432 aged $>20$ yrs	OR=1.42	Prevalence=7.6%	1,580/stratum
Osteoarthritis	Uhl 2013, NHANES 4,102 aged 20-84	PFOA mean serum level = 5.4 $\mu\text{g/L}$ , 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: OR=1.55  PFOS mean serum level = 24.6 $\mu\text{g/L}$ , 4 <sup>th</sup> vs 1 <sup>st</sup> quartile: OR=1.77	Assumed similar prevalence as in the C8 study	978/stratum  550/stratum
Ulcerative colitis	Steenland 2013, C8 28,541 community and 3,713 worker cohorts	OR=3.05	Prevalence=0.5%	1,480/stratum

For rare health outcomes such as ulcerative colitis, other autoimmune diseases, or cancer the sample size is too small to detect reasonably expected increases in ORs and those outcomes cannot be effectively studied at one site or even at multiple sites combined (i.e. about 6,000). We should note that we don't know actual differences in clinical or research parameters at Pease or any other sites and there is considerable variability between studies and populations; sample size estimates provide guidance but should be interpreted with caution and in the context of scientific literature.

**Attachment 4** provides further information and details on the derivation of the sample size calculations for Table 2 and also estimates of detectable mean difference and odds ratios for selected clinical tests and health outcomes.



### **3.4 Study Roll Out and Communication Plan**

ATSDR will work with NH DHHS and the Pease CAP to conduct outreach to encourage participation. The Pease CAP has offered to assist in recruitment of Pease and referent children and adults, and CAP involvement will be crucial in conducting outreach for the study. To increase community awareness of the study and to encourage participation, study investigators will work with NH DHHS and the Pease CAP to ensure that outreach about the study is effective. In advance of the start of the study, outreach will involve announcements to local elected officials, local media, community organizations, and the Portsmouth schools (**Attachment 5**). Outreach may also involve meetings with community representatives, medical societies, school officials, and/or public meetings. Although active in outreach, both NH DHHS and the Pease CAP will not directly obtain consent, intervene, or interact with research participants.

As part of the outreach, ATSDR will provide a factsheet to the Pease Development Authority (PDA) and Tenants Association of Pease Tradeport (TAP) so that it is included in newsletters and email notices to subscribing firms at the Pease Tradeport (**Attachment 5, Attachment 7a&b, Attachment 9c**). ATSDR and study investigators may request employers at Pease, PDA and TAP for rosters of current and former employees at the Pease Tradeport to assist with recruitment efforts. The study will also provide the factsheet to the Portsmouth schools, local media outlets, and to community organizations in Portsmouth (**Attachment 5, Attachment 7d&e, Attachment 9c**).

### **3.5 Recruitment**

#### ***3.5.1 Wave One Recruitment from Biomonitoring Recruitment Frame***

ATSDR will first recruit from those who participated in the NH DHHS Pease biomonitoring program in 2015-7. The NH DHHS biomonitoring program consent form did not obtain permission to recontact the participant for future studies. Therefore, ATSDR will not directly contact the biomonitoring program participants for Pease Study recruitment. Instead, NH DHHS, which manages the Pease biomonitoring records and possesses the recruitment frame for the Pease Study, will send out an introductory letter indicating its support for, and encouraging its former participants to contact ATSDR to take part in the ATSDR Pease Study (**Attachments 6a&6b**). The letter will provide a toll-free line that the interested person can call ATSDR to request participation in the study and/or ask any questions about the study. ATSDR will screen each interested caller using a Wave One eligibility screening script (**Attachment 6c**).

### ***3.5.2 Wave Two Recruitment of Additional Exposed Children and Adults***

Given that the Pease biomonitoring program recruited a convenience sample, no statistical sampling will be required. All biomonitoring participants in the recruitment frame will be recruited until the list is exhausted or until the required sample size is reached. If sample size requirements are not met in Wave One, ATSDR will further recruit individuals who were eligible but did not participate in the biomonitoring program. ATSDR will launch a media campaign to announce Wave Two (**Attachment 5**). A recruitment flyer will be sent to parents of other children identified as attending daycare centers at Pease before 2014 who didn't participate in biomonitoring in 2015-7 (**Attachment 7a**). Advertisement in local public media and through PDA and TAP would encourage other eligible adults to participate in the study (**Attachment 5**). The flyers will provide a toll-free number that interested recruits can call ATSDR to request participation in the study and ask any questions about the study (**Attachment 7b**). ATSDR will screen each interested caller using an eligibility screening script (**Attachment 7c**).

### ***3.5.3 Wave Three Recruitment of Referent Children and Adults***

For efficiency, Wave Three may occur concurrently with Waves One and Two. ATSDR will recruit a small referent group of 175 children and 100 adults not exposed to PFAS-contaminated drinking water.

ATSDR will work with school officials to approve this school-based recruitment. ATSDR will also request that the schools distribute a flyer describing the study to students and instruct the students to give the flyer to their parents. Children will be recruited through outreach to Portsmouth schools and day care centers (for those 4–5-year-old) (**Attachment 7d**). Recruitment of adult referents will include outreach to current adult education students among the five colleges at Pease (i.e., students who first attended these schools after closure of the Haven Well), Portsmouth government employees (AFSCME Local #1386), Portsmouth community organizations, and/or specific Portsmouth neighborhoods (**Attachment 7e**). ATSDR will screen each interested caller using an eligibility screening script (**Attachment 7c**).

Both exposure and referent households may have more than one eligible adult and/or child, and some parents may want to enroll in both of the adult and child studies. Trained study staff will use the recruitment tracking form (**Attachment 8**) to track recruitment success and to calculate non-response bias.

### **3.5.4 Enrollment Procedures**

Once potential recruits express interest and are screened for eligibility, study staff will schedule appointments for them at the central study office, or alternatively for a home visit for some who are unable or unwilling to attend an office visit and who live a reasonable distance to the office. The study staff will establish a toll-free telephone line for interested recruits to schedule appointments at their convenience.

#### **3.5.4.1. Waiver of documentation of informed consent**

To minimize the exposure to COVID-19 during epidemic and to reduce in-person interactions between participants and staff, the study will offer an option to administer the questionnaire over the phone instead of at the study office. ATSDR has requested CDC IRB waiver of documentation of informed consent for the questionnaire portion of data collection. The remainder of the consent process and collection of biological samples will still have to occur at the study office as described in Section 3.6.2 (Informed Consent Process).

#### **3.5.4.2. What to expect before your appointment**

Once the appointment is scheduled, study staff will mail an Appointment Packet (containing an Appointment Reminder Card (**Attachment 9a**), the Informed Consent materials (**Attachment 9b**), a Study Fact Sheet (**Attachment 9c**) with a description to arrive fasting, and to bring medications and a urine sample to the appointment. Interested recruits will be mailed urine collection supplies. They will be instructed to collect a first-morning voided urine sample on the day of their appointment. An advance copy of the Informed Consent Form will provide an extra opportunity for the interested recruit to read and more fully understand his or her rights in the study and to ask any questions before the scheduled appointment.

Study staff will give the interested recruit a reminder telephone call and send a text one to two days before the scheduled appointment (**Attachment 10**). The study protocol will provide the flexibility to schedule or re-schedule office or questionnaire appointments. **Home visits will not be conducted due to COVID-19 precautions.** The study staff will make up to five contact attempts to an interested recruit who misses an appointment in order to reschedule the appointment and maximize the number of completed appointments (**Attachment 11**).

### **3.6 Data Collection Procedures**

The study will establish a central office in Portsmouth to obtain informed consent, blood and urine specimens, administering the neurobehavioral batteries to parents and children, and providing a space for completion of the questionnaire. As a COVID-19 precaution, the participants will have an option to schedule questionnaire administration over the phone after their eligibility is determined, and after they provide verbal consent to the questionnaire portion on the phone. Study staff will be available to answer any questions concerning the study. All study staff will receive training on the goals and purposes of informed consent, administration of the questionnaire, administration of the neurobehavioral test batteries, collection methods for the blood specimens, and on proper documentation of data collection procedures. Study staff will receive certified training on Human Subjects Protection (e.g., Collaborative Institutional Training Initiative [CITI] Program) and sign a confidentiality agreement prior to contact with potential recruits and enrolled participants.

Trained study staff will attend dedicated telephone lines to respond to questions and to address concerns from potential recruits, enrolled participants, and the public. Study staff will ask participants to attend their appointment in at least an eight-hour fasting state; therefore, most recruits will likely schedule appointments in the early morning. The steps of the data collection will include:

1. Check-in procedures;
2. Informed consent;
3. Data collection procedures;
4. Exit procedures; including provision of a gift card as a token of appreciation for participation.

#### **3.6.1 Check-in Procedures**

Trained study staff will document the completion of each step from check-in to the provision of gift cards on a hard copy form (**Attachment 11**). This hardcopy form will be stored with the participant's signed Informed Consent Form (**Attachment 9b**) in locked files and in secure rooms. Staff will securely ship all files to ATSDR at the end of data collection. All files and biological samples will be securely stored at the study office prior to shipment.

### **3.6.2 Informed Consent Process**

The informed consent includes a description of study procedures and risks and benefits of participation (**Attachment 9b**), including a Privacy Act Statement (**Attachment 9b1**). A study factsheet will inform the adult participant and the child participant and parent of the chemical tests and clinical outcomes to be measured (**Attachment 9c**). Study staff will emphasize the voluntary nature of participation and will answer any questions the participant, or parent of the child participant, has prior to obtaining signatures. ATSDR has requested CDC IRB waiver of documentation of informed consent for the questionnaire portion of data collection (Section 3.5.3.1).

#### 3.6.2.1 Consent for Specimens and Data

ATSDR will obtain fasting blood specimens from each participant for analyses of PFAS and several effect biomarkers. In addition, all participants will be asked to provide a morning void urine sample on the same day as their blood draw. After all the current laboratory analyses on blood are completed, ATSDR will ask for permission to archive any residual blood specimens and the urines for future analyses.

ATSDR will also enter into an agreement with NH DHHS for conditional access to the Pease biomonitoring program data and residual specimens. The access is conditional because health study participants must consent to allow ATSDR to obtain their existing residual biomonitoring specimens for current or future research purposes. ATSDR will re-consent participants to allow further PFAS and effect biomarker analyses using any remaining residual specimens for research. ATSDR will also seek consent from participants to link their existing laboratory results from the NH biomonitoring program for this new research purpose.

#### 3.6.2.2 Child Consent

Before any data collection can begin in the child study, trained study staff will obtain verbal consent for the questionnaire portion of the data collection which will be completed over the phone and review all other components of the hardcopy Parental Permission and Assent Form (**Attachment 9b2**) including the blood draw and the neurobehavioral testing with the parent who is interested in having the child participate. The study staff will explain to the parent and child the purpose of the study and request that the parent sign the permission forms. If the child is seven years of age or older, the study staff will request that the child give an assent to participate in the study.

ATSDR will request that the parent complete a questionnaire about the child and complete a parental neurobehavioral test battery on behalf of the child. The permission form will request that the parent allow

the child to donate a fasting blood specimen and store any residual specimens for future analyses. The parental permission form will allow the investigators to administer a neurobehavioral test battery to the child, access the child's medical and school records (including special education records) (**Attachments 9b2, 9b3 & 9b5**), and to contact the child and parent for possible future studies. Once the parent signs the consent and permission forms (and the child aged  $\geq 7$  years gives assent to participate), the parent and/or the child become study participants.

For those children who participated in the Pease biomonitoring program, the consent form will request permission from the parent to access the child's residual biomonitoring blood specimen for the analysis of additional PFAS chemicals and immune biomarkers. The consent form will also permit NH DHHS to provide the child's PFAS serum results from the biomonitoring program for use in this research study.

#### 3.6.2.3 Adult Consent

Before any data collection can begin in the adult study, trained study staff will obtain verbal consent for the questionnaire portion to be administered over the phone and review the hardcopy Adult Consent Form for all other components of informed consent with the interested recruit (**Attachment 9b4**). The study staff will explain the purpose of the study and obtain written informed consent for the completion of a questionnaire, the collection of a new fasting blood specimen, the storage of this blood specimen for future analyses, access to medical records (**Attachment 9b5**), and permission to contact the participant in the future for a possible study. After signing the consent form, the adult will become a study participant.

For those adults who participated in the Pease biomonitoring program, the consent form will request permission to access the individual's residual blood specimen for the analysis of additional PFAS chemicals. The consent form will also permit NH DHHS to provide his/her PFAS serum results from the biomonitoring program for use in this research study.

#### 3.6.2.4 Risks and Benefits

The main risk in this study is the potential for inadvertent disclosure of participants' personally identifiable information (PII). ATSDR has taken steps to minimize this risk. Study staff and contractors will be trained on the requirements to protect identifiable and sensitive data and will be required to sign a Rules of Behavior Agreement (**Attachment 14**). As further described in **Section 3.8.1**, ATSDR will inform the participant that his or her participation is protected by a Certificate of Confidentiality under Section 301(d) of the Public Health Service Act (PHSA) as amended by Section 2012 of the 21<sup>st</sup> Century Cures Act. ATSDR

will further inform the participant that identifiable occupational history, private medical records, and to school records are protected from certain disclosures under Section 301(d) of the PHSA.

The risks of participation in this study are minimal (defined in 45 CFR 46.110). In-home urine collections are minimal risk. This study plans for a one-time 30-ml (6 teaspoons) volume of fasting blood collected from the child and a one-time 40-ml volume of fasting blood collected from the adult. These amounts of blood are the minimum necessary to conduct analyses for PFAS and the effect biomarkers (**Attachment 3**). After the blood draw, the participant will be offered a small snack, thereby allowing monitoring of adverse events due to phlebotomy.

Participants in this study will not receive any direct benefit from taking part in this research. Their taking part in this research will provide the scientific community and the public a better understanding of how exposures to PFAS-contaminated drinking water may affect human health. Each adult participant and the parent of the child participant will receive the results of the analyses of serum PFAS levels and effect biomarkers.

### ***3.6.3 Update Contact Information and Medication List***

The adult participant and the parent of the child participant will be asked to verify and update his or her current contact information for results reporting and potential future contact (**Attachment 12**).

The study staff will request that the adult participant and the parent of the child participant bring all current prescription and over the counter medications prior to the study office. This will help the study staff to complete the medications list (**Attachment 13**).

### ***3.6.4 Body and Clinical Measurements***

Trained study staff will perform the body and clinical measurements and specimen collections as described in the Manual of Procedures (**Attachment 14**).

*Body Measurements:* Trained study staff will perform body measurements, blood pressure measurements, and blood draws. Three blood pressure (BP) measurements will be taken and averaged. The measured BP level is subject to biological and observer variability; therefore, the study will use three different sizes of the manual cuffs in the measurements; the appropriate cuff size will be selected for each participant and administered 3 times. The purpose of a specific measurement protocol, or training and certifications of technicians and of ongoing quality control is to minimize variability due to known

exogenous factors and to reduce imprecision and biases in measurement. Measurement of resting blood pressure, height, weight, and waist and hip circumference can occur in any order, but the BP measurement should occur after the subject has been in the seated position for at least five minutes. BP measurement will occur before venipuncture if the activities are scheduled consecutively. Trained study staff will record the measurements in the Body and Blood Pressure Measures Form (**Attachment 15**).

*Fasting Blood Specimen and First Morning Urine Void Collection:* Participants will transport their urine sample to study office for collection. Trained staff will collect and record the urine specimen intake (**Attachment 16**). The blood collection procedure consists of administering and recording responses to a blood draw screening questionnaire for conditions that exclude the participant from the blood draw (hemophilia, skin condition, or chemotherapy in the past four weeks, and pregnancy), ask about having diabetes, taking blood thinning medications, participant's weight, and fasting status (**Attachment 16**). Next, phlebotomists will draw 30-ml (1.0 ounce or about 6 teaspoons) of blood from the child participant and 40 -ml (1.4 ounces or about 8 teaspoons) of blood from the adult participant using standard venipuncture techniques (**Attachment 14**) and record the outcome (**Attachment 16**). If a person is unable to provide the desired volume of blood, a smaller amount can be drawn and documented. Trained study staff will record the phlebotomy and urine collection result on the Blood Draw and Urine Collection Form (**Attachment 16**).

Common adverse events from blood draws include bruising, bleeding, and fainting. No serious adverse events are anticipated in drawing these volumes of blood. Fasting diabetic participants who use insulin will receive priority appointments for their blood draw. Light snacks will be provided following blood collection. While we ask participants to provide fasting sample, we realize that some may not be able to fast. As PFAS are bound to proteins that will affect measured levels less than measurements affected by lipid levels. In the C8 Science Panel studies, about 25% of participants fasted – but they were not asked to do so (Frisbee 2009).

Phlebotomists will extract serum, and label and prepare the serum, whole blood, and urine specimens for secure storage and transport from the study office to the CDC NCEH laboratory in Atlanta, GA, and clinical laboratories (**Attachment 14**).

The NCEH laboratory will perform the analyses of serum PFAS according to the biochemical analytical plan (**Attachment 3**) and approved laboratory methods (Kuklennyik 2015). The study staff s will also aliquot and ship blood and serum specimens to participating laboratories for the analyses of the effect biomarkers



according to the plan. ATSDR will wait to analyze urines until more knowledge is gained about urinary PFAS. Residual blood and urines will be archived at CDC Biorepository.

### **3.6.5 Questionnaire**

Each adult participant, and a parent of the child participant, will complete a questionnaire. As a COVID-19 precaution, the participants will have an option to schedule questionnaire administration over the phone instead of during the appointment for the blood draw.

#### **3.6.5.1 Children and Parents**

Study staff will request that the parents of the child participant complete the questionnaire. The questionnaire will obtain demographic information (e.g., education, primary occupation), medical history of the mother and child, the child's medications, the mother's reproductive history (including maternal age at birth of the participating child) and any occupational exposures the mother may have had to PFAS. The questionnaire will be administered in two formats: a form for the child whose parent is not also a participant (**Attachment 17**), and an abbreviated form for the child whose parent is also an adult participant (**Attachment 17a**).

The questionnaire will ask about the dates the child's mother worked at the Pease Tradeport, her water consumption (including bottled water) while at Pease, and the dates the child attended daycare at Pease and the child's water consumption (including use of water for formula, juices, etc., bottled water use) while attending a daycare center at Pease. The questionnaire will also ask the dates and length of time of the pregnancy and breastfeeding of the child.

The questionnaire will request information on the child's height and weight, vaccination history, and whether the child regularly exercises, currently smokes (and the number of cigarettes/day) or consumes alcohol (and the number of drinks/week). The questionnaire will ask when the female child first began to menstruate. The questionnaire will include specific questions addressing health outcomes of interest. For example, for ADHD, the questionnaire will ask, "Has a doctor or health professional ever told your child that your child has/had ADD or ADHD?" If the answer is "yes," a second question will ask for a list of medications the child took for the condition. The questionnaire will ask if the child had learning or behavioral problems, and if so, the type of problem and the treatment used. Questions would be included for the hypersensitivity-related outcomes, asthma, atopic dermatitis (or atopic eczema), and allergies. The

study will attempt to confirm diseases and conditions reported in the questionnaire by accessing medical records (**Attachments 19,19a&19b**).

#### 3.6.5.1.1 Child/Parent Neurobehavioral Assessments

**Table 3** provides the neurobehavioral test battery for children enrolled in the Pease Study.

Trained professionals will administer the following tests to children:

- The Wechsler Abbreviated Scale of Intelligence – 2<sup>nd</sup> Edition (WASI – II) test will be administered to measure Full Scale IQ (FSIQ) among children 6-17 years (15 minutes). Intelligence testing of children aged 4 – 5 years will not be conducted.
- Each child 4-16 years will complete the NEPSY-II selected tests. Except for Theory of Mind, these additional tests are short and useful to assess memory and inhibition. For all the NEPSY – II tests, children 4 years would take about 52 minutes, and children ≥5 years, about 70 minutes.
- Children aged 4 – 7 years will complete the Connors Kiddie Continuous Performance Test (K-CPT – 2) (8 minutes), and children aged >7 years will complete the Connors CPT – 3 (14 minutes).

Trained professionals will administer the following tests to parents about their children:

- Strengths and Difficulties Questionnaire (SDQ) (5 minutes).
- Behavior Rating Inventory of Executive Function® (BRIEF®) to assess the child’s emotional, conduct, and peer relationship problems as well as problems with hyperactivity, inattention and executive function.
  - Parents of children aged 4 – 5 years will complete the preschool version (BRIEF®-P) (10 minutes).
  - Parents of children aged >5 years will complete the BRIEF® (10 minutes).

A summary of the neurobehavioral test battery is found in **Attachment 20**. Each child will spend an average of 90 minutes to complete the child battery of tests. Each parent will spend an average of 15 minutes to complete the parent battery of tests. Overall, each parent/child pair will take 105 minutes to complete the neurobehavioral test battery (**Attachment 20a**).

**Table 3. Neurobehavioral Test Battery for Children**

Neurobehavioral Test	Domain	Age	Administration	Time to Administer
Wechsler Abbreviated Scale of Intelligence – 2 <sup>nd</sup> Edition (WASI - II)	Two Subtest Form (FSIQ)	6 – 17*	Child	15 minutes
A Developmental Neuropsychological edition (NEPSY – II) subtests  * from Core Assessment	Comprehension of Instructions* (receptive language, trouble following multi-step commands)	4 – 16	Child	6 – 8 minutes
	Speeded Naming* (expressive language, processing speed)	4 – 16	Child	2 – 7 minutes
	Narrative Memory* (comprehension, verbal memory)	4 – 16	Child	6 – 11 minutes
	Design Copying* (visuospatial processing)	4 – 16	Child	7 – 10 minutes
	Affect Recognition (social perception)	4 – 16	Child	5 – 7 minutes
	Statue (inhibitory control)	4 – 6	Child	3 minutes
	Word Generation (expressive language, executive control)	4 - 16	Child	4 – 6 minutes
Conners Kiddie Continuous Performance Test, 2 <sup>nd</sup> Edition (Conners K-CPT 2)	Inattentiveness, Impulsivity, Sustained Attention, Vigilance	4-7	Child	8 minutes
Conners Continuous Performance Test 3 <sup>rd</sup> edition (CPT 3)	Inattentiveness, Impulsivity, Sustained Attention, Vigilance	8-17	Child	14 minutes
Strengths and Difficulties Questionnaire <sup>®</sup> (SDQ <sup>®</sup> )	Double-sided form with impact supplement (behavioral problems)	4 – 17	Parent about Child	5 minutes
Behavior Rating Inventory of Executive Function <sup>®</sup> (BRIEF <sup>®</sup> )	Executive Function	6-17	Parent about Child	10 minutes
Behavior Rating Inventory of Executive Function <sup>®</sup> – Preschool Version (BRIEF <sup>®</sup> -P)	Executive Function - Preschool	4-5	Parent about Child	10 minutes

For each child, ATSDR will also review school records, including special education records, to identify learning problems and behavioral problems as requested by ATSDR (**Attachment 20b**) and as abstracted by the schools (**Attachment 20c**). If the parent reports that the child has a developmental disability (e.g.,

ADHD, autism, or a learning disability), then the contractor shall obtain and abstract the special education records for the child including the individualized education program (IEP), the IEP evaluation report (“Full Individual Evaluation” or “FIE”), and if available, the Independent Educational Evaluation.

#### 3.6.5.2 Adults

Each adult participant will complete a questionnaire requesting demographic information, occupational history, medical history and reproductive history (**Attachment 18**). In particular, the questionnaire will ask if the participant ever had kidney disease, liver disease, cardiovascular disease, hypertension, thyroid disease, diabetes, autoimmune diseases, osteoporosis, osteoarthritis, pregnancy-induced hypertension, and endometriosis. For each reported disease or condition, the questionnaire will ask about the date of diagnosis, the medical provider who made the diagnosis, and the medications used for treatment. The study will attempt to confirm each disease or condition by accessing medical records. The questionnaire will ask for the dates the participant worked at Pease and water consumption habits (including bottled water use) while working at Pease. The questionnaire will ask the participant about conditions that might affect PFAS serum levels such as date of menopause, menstrual cycle information, blood transfusions, and blood donations. The study will attempt to confirm diseases and conditions reported in the questionnaire by medical records review (**Attachments 19,19a&19b**).

#### **3.6.6 Exit Procedures**

At the end of the data collection, study coordinators or staff will review recorded items in the participant’s Appointment Tracking Form for completeness (**Attachment 11**).

The adult participant or the parent of the child participant will receive a copy of the participant’s Body and Blood Pressure Measures Report (**Attachment 21**). These results will be immediately available and will require no further evaluation or interpretation with two exceptions. The adult participant or the parent of the child participant will receive a supplemental notice if the participant has a critical blood pressure measure (diastolic blood pressure > 120 mm Hg, or systolic blood pressure >180 mm Hg). In this case, a Critical Hypertension Notice will be appended to the Body and Blood Pressure Measurements Report along with written and verbal recommendations to obtain immediate medical attention. If the participant does not have a personal physician, the study coordinator will provide a referral. If the participant has an elevated but non-critical blood pressure measure (resting blood pressure > 140/90), an Elevated Hypertension Notice will be appended to the Body and Blood Pressure Measures Report with written and verbal recommendations to obtain clinical follow-up.

### 3.6.6.1 Gift Cards as a Token of Appreciation for Participation

As a token of thanks for participation, ATSDR will offer gift cards according to the following schedule:

- \$25 for body and blood pressure measures, and for blood and urine collection;
- \$25 for completed questionnaire; and
- \$25 for child/parent completion of the neurobehavioral test battery

Trained study staff will document provision of gift cards on the hard copy form (**Attachment 11**). As part of the exit procedures, the participant will sign this form to document receiving the gift card.

### 3.6.7 Adverse Events

The risks associated with this study are minimal. There is a small chance of unexpected or adverse events occurring during the course of this project. Unanticipated problems involving risk to the subjects or others will be reported to the CDC Human Institutional Review Board (IRB) in accordance with institutional policies and procedures.

The most likely adverse event is a participant feeling lightheaded or fainting during blood collection. The phlebotomist will receive training to respond to such situations. The tests and procedures conducted by trained study staff are for research purposes only and are not diagnostic exams. They are not a substitute for an evaluation by a medical professional. The study will not perform any clinical treatments or health interventions as part of the study.

If a participant loses consciousness, falls, is unable to stand, or experiences chest pain the study staff will decide whether to advise the adult participant or the parent of the child participant to seek immediate medical treatment or to contact emergency medical services. Study staff have identified appropriate local medical care providers that participants may be referred to if clinical results suggest medical attention is needed (**Attachment 14**).

### 3.7 Biochemical Analyses

*Serum PFAS:* The ATSDR biochemical analytical plan is found in **Attachment 3**. The study will analyze PFAS in fasting serum including linear PFOA, the sum of branched isomers of PFOA, linear PFOS, branched PFOS, and PFHxS (Kato 2018). Other PFAS analyzed will include: 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), , perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and

perfluoroundecanoic acid (PFUnA). For NHANES 2017-8, three PFAS analytes were added: 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid, Adona (ammonium salt of 4,8-dioxa-3H-perfluorononanoic acid, and GenX (ammonium salt of 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid (HFPO-DA), but their reference ranges were all below the limit of detection (CDC 2021) and will not be measured in Pease.

*Urinary PFAS:* The study will store urine samples for potential analyses of PFAS in the future. Urine is an important excretion pathway for human metabolism and PFAS urine elimination may be important influencing serum concentrations (Harada 2005, Zhang 2015).

### **3.7.1 Children**

The study will analyze fasting serum for the following biomarkers of lipids, thyroid, glycemic, liver, and kidney function, sex hormones, and immune function (**Attachment 3**):

- Total cholesterol, low density lipoprotein, high density lipoprotein, total triglycerides,
- Uric acid, creatinine,
- Thyroxine (T4), T3, thyroid stimulating hormone (TSH),
- Glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide, pro-insulin,
- Alanine transaminase (ALT),  $\gamma$ -glutamyltransferase (GGT), direct bilirubin, and cytokeratin-18 (CK-18),
- Testosterone, estradiol, sex hormone-binding globulin (SHBG), follicle stimulating hormone, insulin-like growth factor,
- Immunoglobulin G (IgG), IgA, and IgM; antibodies to measles, mumps, rubella, tetanus, and diphtheria.

The child study will use the cut points of 50 ng/dL of total testosterone and 20 pg/mL of estradiol to identify sexual maturation in boys and girls, respectively (Lopez-Espinosa 2011). The child study will measure IgG antibodies for measles, rubella, and diphtheria to determine vaccine responses. It will analyze allergen-specific IgE (mold, dust mites, dog, cat, cow's milk, peanut, hen's egg, and birch). The study will analyze serum levels of thyroid stimulating hormone (TSH) and total T4 separately and use these measurements to determine clinical and subclinical hypothyroidism and hyperthyroidism. The study will

measure uric acid, total cholesterol, low-density and high-density lipoprotein, and triglycerides. We also propose to measure liver enzymes and CK-18 (Feldstein 2013, Mora 2018, Santoro 2013).

### **3.7.2 Adults**

The study will analyze the following biomarkers in the adult fasting serum (**Attachment 3**):

- Total cholesterol, low density lipoprotein, high density lipoprotein, total triglycerides,
- Uric acid, creatinine,
- Thyroxine (T4), T3, thyroid stimulating hormone (TSH),
- Glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide, pro-insulin,
- Alanine transaminase (ALT),  $\gamma$ -glutamyltransferase (GGT), direct bilirubin, and cytokeratin-18 (CK-18),
- Immunoglobulin G (IgG), IgA, IgE and IgM; C reactive protein, and antinuclear antibodies (ANA),
- Cytokines and adipokines (e.g. IL-1 $\beta$ , IL-6, IL-8, MCP-1, TNF $\alpha$ , leptin, adiponectin, resistin, PAI-1).

### **3.7.3 Quality Control/Quality Assurance**

To maintain the integrity of the lab results, a backup generator will be available for the refrigerator and freezer at the study office. All blood specimens will be securely stored at the study office until shipped to the NCEH laboratory.

The NCEH and other participating laboratories will fulfill quality assurance/quality control criteria (QA/QC) including a documented quality assurance plan and adherence to required quality control procedures specified in an approved method. The laboratories will ensure that the analytical data are scientifically valid, defensible, and of known and acceptable precision and accuracy. QA/QC procedures, including appropriate calibration of instruments, running standards and blanks, reporting limits of detection, and other parameters will be in place before specimens are tested. Specimen collection, storage, and transportation techniques are specified in the Manual of Procedures to ensure the integrity of the specimens (**Attachment 14**). Specimens will be stored at the proper temperature and isolated from potential sources of contamination.

The Standard Operating Procedure (SOP) for each analytical method is kept on file by the PI, and is available for review upon request.

### **3.7.4 Reference Values**

The participating laboratories will provide reference values and action levels for the effect biomarkers which will be reported in **Attachments 22&23**. ATSDR will report the participant's PFAS results using reference values from the most recent NHANES report (**Attachment 24**). Currently, the 2013-14 report is available and provides reference values for children. **Section 4** provides additional descriptions of the procedures for advance and final results reporting.

## **3.8 Data Handling**

### **3.8.1 Certificate of Confidentiality**

Section 301(d) of the Public Health Service (PHS) Act, as amended by Section 2012 of the 21st Century Cures Act, P.L. 114-255 (42 U.S.C. 241(d)), states that the Secretary shall issue CoCs to persons engaged in biomedical, behavioral, clinical, or other research activities in which identifiable, sensitive information is collected. In furtherance of this provision, CDC research commenced or ongoing after December 13, 2016 and in which identifiable, sensitive information is collected, as defined by Section 301(d), is deemed issued a CoC and therefore researchers are required to protect the privacy of individuals who are subjects of such research in accordance with Section 301(d) of the PHSA.

Consistent with Section 301(d), ATSDR determined that a CoC applies to this research by answering the following questions:

1. Is the activity biomedical, behavioral, clinical, or other research? YES
2. Does the research involve Human Subjects as defined by 45 CFR Part 46? YES
3. Is ATSDR collecting or using biospecimens that are identifiable to an individual as part of the research? YES
4. If collecting or using biospecimens as part of the research, is there a small risk that some combination of the biospecimen, a request for the biospecimen, and other available data sources could be used to deduce the identity of an individual? YES
5. Does the research involve the generation of individual level, human genomic data? NO

Therefore, in accordance with subsection 301(d) of the Public Health Service Act, ATSDR and any of its contractors shall not:

- Disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or



biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains; or

- Disclose or provide to any other person not connected with the research the name of such an individual or any information, document, or biospecimen that contains identifiable, sensitive information about such an individual and that was created or compiled for purposes of the research.

Disclosure is permitted only when:

- Required by Federal, State, or local laws (e.g., as required by the Federal Food, Drug, and Cosmetic Act, or state laws requiring the reporting of communicable diseases to State and local health departments), excluding instances of disclosure in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding;
- Necessary for the medical treatment of the individual to whom the information, document, or biospecimen pertains and made with the consent of such individual;
- Made with the consent of the individual to whom the information, document, or biospecimen pertains; or
- Made for the purposes of other scientific research that is in compliance with applicable Federal regulations governing the protection of human subjects in research.

ATSDR and its contractors conducting this research are required to establish and maintain effective internal controls (e.g., policies and procedures) that provide reasonable assurance that the research contract is managed in compliance with Federal statutes, regulations, and the terms and conditions of the award (**Attachment 14**). Contractors are also required to ensure: 1) that any investigator or institution not funded by CDC/ATSDR who receives a copy of identifiable, sensitive information protected by this CoC, understands that it is also subject to the requirements of subsection 301(d) of the PHS Act; and 2) that any subrecipient that receives funds to carry out part of this CDC award involving a copy of identifiable, sensitive information protected by a Certificate understands that it is subject to subsection 301(d) of the PHS Act.

For studies in which informed consent is sought, ATSDR and its contractors shall inform research participants of the protections and the limits to protections provided by this CoC (**Attachment 4b**). Therefore, all study staff will receive training on the importance of protecting the confidentiality of human research subjects and of personal information acquired, including the collection of biological specimens.

The study will minimize the risk of loss of confidentiality and privacy through careful attention to procedures for such protections in the collection, handling, and reporting of individually identifiable and sensitive data (**Attachment 14**).

### **3.8.2 Data Management and Security**

Data management for this study described below includes guidance on:

1. Use and protection of information in identifiable form (IIF);
2. Security access (physical, technical, and administrative) controls for ATSDR and its contractor;
3. Appropriate data delivery; and
4. Data ownership and data sharing.

*Collection of IIF.* The study staff will collect, manage and store IIF in an already established record system (System of Records Notice [SORN] No. 09-19-0001 titled “Records of Persons Exposed to Toxic or Hazardous Substances”). ATSDR will use IIF to report results to each parent of a child participant or adult participant. ATSDR will be the final recipient of the IIF (to keep for potential re-contacting of participants).

The study staff will deliver all field-collected records to ATSDR headquarters at the end of the study. ATSDR will retain IIF such as name, Social Security Number (SSN), current address, phone number, email address, date of birth, and the date of the participant’s blood draw and questionnaire completion. ATSDR will store the IIF in a separate master key dataset along with a study-generated ID. This dataset will be separate from the dataset containing the questionnaire data and other data used in the statistical analyses. The study-generated ID will be the variable that can link the two datasets if necessary. IIF will not be linked with files used for statistical analysis and will not appear in any reports generated from this data set.

### **3.8.3 Impact on Privacy**

Because the study staff will collect, store, manage, and maintain IIF on an already established record system, there would be a likely effect on the participant’s privacy if a breach of data security occurred. Therefore, its established record system has stringent safeguards in place as described in the following section.

Research datasets will include only coded information that might be sensitive, such as questions on reproductive outcomes, fertility, or fecundability. These files will not have associated information that might directly identify these participants. IIF will be stored in a separate master key dataset, which will

enable ATSDR investigators to link the participant's research data with his or her IIF via a study-generated ID. Maintaining this contact information is necessary to provide results of the tests or re-contact them in the future if a longitudinal study becomes feasible. Therefore, stringent data security measures will be in place, including administrative, physical, and technical controls as described below.

All laboratories involved in biochemical analyses will receive biological specimens with participants' study-generated ID only. Nondisclosure agreements will be executed between ATSDR and laboratories that will not be engaged in research.

#### 3.8.3.1 Access Controls and Security

The ATSDR PI and Project Manager will be responsible for all required staff training and certification, periodic checks of procedures and data collection methods, privacy, and security of data, as well as access of assigned personnel to different types of data. For this information collection, all study staff will be under the direct supervision of the ATSDR on-site supervisor. The study staff will obtain appropriate office space for the blood draws, clinical assessments, questionnaire, neurobehavioral batteries administration, secure storage of questionnaires, medical and school records, and storage of blood specimens (including refrigeration) prior to shipment to the NCEH laboratory. All data and biological specimens collected in the study are the property of ATSDR. Methods to ensure least privilege access to the study information will be in place; therefore, access to identifiable information will be role-based on a need-to-know basis for ATSDR investigators.

The study staff will provide details on its data security technology and methods including password protection, desktop firewalls, daily backups and server based storage, intrusion detection, vulnerability scans of personal computers and server, laptop security, and computer encryption procedures.

Once collected from the participant, all hardcopy informed consents and data collection forms will be stored in locked files in locked rooms in the study office and at ATSDR.

Upon completion of the project and once the ATSDR has received all approved study related paper documents, ATSDR will destroy those hardcopy documents not necessary to complete the study analyses or to contact study participants.

Data security measures at ATSDR will comply with the *CDC/ATSDR Protection of Information Resources Policy* and the *CDC/ATSDR IT Security Program Implementation Standards*. These policies apply to all

authorized ATSDR employees. All incidents involving a suspected or confirmed breach of IIF must be reported to OCISO according to the policy titled *OCISO/CDC Standard for Responding to Breaches of Personally Identifiable Information (PII)*.

*Physical controls* – The CDC/ATSDR issues identity credentials based on the Federal Information Processing Standards (FIPS) Publication 201 for Personal Identity Verification (PIV) authentication of government employees' identities. Security measures for physical access to secured facilities include the use of PIV Cards, security guards, and closed circuit TV monitoring.

*Technical Controls* – CDC/ATSDR policy requires employees to gain authorized logical access to its information systems through a unique electronic identity (User ID). The computer-controlled limits on what can be done by the user are assigned based on program roles and privilege requirements.

*Administrative Controls* – Authorized CDC/ATSDR employees are required to:

- Complete required privacy and information security refresher training.
- Read, acknowledge, sign (if online completion is not available), and comply with the HHS Rules of Behavior, as well as other applicable CDC/ATSDR- and system-specific rules of behavior before gaining access to the CDC/ATSDR's systems and networks.
- Adhere to the requirements set forth in the *CDC/ATSDR IT Security Program Implementation Standards*, and other security policies and procedures that minimize the risk to CDC systems, networks, and data from malicious software and intrusions.
- Abide by all applicable acceptable use policies and procedures regarding use or abuse of CDC/ATSDR IT resources.

All study records are subject to the ATSDR Comprehensive Record Control Schedule (CRCS), B-371, which contains authorized disposition instructions for ATSDR's administrative and program records. ATSDR is legally required to maintain its program-related records in accordance with disposition instructions contained in this comprehensive records control schedule. These retention periods have a direct impact on completing Freedom of Information Act (FOIA) requests and in applying the requirements of the Privacy Act. The current schedule requires ATSDR to retain and archive program records for a period of 75 years after the end of the study activities.

### **3.8.4 Data Delivery**

Study staff will follow checks and quality control procedures for data entry. Only authorized study staff will receive permission to enter or manipulate the study data. Data entry from hardcopy documents will involve double entry with discrepancies compared and corrected.

Study staff will prepare draft datasets to record questionnaire responses and medical record/school record data to send to ATSDR for review and approval. ATSDR will work with the study staff to resolve missing values and other data issues. The study staff will also keep and deliver a shipping log of blood specimens sent to the NCEH laboratory in Microsoft Excel format (**Attachment 14**). The log will include the include vial type, volume, ID code, date, and carrier details. ATSDR will receive lab results from the participating laboratories. The lab dataset will be merged by study ID with the questionnaire data to create a combined questionnaire and lab dataset.

All dataset formats will be transformed to SAS datasets (SAS 9.3, Cary NC). All final data management will be performed on this platform. Final datasets will be sent to ATSDR using encrypted, password coded spreadsheets through a password protected data sharing facility. The contractor will deliver to ATSDR the code and the master key dataset by which the response data are potentially relinkable to PII.

Consent forms that collect the signatures of participants will be paper instruments and the adult participant or parent of the child participant will receive a copy of the consent form. Height, weight, and other applicable body measures and blood pressure will be recorded on a paper form and transferred to an electronic form.

### **3.8.5 Data Ownership and Data Sharing**

Coded research datasets will be available to all ATSDR study investigators listed in **Attachment 1**. We will produce coded datasets by removing the following: name, SSN, date of birth, address, former address (es), phone number, and date of completion of the blood draw and questionnaire. SSN will be collected at enrollment for linkage to medical records and school records. Once the linkage has occurred, the SSN will be kept with other PII in a separate access restricted secure database. Age will replace date of birth in the data analysis file because it is the necessary variable in exposure and health outcome analyses.

Release of de-identified data to outside investigators must be approved by ATSDR. A data use agreement (DUA) will be prepared, detailing the condition of use of the data and proposed analyses for each outside

project. The DUA condition of use will specify that ATSDR will not release the link between the study IDs and the participants' PII to the outside researchers. The DUA will also specify that:

1. Our data cannot be merged with public data in such a way that individuals may be identified;
2. Our data cannot be enhanced with public data sets with identifiable, or potentially identifiable, data;
3. One of the study investigators listed in **Attachment 1** must be a co-investigator on any outside research project to guarantee adherence to the agreed conditions of use; and
4. Each data release will be cleared by a specific IRB request to the investigator's home institution prior to data release.

After the approved project with the outside researchers is completed, further or secondary analyses of electronic datasets can only be undertaken with additional approval(s) from ATSDR. Written confirmation of understanding the conditions of use will be required from the lead scientist and institution. Copies of statistical code and datasets used in statistical analyses by the outside investigators will be kept by ATSDR.

### ***3.8.6 Storing Residual Blood for Future Use***

After performing the chemical and clinical tests, there may be some residual blood. In the consent form, we will ask participant's permission to save this residual blood for additional future analyses of PFAS and possibly additional effect biomarkers. We will only store blood of those participants who will consent to have their blood archived for additional PFAS and effect biomarker analyses. Some enrolled participants would have also participated in the 2015-17 Pease PFC Blood Testing Program and have leftover blood stored at NH DHHS (NH DHHS 2016). We will ask those Pease study participants if they consent to have those samples stored by ATSDR to be used in future analyses related to PFAS research. If they do not consent or if they decided not to participate in the Pease study, those samples will be discarded at the end of the enrollment by NH DHHS.

All residual blood specimens will be stored with the study-generated ID only. ATSDR will keep a separate dataset that can link the study ID with the participant's name. If participants change their minds later about letting their blood used for additional analyses, they can contact ATSDR and we will remove their specimens. We do not plan to provide participants the results of these future tests, but we may contact them if we learn something that is important.

We will consent participants at enrollment and will not recontact them for the additional analyses of stored biospecimens related to this PFAS research: Because new scientific knowledge, tests, or methods may arise, we would like to save this leftover biospecimens for additional analyses on exposures or health conditions related to PFAS. In addition, ATSDR or recipients may release de-identified research datasets or de-identified biospecimens for future studies related to PFAS to outside investigators under a data use agreement that will prohibit any attempt to identify you or your child as a research subject. In this case, your individual test results will not be reported to participants.

After we complete this study ATSDR may conduct new research studies. At that time, we may ask for additional consent to include participants' data or leftover biospecimens from this current study.

For all future use, the stored biospecimens will not be used for any commercial activities for profit. In addition, we do not anticipate the collected biospecimens to be used for whole genome sequencing (you would need to be recontacted to consent for such tests). All future analyses and studies must adhere to IRB review requirements.

### ***3.8.7 Future Exploratory Analyses***

CDC IRB approval will be sought for this additional research either as a protocol amendment or under a new research protocol prior to undertaking this plan.

## **3.9 Exposure Estimation**

The study will use the fasting serum PFAS measurements obtained from study participants to estimate exposures. In addition, the study will estimate each participant's cumulative PFAS serum level, using:

- PFAS serum measurements obtained in the study,
- PFAS serum measurements from the Pease biomonitoring program,
- Historical reconstruction of PFAS concentrations in the drinking water consumed by the participant,
- Questionnaire data on the participant's consumption of PFAS-contaminated drinking water and factors that might affect PFAS serum levels,
- Age-, sex-, and calendar year-specific "background" PFAS serum levels from NHANES, and
- Physiologically based pharmacokinetic (PBPK) models.

The C8 studies used PBPK modeling to estimate cumulative serum levels of PFOA and PFOS (Shin 2011). The model incorporated information from the historical reconstruction of PFAS concentrations in the drinking water serving the C8 areas, questionnaire data on each participant's water consumption, and the serum levels of PFOA and PFOS obtained from study participants. A recent effort to reconstruct historical exposures worked well for PFOA and PFOS; the model for PFHxS serum levels using biomonitoring data in the US and Australia was not successful (Gomis 2017). However, recently a study was published that successfully developed a PBPK model for PFHxS (Kim et al 2018). Low environmental concentrations, lack of decline in older population, possible ongoing exposure in children/younger adults, and scarcity of time-trend data in consumer products were cited as reason for poor prediction characteristics of PFHxS models (Gomis 2017). However, if there are high correlations in serum levels between PFHxS and PFOS and/or PFOA, then it may be possible to estimate cumulative PFHxS serum levels based on the historical estimates for serum PFOS and/or PFOA.

Recently, an online serum PFOA calculator for adults became available using a modified one-compartment exponential decay model to estimate PFOA serum levels from PFOA concentrations in drinking water (Bartell 2017). Developing a similar calculation for serum PFOS is possible. The studies of children and adults will explore this approach to estimate serum PFOA, PFOS, PFHxS and PFNA levels and make comparisons with serum levels from the blood specimens as well as the results of the Pease biomonitoring program. We propose to use a one-compartment PBPK model similar to one used by Shin (2011) and Avansi (2016), and also used as the basis for a recent PFOA serum calculator (Bartell SM 2017).

A number of improvements in PBPK modeling approaches, especially as related to multi-compartment models, have been developed recently and we will take those into consideration (Loccisano 2013, Fabrega 2014, 2016; Verner 2015, 2016).

In our efforts, we will attempt to integrate broad range of information on individuals' sociodemographics (birth year, age, sex, ethnicity), PFAS pharmacokinetics (e.g. tissue partitioning and distribution volumes, elimination rates), as well as exposure sources as pertain for the general population (e.g. breastfeeding, water consumption, blood transfusion) and secretion routes (e.g. parity, breastfeeding history, and menstruation in women; donating blood) which will be collecting in the adult and child questionnaire. Questionnaires also includes detailed information on menstruation cycles for women (regular/irregular, length, heavy/light flow, last menstruation before blood draw; Wong 2015, Verner and Longnecker 2015). We assume the contributions from dietary intake, cookware, cleaning supplies, etc. to be similar to the background US population (Domingo 2012, Christensen 2017). We will also assume that NHANES calendar



year-, age- and sex-specific PFAS serum concentrations reflect these background exposures (Calafat 2007, Ye 2017).

In order to model the migration of PFAS chemicals contained in the AFFF from the source of contamination (e.g., fire training areas) on base through the soil and groundwater to the drinking water supply wells, the sub-studies will need the following information:

- The annual amount of AFFF used at Pease Air Force Base prior to its closing,
- Information on any accidental releases at the base,
- Location of fire training areas at the base,
- Soil and groundwater characteristics in the vicinity of AFFF use and accidental spills on base, and
- Production logs and other information on the drinking water supply wells on base.

ATSDR will request this information from the U.S. Air Force (USAF) and the NH Department of Environmental Services.

The studies of children and adults will recruit from those who participated in this biomonitoring program and will use the serum results from the program in the estimation of cumulative PFAS serum levels. Study participants who didn't bring their biomonitoring results collected by NH DHHS in 2015-2019 to the study office can authorize NH DHHS to transfer those directly to ATSDR (**Attachment 24b**). In addition, the studies will ask study participants to consent to the analyses of their stored blood specimens for additional PFAS chemicals not measured in the biomonitoring program.

### **3.10 Statistical Analyses**

ATSDR staff will perform statistical analyses using SAS, R and STATA. ATSDR staff may also use SPSS for data management. ATSDR staff will calculate descriptive statistics (including means, geometric means, medians, standard deviations, and percentiles) to identify the presence and distribution of PFAS and effect biomarker analytes in the Pease participants and their referent groups. Statistical methods will include multiple linear regression of continuous (untransformed and natural log transformed) effect biomarkers on continuous (untransformed and natural log transformed) PFAS serum levels and categorized PFAS serum levels, and logistic regression of categorized effect biomarkers (e.g., hypercholesterolemia) or disease prevalence on continuous (untransformed and natural log transformed) and categorical PFAS serum levels. ATSDR staff will use restricted cubic spline methods (or generalized additive models using cubic regression splines) for linear and logistic regression to obtain flexible, smoothed exposure-response

curves. To identify risk factors that may act as confounders for a particular health outcome, the analysis will implement a “10% change in the estimate” rule (Maldonado 1993).

Primary analyses will focus on estimated cumulative PFAS serum levels. Supplemental analyses will evaluate PFAS serum levels in the new blood specimens obtained in the study as well as estimated maximum and average PFAS serum levels. The primary analyses will evaluate each PFAS chemical separately. However, ATSDR will explore the use of methods for evaluating multi-pollutant mixtures, such as the hierarchical Bayesian model, to analyze the effects of exposures to the PFAS mixtures. ATSDR will use quantitative methods to assess the impact of possible selection bias and possible confounding due to unmeasured risk factors (Lash 2009). There are several caveats and recommendations in conducting analyses of mixtures to determine the optimal method that avoids amplifying bias due to confounding (Weisskopf et al 2018). For the bias analyses, ATSDR will identify “negative control” diseases with no known association with PFAS exposures (Lipsitch 2010). ATSDR will conduct a literature search to identify these negative control diseases and include them in the questionnaire. To gauge the potential and magnitude of possible selection bias and information biases, two approaches will be taken. First, quantitative methods described in Lash et al (2009) will be used to estimate the possible magnitude of selection and informational biases. Second, “negative control” diseases will be used to also estimate the potential and magnitude of these biases (Lipsitch et al 2010). Negative control diseases are those diseases not known to be associated with the exposures of interest. In the Pease study, the exposures of interest are PFAS serum levels. The negative control diseases for children included in the questionnaire are celiac disease, scleroderma, lupus, and Crohn’s disease. In addition to these diseases, negative control diseases for adults include Parkinson disease, emphysema, chronic bronchitis, multiple sclerosis, and fibromyalgia.

ATSDR will interpret the findings from this study based on the magnitude of the effect estimates (e.g., the linear regression coefficient for continuous outcomes or the odds ratio for categorical outcomes) of the exposure-response relationship, consistency with findings from other studies, and the possible sources of bias (Rothman 2014). The analyses will construct confidence intervals to indicate the level of precision (or uncertainty) in the effect estimates.

The studies will use statistical significance testing to interpret findings but will not use it as a sole factor in determining scientific and public health significance (Rothman et al. 2008, 2010; Stang et al. 2010). A finding that fails to achieve statistical significance can still provide evidence for a causal association, and a finding that achieves statistical significance can lack any such significance (Porta 2014).

## **4. RESULTS REPORTING**

### **4.1 Notification of Individual Results**

Some of the clinical tests may include results that indicate disease or serious medical condition. Due to the scheduled timespan between blood specimen collection and the actual laboratory analyses, we are unable to report study results in a short period. Study staff will report to the participant the result of a clinical test that clearly indicates the potential for a serious health consequence immediately after receiving the result from the laboratory. An advance notification phone call from the study investigators (**Attachment 22**) with a subsequent letter of clinical tests results will be sent to the participant when the abnormal result is identified, processed, and checked for accuracy (**Attachment 22a**). Study staff will advise the participants to consult his/her physician, or to contact the physician associated with the study for explanation of clinical findings.

Participants will also receive results of their effect biomarker tests after the study is completed. Contract labs will provide their clinical reference abnormal or 'high' levels, if available, for interpretation of clinical test results (**Attachment 23**). Participants will receive their PFAS test results. ATSDR will provide to the 50<sup>th</sup> and 95<sup>th</sup> percentiles from NHANES for comparison to the U.S. population (CDC, 2018; **Attachment 24**). Study staff will advise participants to consult ATSDR with questions about their results if they wish to do so.

### **4.2 Disseminating Results to the Public**

The Pease CAP has a permanent and active presence in the Portsmouth community. Therefore, ATSDR will consult with the Pease CAP to determine the most effective method of disseminating the results to the participants and the public. ATSDR will also consult with the local health department and the NH DHHS on methods for results dissemination. ATSDR may consider using a user-centered digital interface developed by the Silent Spring Institute for reporting results to each participant. ATSDR will present study results to the community in public meetings, printed community handout materials, participating in local radio programs and in informal activities. ATSDR also will provide a study website for the Pease community with information about the study findings and general information about any future follow up studies.

Generally, ATSDR will publish study results only as group data analyses in peer-reviewed scientific journals or government reports. If individual data are presented, those will not be linked to participants' identities.

In the event that some other exceptional characteristics would enable personal identification, those would be masked or modified as needed to protect individual privacy. ATSDR will use manuscripts published in peer-reviewed scientific journals and presentations at major scientific meetings to inform the scientific community about the results of the Pease studies.

## **5. STRENGTHS AND LIMITATIONS**

Cross-sectional studies are especially suitable for assessing effect biomarkers and the prevalences of nonfatal diseases, in particular, diseases with no clear point of onset (Checkoway 2004). However, if the cross-sectional study concurrently measures the exposure and the outcome (i.e., the disease or effect biomarker), it might be difficult to determine whether the exposure caused the outcome or whether the outcome influenced the measured exposure level (Flanders 1992, 2016). For example, as discussed above, the concurrent measurement of serum PFAS levels and kidney function biomarkers might raise the question of “reverse causation” because kidney function can affect the levels of PFAS in serum. One approach to minimize the problem of reverse causation or possible confounding due to health outcomes that affect PFAS serum levels is by estimating exposures based on the historical reconstruction modeling of serum PFAS levels. In addition, it might be possible to estimate exposures during critical vulnerable periods (e.g., in utero exposure) through the modeling of historical serum PFAS levels. However, the modeling of historical PFAS serum levels is subject to uncertainties and data limitations, and published methods currently are available only to model serum levels of PFOA and PFOS.

Other issues concerning cross-sectional study designs are similar to those that confront other observational study designs, such as cohort studies. These issues include: 1) the ability to clearly define, enumerate and recruit (without introducing selection bias) the exposed and comparison populations, 2) the comparability of the exposed and comparison populations on risk factors other than the PFAS exposures, 3) accurate exposure assessment, and 4) accurate measurement of effect biomarkers and ascertainment of diseases. In addition, a bias similar to the “healthy worker survival effect” bias could occur in a cross-sectional study because the study population consists of those who remained in the study area (and, for example, did not leave the study area due to health problems caused by exposure to the PFAS contaminated drinking water).

Based on its review of the literature, ATSDR concludes that several health-related endpoints are feasible for studies of the Pease population. It is also clear that exposures to the PFAS-contaminated drinking water have occurred in the Pease population, as documented by the observed serum PFAS levels in the

NH DHHS PEASE PFAS blood-testing program. Therefore, it is reasonable to conduct epidemiological studies of the Pease population. However, whether it is feasible to study a specific health-related endpoint depends on the size of the exposed population that may be recruited into a study.

All epidemiological studies of environmental exposures and health outcomes have limitations and uncertainties. Whether a study will find an association between an environmental exposure and health effects is unknown prior to conducting the study. No single study of the Pease population will provide definitive answers to the community about whether their exposures to the PFAS-contaminated drinking water caused their health problems. The ability of a study of the Pease population to provide useful information will depend largely on the success of recruiting a sufficient number of study participants.

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## 8. LIST OF ATTACHMENTS

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