Human health effects of drinking water exposures to per- and poly-fluoroalkyl substances (PFAS): A multi-site cross-sectional study

Protocol

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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). PFAS contamination of drinking water is widespread in the U.S. For example, one report indicated that at least six million residents were served by 66 public water supplies that had at least one sample at or above the US EPA Lifetime Health Advisory for PFOA and PFOS (individually or combined) of 70 ng/L (Hu 2016). Industrial facilities that manufacture or use PFAS have contaminated drinking water in surrounding communities in West Virginia, Ohio, New York, Minnesota, Alabama, Vermont, New Hampshire, and New Jersey (Kray 2018). An alternative method of estimating PFAS drinking water contamination put the number of people potentially exposed to PFAS at concentration over 2.5 ng/L at about 110 million (Environmental Working Group 2018). PFOS, PFOA, PFHxS and other PFAS chemicals 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, 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 bases and/or surrounding communities (ATSDR 2017a). The Air Force and Navy have identified at least 24 bases with contaminated drinking water in Alaska, California, Colorado, Delaware, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Virginia, and Washington (Kray 2018).

A detailed review of epidemiological studies published up through 2016 was included in the ATSDR Feasibility Assessment for Epidemiological Studies at Pease International Tradeport, Portsmouth, New Hampshire (ATSDR 2017a; released Nov 2017). Health effects of PFAS exposure in children were also recently reviewed by Rapazzo (2017). The scientific evidence linking PFAS exposures with adverse health effects is rapidly growing. Epidemiological 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 (Lopez-Espinosa 2011, Bach 2016) and some cancers (; Barry 2013). However, findings across studies

have been inconsistent for a variety of reasons, including differences in 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.

Most studies of the human health effects from PFAS exposures have focused on PFOA and PFOS. These include studies that evaluated data from the National Health and Nutrition Examination Survey (NHANES), occupational 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. Studies of West Virginia and Ohio residents and workers exposed to PFOA from a chemical plant (the "C8" studies) have provided extensive and high quality information on PFOA (and to a lesser extent, PFOS), studying a large cohort of highly exposed residents (60,000+) and workers living in the vicinity of the production facility. However, other PFAS such as PFHxS and PFNA were not a primary focus of the C8 studies. Except for the C8 studies, there is scant information on the health effects of exposures to PFAS-contaminated drinking water.

1.1.2 Health Study Feasibility Assessment

In 2017, ATSDR published a feasibility assessment of possible future drinking water epidemiological studies at the Pease International Tradeport, Portsmouth, New Hampshire (ATSDR 2017a). Drinking water supply wells serving the Pease Tradeport were contaminated with PFAS from the use of AFFF at the former Pease Air Force Base. As part of this feasibility assessment, ATSDR reviewed the available information on the Pease Tradeport population and exposures (e.g., population size and demographics, PFAS biomonitoring results, and drinking water data) as well as conducted sample size calculations. The ATSDR feasibility assessment concluded that there was a need for additional epidemiological research on the health effects of PFAS exposures to address several research gaps and issues: (1) the small number of studies for some health endpoints, (2) the inconsistency of findings across studies for some health endpoints, (3) the lack of drinking water studies other than the C8 studies, and (4) the need to conduct studies that evaluate PFHxS and PFNA as well as other PFAS chemicals in addition to PFOA and PFOS (ATSDR 2017a).

In addition, ATSDR determined that cross-sectional epidemiological studies of children and adults at one site (e.g., at the Pease Tradeport) were feasible for some health endpoints (e.g., lipids, kidney function), but the size of the populations would be insufficient for other important health endpoints (e.g., thyroid, liver and immune function, autoimmune diseases). Therefore, the feasibility assessment concluded that: (1) a multi-site PFAS study of children and adults was necessary, (2) the study should be cross-

sectional and involve separate evaluations of children (ages 4-17) and adults (ages ≥18), and (3) the study should focus on communities impacted by PFAS-contaminated public drinking water supply wells and/or private wells. A cross-sectional study design was chosen because this design is especially suitable for assessing effect biomarkers and the prevalences of nonfatal diseases, in particular, diseases with no clear point of onset (Checkoway 2004). Additionally, the cross-sectional design can generate data for hypotheses that can be tested in subsequent longitudinal studies.

1.1.3 Summary of Study Goals

The main goal of the cross-sectional multi-site study is to evaluate potential 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 study will attempt to recruit at least 2,100 children and 7,000 adults (equally of both sexes for both children and adults) from communities exposed to PFAS-contaminated drinking water. The criteria for selecting study sites are detailed in **Section 2.3** and include:

1. Documented past or present PFAS drinking water concentrations at the tap,
2. The magnitude of past or present PFAS concentrations at the tap,
3. Size of the population exposed,
4. Geographic coverage;
5. The proposed researchers for a study site were experienced in conducting drinking water epidemiological studies;
6. Amount of information available on the contaminated drinking water system or private wells, and
7. If biomonitoring for PFAS has previously occurred at the site.

Possible candidate sites included communities whose drinking water was impacted by AFFF use at military bases or by industrial PFAS emissions. The site selection process considered the levels of PFAS drinking water concentrations at a site. The aim was to select sites so that a wide range in PFAS exposures levels were included in the study in order to enable the evaluation of exposure-response trends including effects at the lower range of exposures.

For those sites with complex drinking water systems (e.g., where individual supply wells serve particular areas of the distribution system, or when there is uncertainty concerning which areas in the distribution system received contaminated water) or sites with groundwater contamination affecting private wells where there is uncertainty concerning which wells are contaminated, it may be necessary to use modeling methods (e.g., ground water contaminant fate and transport models, water system distribution system models) to identify the areas with contaminated drinking water. A targeted PFAS biomonitoring approach may be needed to confirm results from groundwater and/or distribution system modeling approaches. Modeling may also be necessary to determine the period when the drinking water was contaminated and to historically reconstruct PFAS contaminant concentrations during this period (Shin 2011).

The study will obtain blood samples from participants to measure PFAS serum levels and several effect biomarkers such as lipids, and thyroid, kidney, immune and liver function. The study will also obtain urine samples from participants to measure PFAS levels and kidney function biomarkers. The study will archive serum and urine samples in order to conduct analyses of additional PFAS chemicals and specific effect biomarkers. Adult participants and a parent of the child participant will complete a questionnaire that includes a residential history, medical history, occupational history and water consumption habits. The study will access medical and school records to confirm adverse health outcomes reported in the questionnaire. To facilitate access to these records, the recipient will reach out to local medical societies, the public school system and private schools to enlist their cooperation with the study.

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). Estimated and measured PFAS serum levels will also be evaluated as continuous variables. At sites with preceding PFAS biomonitoring, the study will evaluate changes in PFAS concentration over time. The study 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 confounding and reverse causation due to physiological factors (Dhingra 2017, Weisskopf 2017).

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 or worked in an industry using PFAS chemicals in its manufacturing process). 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.

Based on ATSDR's literature review of epidemiological studies of PFAS, the study will examine potential associations 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, the study will investigate differences in sex hormones and sexual maturation, vaccine response, and neurobehavioral outcomes in children as related to PFAS. In adults, additional outcomes of interest include cardiovascular disease, osteoarthritis and osteoporosis, endometriosis, and autoimmune disease.

These health endpoints were not selected based on power calculations, but rather on epidemiological and scientific bases: (1) endpoints that have been evaluated in previous PFAS research and need follow-up; (2) endpoints observed to be elevated in studies of other chemicals with similar *in vitro/in vivo* activity; and (3) results from toxicological and epidemiological studies of PFAS. With the proposed sample sizes for the multi-site study there should be sufficient power to detect mean differences and odds ratios in the ranges of those observed in other well designed epidemiologic studies.

1.2 Study Investigators and Roles

This cooperative research is being conducted under the ATSDR Notice of Funding Opportunity (NOFO) No. CDC-RFA-TS-19-002, titled "Multi-Site Study of the Health Implications of Exposure to PFAS-Contaminated Drinking Water." The expected number of research recipients[1] is six. The program will be administered by the CDC Extramural Research Program Office (ERPO).

Given that the single IRB mandate under the revised 2018 Common Rule will take effect on January 19, 2020, this research program shall be managed under the review of a single IRB for cooperative research. See §46.114 (Cooperative Research).

Projects that involve the collection or generation of data with federal funds must develop, submit, and comply with a Data Management Plan (DMP) prior to the collection or generation of public health data, and, to the extent appropriate, provide public access to and archiving/long-term preservation of collected or generated data.[2]

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1 A "recipient" is defined as a "non-Federal entity that receives a Federal award directly from a Federal awarding agency to carry out an activity under a Federal program." (see Grants.gov at https://www.grants.gov/learn-grants/grant-terminology.html#R; accessed 02/04/2019).

2 https://www.cdc.gov/grants/additional-requirements/ar-25.html

This protocol also represents CDC-supported research in which identifiable, sensitive information is collected and is issued a Certificate of Confidentiality (CoC). Thus, ATSDR and recipients are required to protect the privacy of individuals who are subjects of such research in accordance with Section 301(d) of the Public Health Service (PHS) Act.[3]

This protocol represents the core research that all recipients must conduct at their sites. Recipients will tailor their site-specific informed consent forms based on the ATSDR template (**Attachment 7b**).

ATSDR and NCEH Roles: The health study team at ATSDR is responsible for the development of and for external peer review requirements for the core protocol for the PFAS multi-site study. The study protocol will be submitted by ATSDR for review and approval by the CDC Institutional Review Board (IRB) under CDC's Federal wide Assurance (FWA) No. 00001413) and by the Office of Management and Budget (OMB). ATSDR will also seek comments from community organizations involved with PFAS.

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 a commercial laboratory as specified in the protocol. Urine specimens will be collected and stored for future analysis and study. ATSDR will conduct data analyses of the combined core data from all the study sites with the recipient participation.

Recipient Role: Data collection at each study site will be conducted by the recipient via cooperative agreement with the ATSDR. The recipient will conduct historical reconstruction of PFAS concentrations in the drinking water at the specific site and will estimate historical PFAS serum levels. The recipient will conduct participant sampling, obtain informed consent, and administer a questionnaire. The recipient will verify reported health conditions with participant's health care providers and approach appropriate school district to abstract special education records. The recipient will obtain a blood and urine sample from each participant and will be responsible for specimen shipment to the CDC NCEH DLS and commercial laboratory. The recipient will deliver the core data and personal identifier information ("PII") such as social security number, full name and date of birth, to ATSDR. Each recipient may conduct analyses of the data from the recipient's site. Each recipient shall maintain PII data in a secure manner and delete PII data after the study is completed.

2. INTRODUCTION

3 https://www.cdc.gov/grants/additional-requirements/ar-36.html

2.1 Authority

ATSDR is authorized to conduct the PFAS multi-site study under Section 316(a) of the 2018 National Defense Authorization Act (Public Law 115-91), as amended by Section 315 of the John S. McCain National Defense Authorization Act for Fiscal Year 2019 (Pub. L. 115-232).

2.2 Background

Starting in the 1950s, PFAS have been used in a wide variety of products and applications including fluoropolymer manufacturing, stain and water repellant coatings, cleaners, and paints. PFAS are also components of aqueous film-forming foam (AFFF) used to extinguishing flammable liquid fires. From approximately the early 1970s, AFFF was used for firefighting training and to extinguish fuel-based fires at a number of military and non-military sites (e.g., airports) around the country. PFAS components of AFFF include perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS).

PFAS contamination of drinking water is widespread with at least six million U.S. residents receiving water having concentrations of PFOA and PFOS (individually or combined) exceeding the EPA's Lifetime Health Advisory of 70 parts per trillion (Kray 2018). Sources of the drinking water contamination include emissions from manufacturing facilities and the use of AFFF at military bases and airports. For example, the Air Force and Navy have identified at least 24 bases with contaminated drinking water in several states including Alaska, California, Colorado, Delaware, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Virginia and Washington (Kray 2018). At these bases, PFAS chemicals in the AFFF likely leached into the soil and ground water and migrated to drinking water supply wells.

An example of a community drinking water supply contaminated via the use of AFFF at a military base is the Pease International Tradeport, Portsmouth, New Hampshire. In 2014, a drinking water supply well had measured PFOS, PFOA and PFHxS concentrations of 2.5 µg/L, 0.35 µg/L, and 0.96 µg/L, respectively. The source of the contamination was use of AFFF at the former Pease Air Force Base. In 2015, NH DHHS established a Pease biomonitoring program for PFAS. The program obtained blood specimens for PFAS analyses from 1,578 persons (NH DHHS 2016, Daly 2018). 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. In analyses conducted by NH DHHS (Daly 2018), geometric mean PFHxS serum levels were higher for persons who drank ≥4 cups of water per day compared to those who drank <4 cups per day (4.76 µg/L versus 3.77 µg/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 ≤19 years (β = 0.31, SE = 0.15, marginal effect = 36.4%). Geometric mean PFOS and PFOA serum levels were also higher among persons who drank ≥4 cups of water per day compared with those who drank <4 cups per day (NH DHHS 2016, Daly 2018). 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, Daly 2018).

2.3 Selection of Sites

Possible candidate sites included communities whose drinking water was impacted by AFFF use at military bases or by industrial PFAS emissions. The criteria for selecting study sites included:

1. Documented past or present PFAS drinking water concentrations at the tap,
2. The magnitude of past or present PFAS concentrations at the tap,
3. Size of the population exposed,
4. Geographic coverage;
5. The proposed researchers for a study site were experienced in conducting drinking water epidemiological studies;
6. Amount of information available on the contaminated drinking water system or private wells, and
7. If biomonitoring for PFAS has previously occurred at the site.

In order to determine the feasibility of a site for inclusion in the multi-site study, information on the following parameters were included in the application

1. For public water systems using ground water sources, enumeration of supply wells that provided drinking water to the site. Information on each supply well should include years of operation, well capacity, and daily or monthly pumping rates. This information can be used to

determine the monthly proportion of the total water supply provided by each well during the period when PFAS contamination occurred. Information is also necessary about changes to the water system (e.g., closure of contaminated supply wells) after the contamination was detected.

2. For a water system supplied by surface water, characteristics of this source.
3. For a water system purchasing water from another system, characteristics of this source, the period of time purchased, and daily or monthly amount purchased in order to determine the proportion of the total water supply provided by the purchased water.
4. Characteristics of the drinking water distribution system. For example, for systems using supply wells, it is important to obtain information on whether mixing from the supply wells occurred at the treatment plant before entering the distribution system or if each supply well served a specific area in the system. If water was purchased from another system, then information on the area of the distribution system served by purchased water is necessary. For systems in which PFAS concentrations throughout the distribution system cannot be assumed to be similar (e.g., if all water is not mixed at the treatment plant before distribution), then It may be necessary to obtain sufficient information on the distribution system (e.g., pipe network, elevation and water demand at each node, pipe length and diameter, etc.) so that preliminary modeling using software such as EPANET can be used to estimate PFAS concentrations at various areas in the distribution system.
5. Description of when and how PFAS samples from monitoring or supply wells (or surface water) were obtained, the location of the wells, and the measured concentrations of PFAS including description of analytical methods used by the laboratory.
6. If the distribution system was sampled, which PFAS were detected, when, and the measured levels of concentration.
7. For sites involving private well contamination, the number and locations of the wells, periods of operation, any information on the source of contamination and the PFAS groundwater plume, and the dates of PFAS sampling and the measured concentrations.
8. Any information on the historical use of AFFF (e.g., amount purchased/used, location and frequency of training exercises, fire incidents, spills, etc.) at the site or in the vicinity of the site (e.g., military base airstrip) which was the source of the drinking water contamination. Any information on the soil and ground water characteristics in the vicinity of AFFF use. Any information on the groundwater PFAS plume.
9. If previous human PFAS biomonitoring program was conducted, the PFAS serum results, dates of blood or urine collection, and possible descriptive/predictive factors of the serum

concentrations (e.g. volume of water consumed, length of residence at site, differences in age, race, or other population characteristics).

For those sites with complex drinking water systems (e.g., where individual supply wells serve particular areas of the distribution system, or when there is uncertainty concerning which areas in the distribution system received contaminated water) or sites with groundwater contamination affecting private wells where there is uncertainty concerning which wells are contaminated, a targeted PFAS biomonitoring approach may be useful to confirm results from groundwater and/or distribution system modeling approaches. Possible candidate sites included communities whose drinking water was impacted by AFFF use at military bases or by industrial PFAS emissions.

On September 23, 2019, ATSDR awarded cooperative agreements with seven partners to study the human health effects of exposures to PFAS through drinking water at locations across the nation. Information regarding the multi-site study cooperative agreement partners and the location where they each will conduct their work are as follows:

•Colorado School of Public Health, University of Colorado Anschutz Medical Campus, to look at exposures in El Paso County, CO

•Michigan State Department of Health and Human Services to look at exposures in Parchment/Cooper Township, MI, and North Kent County, MI

•RTI International and the Pennsylvania Department of Health to look at exposures in Montgomery and Bucks Counties, PA

•Rutgers Biomedical and Health Sciences – School of Public Health to look at exposures in Gloucester County, NJ

•Silent Spring Institute to look at exposures in Hyannis, MA, and Ayer, MA

•University at Albany, SUNY and New York State Department of Health to look at exposures in Hoosick Falls, NY, and Newburgh, NY

•University of California – Irvine to look at exposures in communities near the UC Irvine Medical Center

2.4 General Approach for 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 the multi-site study 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 study should be consistent with methods used in previous epidemiological research of PFAS exposures, given adequate sample size and power. In the future, the follow up to the cross-sectional studies of health-related outcomes proposed to be studied in the longitudinal studies.

The recipient should work closely with local and state agencies (e.g., public school systems, local and state health departments), local community organizations, and local media to conduct outreach about the study to encourage participation and community engagement with all local stakeholders. For those sites involving a contaminated public water system, the recipient should request that the water purveyor include a flyer about the study in its billing mailings and email notices.

If feasible, the recipients were encouraged to identify and enumerate all households served by the contaminated drinking water supply in the selected community in order to recruit potential participants to meet the sample size requirements for children and adults. If the selected community is served by a PFAS-contaminated public water system, then the recipient was encouraged to obtain a list of households served by the water purveyor from its billing records, if available. If the community is served by contaminated private wells, then the recipient was encouraged to obtain a list of households with contaminated wells from the local and/or state health and environmental agencies, if available.

Recipients could use statistical sampling methods (e.g., a two stage cluster sample) for recruitment of study participants if all the affected households can be enumerated. However, it was recognized that a simple random sample may not be appropriate if the PFAS drinking water concentrations vary widely across the community. In these situations, a random sample of households stratified by PFAS concentration levels might be more appropriate in order to ensure a sufficiently wide distribution of PFAS serum levels among study participants to evaluate exposure-response trends effectively.

However, although a recruitment process based on a statistically-based sampling approach may be theoretically ideal, in practice it may not be feasible. For example, enumeration of all households may not be possible. Moreover, if participation rates are expected to be low, then in order to achieve the sample size objective, the recipient should consider non-probabilistic sampling approaches such as "judgement" and "snowballing" sampling approaches (Tyrer 2016).

As stated above, regardless of sampling method used, the recruitment strategy should achieve a wide distribution of exposure levels among study participants, i.e., it should be exposure-driven, in order to effectively assess exposure-response relationships. Therefore, the recipient should consider a targeted sampling approach, e.g., oversampling areas with higher PFAS drinking water concentrations. If the PFAS concentrations in drinking water are generally uniform throughout the community (e.g., if drinking water from all sources is mixed at the treatment plant prior to distribution), then a targeted sampling approach may not be necessary. On the other hand, if PFAS concentrations are not likely to be uniform throughout the distribution system or among private wells in the affected area, then a targeted sampling approach will probably be necessary with oversampling in areas with higher PFAS drinking water concentrations. To enable a targeted sampling approach, the recipient should use available information and, if necessary, preliminary modeling methods, to classify households in the community by past or present PFAS concentration levels in the drinking water. For contaminated public water systems, the recipient should request distribution system information from the water purveyor in order to identify areas with higher and lower PFAS concentrations in the drinking water. For contaminated private wells, the recipient should request information on the ground water PFAS contamination plume affecting the wells from the local or state environmental agency.

In response to Notice of Funding Opportunity and following guidelines of the draft multi-site study protocol, recipients developed detailed recruitment protocols specific for each site. Those were reviewed by external peer review and approved by ATSDR when awarding the cooperative agreement grants. Non-random approaches were made available to site investigators, because association/etiologic studies (as opposed to descriptive studies like NHANES, for which estimation is targeted at individual variables rather than association parameters), selection bias results only when study participation is affected by both the exposure status and disease status (Hernan et al., 2004). The multi-site study is aimed at measuring exposure-disease associations, rather than estimating community-wide disease rates. Thus, non-random participation is only a concern if the two conditions for selection bias are met.

Investigators at five sites were able to enumerate the households and will proceed with statistically based sampling (or inviting all residents in the sampling frame area). However, as outlined above, the statistically representative sampling is needed for surveys generating normative data, such as quantifying exposures in the community (e.g. ATSDR Exposure Assessment), but not for ensuring the validity of studies of disease etiology (sic). Furthermore, the low response rates in communities - which are typical of studies of this nature - often preclude having meaningful probability samples.

- If the proposed efforts result in response rates below 15% after exhausting mail, phone, social media, and door to door attempts to contact (no more than 15 attempts total for selected household); the site will request ATSDR for deviation of the protocol and pursue non-probability sampling as described above.
- In addition, use of targeted sampling in high exposure area (e.g private wells), and volunteers to complement site specific exposure scenarios complement the statistically based sampling at three of those five sites

Two remaining sites concluded that the statistically based sampling was not feasible and elected to use snowball/referral based sampling and quota sampling methods.

- If the sites are unable to reach 60% of their recruitment goals using those techniques within one year of starting recruitment, they can request ATSDR to allow enrolling volunteers that meet study eligibility criteria.

All sites will fully document their methods and address how the final samples are likely to deviate from a true probability sample, drawing on relevant empirical data as feasible. Each site s will make adjustments as needed to attain the required study size per guidelines above and in coordination with ATSDR. Site investigators will work diligently to document all steps of the process and will commit to the technical oversight and quality control through the Sampling and Recruipment Working Group established from the Personnel Responsible for Collection and Analysis of Information (Supporting Statement B).

The primary issue in combining data from different sites is the sufficient comparability of the data in respect to conceptual framework and overall objectives of the study (Bangdiwala et al., 2018). Comparability will be ensured in this multi-site study by the implementation of common protocol that requires the same application of: a) eligibility criteria and characteristics; b) computer assisted interviews in study office (RedCap), c) outcomes of interest; d) sample collections/processing/storage

procedures; e) timelines of implementation per funding mechanism; f) centralized laboratories for exposure; effect biomarker and clinical tests; g) data quality assurance and management through unified contract mechanism; and h) shared tools for staff training.

Meta-analysis is a well-known approach for obtaining common effect from several similar studies. In order to protect against bias in the pooled analyses, it may be necessary to adjust some pooled epidemiological models for study sites. For example, meta-analyses often use either indicator variables or random effects approaches to take into account differences across sites due to the effects of geographical location (i.e., the study site is likely to have direct effects on PFAS water concentrations and participation, as well as possible direct effects on some health outcomes). A weighted pooled estimate is obtained, considering the inverse of each study 's variance. Multi-level meta-regression or modeling structural relationship are further options in analyzing aggregated data (Bangdiwala et al., 2018; Basagana et al. 2016).

To aggregate pooled data effectively and to guide statistical approaches for pooled data analysis we will use formal tests of heterogeneity across study sites (Friedenreich, 1993). Standardized study sampling/recruitment protocols in itself might or might not prevent substantial heterogeneity in observed exposure-disease associations, but if such heterogeneity is observed, key features of the different sites that bear on comparability will be tabulated and examined by the study team (Supporting Statement B, Table B.5.2), with sensitivity analyses to consider the impact of excluding sites from some analyses based on those features (Roetzheim et al. 2012).

The recipient should request assistance from local and state health departments in its recruitment efforts. In addition, the recipient should engage community organizations to assist in conducting outreach about the study and recruitment of participants. In addition, the recipient may establish a community assistance panel ("CAP") to review and provide comments on the study protocol and to facilitate the involvement of the affected community in decisions related to outreach about the study, participant recruitment strategies, and study logistics. The CAP would also assist the recipient in the dissemination of study findings to the community.

2.5 Study Objectives and Study Questions

The main goal of the multi-site study of children and adults is to evaluate the potential associations between specific health effects and serum PFAS concentrations among those exposed to PFAS-contaminated drinking water.

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 via contaminated drinking water and occupationally, respectively (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. PFHxS and PFOS are often major contaminants in drinking water impacted by AFFF. Except for the C8 studies, there is scant information on the health effects of exposures to PFAS-contaminated drinking water.

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 studies of populations exposed to the PFAS-contaminated drinking water should be initially be cross-sectional to be comparable with other studies and to establish a baseline for potential follow-up longitudinal studies. Studies should also evaluate measured serum PFAS measurements as well as estimated cumulative PFAS serum levels and use methods for the evaluation of health-related endpoints that are 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 birth size for gestational age, measures of intrauterine growth retardation, and preterm birth. In particular, several 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 1.3 and 1.6, so there was some evidence of an increased risk, although not strong. A study using NHANES data obtained an OR of 1.6 for 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 individual 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, 2016), 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 recent 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).

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 2010; Nelson 2010; Fletcher 2011; Steenland 2015), as were increased uric acid levels (Costa et al., 2009; Steenland 2010; Shankar 2011; Geiger 2013; Gleason 2015), concentrations of thyroid and sex hormones (Olsen and Zobel 2007; Knox 2011; Jain 2013; Wen 2013; Winquist and Steenland 2014), immune parameters (Dalsager2016), and reproductive effects (Joensen 2013; Kristensen 2013; Crawford 2017).

Associations with liver enzymes were found with PFAS in most cross-sectional studies (Olsen 2000; Sakr2007; Lin 2010; Gallo 2012; Gleason 2015) but were weaker or found no association in the cohort studies of liver enzymes (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 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 (C8 Science Panel 2012a). 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 unless there is evidence that confounding or reverse causation can explain these results.

In evaluation of kidney function, data from Watkins (2013) and Dhingra (2017) showed that while measured PFOA showed positive association, modeled PFOA concentrations had no relation to eGFR illustrating example of potential reverse causality. C8 Science panel found no association with the nonmalignant renal disease in their cohort study (2021b)

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); strengthening the case for adverse metabolic activity of these compounds.

Roles of inflammatory cytokines and adipokines have been explored several studies of liver disease such as non-alcoholic fatty liver disease/steatohepatitis and in atherosclerosis (Hennig 2007, Wahlang 2016, Clair 2018). Proinflammatory responses, alteration 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 strong associations for liver, kidney, and testicular cancer (Alexander and Olsen 2007; Barry2013; Bonefeld-Jorgensen2014; Hardell2014; Steenland2015).

Some studies have found 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 counfounding, bias, and chance on observed associations with PFAS compounds were explored in reviews of immune and cancer outcomes (Chang 2014, Chang 2015) and in studies of PFAS and menopause and endometriosis (Dhingra 2017, Ruark 2017, Ngueta 2017).

Few studies have evaluated PFHxS exposures and the risk of cancers and other adult diseases. Although epidemiological studies have primarily evaluated PFOA and PFOS, there remain considerable data gaps concerning the health effects of exposures to these chemicals in adults. There have been inconsistencies in findings across studies and limited sample sizes in some studies. For some adverse outcomes, only one or a few studies have been conducted. Finally, except for the C8 studies, there are no published individual-level epidemiological studies in adults that have evaluated the health effects from exposures to PFAS-contaminated drinking water. Therefore, additional research is necessary to determine whether drinking water exposures to PFHxS, PFOS, and PFOA increase the risk of non-cancer

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 Multi-site Study will evaluate the following main hypotheses, following the outline of the biochemical analytical plan (**Attachment 2**):

Higher serum levels of PFOA, PFOS, PFHxS, or other PFAS are potentially 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), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), albumin, 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, free T4, and total triiodothyronine (TT3); thyroglobulin antibody, thyroid peroxidase antibodies (TPO); higher prevalence of hypothyroidism/hyperthyroidism).
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, IgE, 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 ≥18 years), the Multi-site Study will evaluate the following main hypotheses.

Higher serum levels of PFOA, PFOS, PFHxS, or other PFAS are potentially 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 hypertensive disorders of pregnancy).
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, free T4, and TT3, thyroglobulin antibody, thyroid peroxidase antibodies (TPO); and higher prevalence of hypothyroidism/hyperthyroidism.
6. Liver function/damage biomarkers (e.g. alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), albumin, 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), rheumatoid factor, antinuclear antibodies (ANA), inflammatory cytokines and adipokines (interleukin 1-β (IL-1β), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 12 (IL-12), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor α (TNFα), 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 drinking water exposures to PFAS other than PFOA is at an early stage, the Multi-site Study should make an important contribution to the scientific literature, expand knowledge in this field, and help addressing concerns about past exposure.

Additionally, the Multi-site Study will provide the PFAS serum level and the results of the clinical tests and effect biomarker tests to each study participant. The participant can use this information for medical decision-making. Advice and assistance (e.g. workshops and or training programs) to clinicians in each community be provided by recipients and ATSDR as a part of the community engagement efforts to be able to answer questions about the potential effects of elevated PFAS levels on health, interpreting results, additional test or treatments. ATSDR will provide summaries of the study findings to the participating affected communities and will also provide assistance in interpreting each of these results.

3. METHODS

3.1 Study Design

The Multi-site study will be cross-sectional with separate evaluation of children (ages 4 – 17 years) and adults (aged ≥18 years). The participants will be recruited from lists of residences served by PFAS-contaminated drinking water.

- The recipient will obtain adult consent and parental permission (ages 4-17) and child assent (ages 7 -17), to participate in this research study (including consent to be contacted for any future studies).
- The recipient will administer adult and child questionnaires and seek medical records verification of self-reported diseases and medical histories (including neurobehavioral diseases).
- The recipient will administer neurobehavioral test batteries to the children and their parents and seek to abstract children's school records, in particular, special education records.
- The recipient 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 of PFAS and relevant effect biomarkers. The recipient will ship the urine

samples to CDC biorepository for analysis at a later time when more knowledge is gained about urinary PFAS and effect biomarkers and until the laboratory methods are developed.

- The recipient will seek consent to store residual blood and urine samples for future analyses of other PFAS and/or relevant effect biomarkers yet to be identified.

3.2 Study Populations and Eligibility

The target areas for the Multi-site Study are those served in the present or past by public water systems and/or private wells with documented past or present PFAS concentrations at the tap. The target populations consist of those residing in households in the target areas. Those eligible for the study include individuals aged ≥4 years at the start of the study who reside in a household in the target area and whose last exposure to drinking water exceeding the EPA Lifetime Health Advisory Level for PFOS and PFOA was no more than 15 years prior to the start of the study. In addition to those who resided in households served by contaminated drinking water, individuals exposed in utero and during breastfeeding when the mother resided in the household would also be eligible if the exposure occurred within 15 years of the start of the study. The limit of 15 years since last exposure was chosen to take into account the estimated half-lives in the body of PFOA, PFOS and PFHxS and to ensure that exposures to the contaminated drinking water are relatively recent.

Firefighters and others with occupational PFAS exposure from sources other than the drinking water will not be included in the 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 2,100 children (ages 4-17) and 7,000 adults aged ≥18 years with drinking water exposure to PFAS.

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 in areas with documented past or present PFAS drinking water concentrations at the tap, or were exposed in utero or during breastfeeding when the mother consumed the contaminated drinking water,
3. Drinking water exposure occurred within 15 years of the start of the study.
4. Children will be excluded if their 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.

The requirement that the child's last exposure be within 15 years of the start of the study 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). Slightly longer half-lives for individual PFAS (5 to 8 years) were derived in the draft ATSDR toxicological profile (ATSDR 2018). 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.

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 ≥4 years of age because most of the neurobehavioral tests that will be used in the study are appropriate for children aged ≥4 years of age.

3.2.2 Adults

The eligibility criteria for adults is as follows:

1. Aged ≥18 years at the start of the study.
2. Resided in areas with documented past or present PFAS drinking water concentrations at the tap,
3. Drinking water exposure occurred within 15 years of the start of the study.
4. 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 will be excluded.

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 ("α error") of .05 and type 2 error ("β 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 using NHANES data. 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 might occur at other sites. For outcomes not evaluated by NHANES or C8 studies, it was necessary to use

studies conducted in other countries. 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 3 includes additional information and assumptions pertinent to selected health outcomes to be studied.

3.3.1 Children

For children, **Table 1** (and **Attachment 3a**) provide the sample size calculations for several health outcomes of interest assuming a type 1 ("α error") of .05 and type 2 error ("β 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 minimum sample size of 2,100 children (equally of both sexes) is large enough to effectively evaluate many of the health outcomes identified in the Pease Feasibility Assessment literature review and the recent systematic review (Rapazzo 2017) as potentially associated with PFAS in children. 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 differences in risk for obesity and atopic dermatitis. A sample size of 2,100 children would be larger than many of the PFAS studies that evaluated neurobehavioral outcomes such as IQ and ADHD (Wang 2015, Stein 2013, 2014, Fei 2011, Hoffman 2010, Strom 2014).

An NHANES study of estimated glomerular filtration rate observed statistically significant findings with a total sample size of just under 2,100 children (Kataria 2015). For thyroid function, estradiol, delayed puberty, and asthma, a total sample sizes of 2,100 children may be sufficient, although larger sample sizes would be optimal (Lopez-Espinosa 2011, 2012; Stein 2016).

In summary, a total sample size of ≥2,100 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. Each cooperative agreement recipient will attempt to meet a target recruitment of 300 children.

Table 1. Sample size estimations for selected health-related endpoints in Child Study (ages 4-17 years)

Health-related Endpoint	Relevant Study	Observed Effect Size	Assumptions	Sample Size/Stratum α error = .05 β error = .20
Total Cholesterol (mg/dL) High cholesterol	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: 5th vs 1st quintile Age: <12 yrs 12-18 Boys: +6.2 +9.3 Girls: +4.6 +9.4 OR = 1.6	Mean PFOS serum levels were about 20 µg/L. SD for total cholesterol=29.3 mg/dL Prevalence=34.2%	+4.6: 637/stratum +9.3: 156/stratum 300/stratum
Thyroid function TT_4 Thyroid disease	Lopez-Espinosa 2012, C8 1,078 1-5 yrs 3,132 6-10 yrs 6,447 >10 – 17 yrs	PFOS, 4th vs 1 quartile: 2.3% change (mean difference = 0.17 µg/dL) PFOA: OR=1.44 (PFOS: OR < 1.0)	Mean PFOS serum levels were about 20 µg/L. SD for TT_4 as estimated at 1.4. Percent change in TT_4 was converted to mean difference assuming the median TT_4 was ref. level. Prevalence=0.6% (used PFOA results)	1,080/stratum >16,000/stratum
Uric Acid	Kataria 2015, NHANES 1,960; 12-18 yrs	PFOS: 4th vs 1st quartile = +0.19 mg/dL	Mean PFOS serum level = 12.8 µg/L. SD = 1.19.	556/stratum
Hyperuricemia	Geiger 2013, NHANES 1,772; 12-18 years	PFOS: 4th vs 1st quartile, OR=1.65	Mean PFOS serum level =16.6. Prevalence=16%	400/stratum
eGFR	Kataria 2015	PFOA mean serum level	Standard	275/stratum

	1,960; 12-18 yrs	=3.5 µg/L. mean difference= -6.6	deviation=27.6	
Testosterone	Lopez-Espinosa 2016, C8 1,169 boys; 6-9 yrs 1,123 girl; 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. 4th vs 1st 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., 4th quartile OR=2.19	Prevalence=10.7%	220/stratum
Obesity	Karlsen 2017 (Faroes)	PFOA mean serum level=2.22 µg/L. OR=1.88	Prevalence=17%	250/stratum

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

¶ 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 3b**) provide the sample size calculations for several health outcomes of interest assuming a type 1 ("α error") of .05 and type 2 error ("β 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.

The proposed minimum sample size of 7,000 adults (equally of both sexes) is large enough to effectively evaluate many of the health outcomes identified in the Pease Feasibility Assessment literature review. For example, for 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 7,000 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 7,000 (Melzer 2010; Wen 2013). NHANES studies of liver function also obtained statistically significant findings with total sample sizes considerably less than 7,000 (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 7,000 should be sufficient to evaluate these biomarkers as we assumed similar endpoint differences of those outcomes.

For ulcerative colitis, a sample size of 7,000 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 7,000 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 smaller sample sizes than the one proposed for the Multi-site 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 ≥7,000 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. Each cooperative agreement recipient will attempt to meet a target recruitment of 1,000 adults.

Table 2. Sample size estimations for selected health-related endpoints in Adult Study.

Health-related Endpoint	Relevant Study	Observed Effect Size	Assumptions	Sample Size/Stratum α error = .05 β error = .20
Total Cholesterol (mg/dL)	Steenland 2009, C8 46,294 aged ≥18 yrs	PFOS, mean serum level = 19.6 µg/L, 10th vs 1st decile:+11 mg/dL	SD=41.9	228/stratum
High cholesterol		4th vs 1st quartile, OR=1.51	Prevalence=15%	660/stratum
High Cholesterol	Fisher 2013, Canada	PFHxS, mean serum level = 2.2 µg/L, 4th vs 1st quartile, OR=1.57	Prevalence=44%	290/stratum
Cardiovascular disease	Shankar 2012, NHANES 1,216 aged ≥40 years	PFOA mean serum level = 4.2 µg/L, 4th vs 1st quartile: OR=2.01	Prevalence = 13%	250/stratum
Uric Acid	Steenland 2010, C8 53,458 aged ≥20 yrs	PFOS mean serum level = 20.2 µg/L, 10th vs 1st decile: +0.22 mg/dL	SD=1.55	780/stratum

		Hyperuricemia, 5th vs 1st quintile: OR=1.26	Prevalence:24%	1,525/stratum
Uric Acid	Shankar 2011, NHANES 3,883 aged ≥20 yrs	PFOA mean serum level = 3.5 µg/L, 4th vs 1st quartile: +0.44 mg/dL Hyperuricemia, 4th vs 1st quartile: OR=1.97 PFOS mean serum level = 17.9 µg/L Hyperuricemia, 4th vs 1st quartile: OR=1.5	SD = 2.5 Prevalence: 19.2%	507/stratum 200/stratum 550/stratum
Liver function Elevated ALT	Gallo 2012, C8 46,452 aged ≥18 yrs	PFOA and PFOS mean serum levels were 28 µg/L and 20.3 µg/L, respectively. PFOA: OR=1.54 PFOS: OR=1.25	Prevalence = 11.2%	725/stratum 2,917/stratum
Liver function ALT (µIU/mL)	Gallo 2012, C8 46,452 aged ≥18 yrs	The top quintile of serum PFOS in the Pease population was 15 µg/L. This would approximately correspond to a mean difference in ALT of +1.8 µIU/mL	SD=1.47	1,958/stratum
Liver function Elevated ALT	Gleason 2015, NHANES 4,333 aged ≥12 yrs	PFHxS mean serum level = 1.8 µg/L. 4th vs 1st quartile: OR=1.37	Assumed similar prevalence as in the C8 study	1,570/stratum
Thyroid disease	Melzer 2010, NHANES 1,900 men, aged ≥20 yrs 2,066 women, aged ≥20 yrs	PFOA, mean serum level=3.5 µg/L, 4th vs 1st quartile: Thyroid disease ever: Women, OR=1.64 Men, OR=1.58 Thyroid disease with current meds	Prevalences: 16.18% 3.06%	410/stratum 2,035/stratum

		Women, OR=1.86 Men, OR=1.89	9.89% 1.88%	365/stratum 1,575/stratum
Subclinical hypothyroidism	Wen 2013, NHANES 672 males aged ≥20 yrs 509 females aged ≥20 yrs	PFHxS mean serum level averaged about 2 µg/L. Unit increase in Ln(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 µg/L , 4th vs 1st quartile: OR=1.55 PFOS mean serum level = 24.6 µg/L, 4th vs 1st 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 ulcerative colitis, other autoimmune diseases, or cancer the sample size of 7,000 adults is too small to detect reasonably expected increases in the ORs.

It should be noted that the number of PFAS epidemiological studies available for each of the outcomes is limited, and the actual differences in clinical and research parameters may be quite different in the Multi-site study than have been observed in the PFAS literature. Sample size estimates provide guidance and may be useful for planning purposes but should be interpreted with caution, especially given the limited nature of the PFAS literature.

Attachment 3 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

The recipient will work with local and state health and environmental agencies as well as local and state-wide community groups in conducting outreach to encourage participation in the study. The recipient may establish a community assistance panel (CAP) at each site, (or covering several nearby sites), to assist in outreach efforts. The recipient may also establish a multi-site "umbrella" CAP, with community representatives from each of the sites included in the study, to develop a coordinated, across-site, approach to conducting outreach about the study.

Community involvement via a CAP or an alternative participatory mechanism will be crucial in achieving a high participation rate at each site and the sample size requirements of the study. In advance of the start of the study, outreach and engagement will involve announcements to local elected officials, medical societies/community health clinics, local media, community organizations, local unions, the public school system, and local private schools (**Attachment 5**). Outreach may also involve meetings with community representatives, medical societies, school officials, and/or public meetings. Although active in outreach, state and local agencies, CAPs, unions and community organizations will not directly obtain consent, intervene, or interact with research participants. As part of the outreach, the recipient will prepare a factsheet for distribution to state and local agencies, unions, and community groups (**Attachment 5, Attachment 7c**).

3.5 Recruitment

For sites with a contaminated public water supply, the recipient will request a list of residences served by the water purveyor (Attachment 3c). The information requested will include the name of the person on the residential account and the street address of the residence. The recipient will also request information from the water purveyor on the distribution system characteristics, in particular, whether the PFAS concentrations can be assumed to be relatively uniform throughout the system or whether the system had specific areas with substantially higher or lower PFAS concentrations. If uniform PFAS concentrations can be assumed, then a random sample of households may be conducted and recruitment letters mailed to these households. If the system has specific areas with substantially higher PFAS concentrations, then households in these areas will be targeted (oversampled) for recruitment letters.

For sites with contaminated private wells, the recipient will request information on the impacted residences and the results of PFAS sampling of their private wells from the state and/or local health and

environmental agencies (Attachment 3d). Sampling will target households based on the magnitude of the PFAS concentrations in their private wells – i.e., wells with higher concentrations will be oversampled – in order to ensure a sufficiently wide range of PFAS serum levels to evaluate exposure-response trends effectively.

Recruitment letters will provide a phone number to call for information about the study and to accept the invitation to participate in the study. The recipient will screen each interested caller using an eligibility screening script (**Attachment 4**). If necessary to achieve a high participation rate and the sampling size goal for the site, study staff may visit the sampled households to recruit participants.

Sampled 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 6**) to track recruitment success and to calculate non-response bias.

3.5.3 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. Once the appointment is scheduled, study staff will mail an Appointment Packet (containing an Appointment Reminder Card (**Attachment 7a**), the Informed Consent materials (**Attachment 7b**), a Study Fact Sheet (**Attachment 7c**) 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 8**). The study protocol will provide the flexibility to schedule or re-schedule office or home visits within the study period. Interested recruits who are unable or unwilling to come to the study office and live within a one-hour drive of the study office, will be offered an in-home appointment by trained study staff to complete the study. Interested recruits who request or require a home interview, blood draw, and urine collection, should reside within a one-hour

drive from the study office. 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 9**).

3.6 Data Collection Procedures

The study will establish a central office in each study site 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. 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 9**). This hardcopy form will be stored with the participant's signed Informed Consent Form (**Attachment 7b**) 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 7b**), including a Privacy Act Statement (**Attachment 7b1**). 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 7c**). 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.

3.6.2.1 Consent for Specimens and Data

The recipient 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, the recipient will ask for permission to archive any residual blood specimens and the urines for future analyses of PFAS and/or effect biomarkers.

If a study participant previously had a PFAS serum measurement, the recipient will ask the participant for the results.

3.6.2.2 Child Consent

Before any data collection can begin in the child study, trained study staff will review the hardcopy Parental Permission and Assent Form (**Attachment 7b2**) 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.

The recipient 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 7b2, 7b3 & 7b5**), and to contact the child and parent for possible future studies. Once the

parent signs the consent and permission forms (and the child aged ≥7 years gives assent to participate), the parent and/or the child become study participants in the future.

3.6.2.3 Adult Consent

Before any data collection can begin in the adult study, trained study staff will review the hardcopy Adult Consent Form with the interested recruit (**Attachment 7b4**). 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 7b5**), 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.

3.6.2.4 Risks and Benefits

As further described in **Section 3.8.1**, the recipient 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 as amended by Section 2012 of the 21st Century Cures Act. The recipient will further inform the participant that access to 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 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 2**). 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. They will receive the results of their urine PFAS and effect biomarker levels, if ATSDR identifies meaningful urinary analyses to perform.

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 10**).

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 11**).

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 12**).

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 13**).

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 14)**. 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 fours), ask about having diabetes, taking blood thinning medications, participant's weight, pregnancy, and fasting status (**Attachment 14**). Next, phlebotomists will draw 30-ml (about 1.0. ounce or about 6 teaspoons) of blood from the child participant and 40-ml (1.3 ounces or about 8 teaspoons) of blood from the adult participant using

standard venipuncture techniques (**Attachment 12**) and record the outcome (**Attachment 14**). 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 14**).

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 each participant will be asked to provide a fasting sample, it is recognized that some may not be able to fast. Variations in lipids levels due to fasting will affect PFAS compounds measurements to a lesser extent as PFAS in serum are bound to proteins not the lipid fraction. . 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 and urine specimens for secure storage and transport from the study office to the CDC NCEH laboratory in Atlanta, GA (**Attachment 12**).

The NCEH laboratory will perform the analyses of serum PFAS according to the biochemical analytical plan (**Attachment 2**) and approved laboratory methods (Kuklenyik 2015). The NCEH laboratory staff will also aliquot and ship blood and serum specimens to a centralized laboratory for the analyses of the effect biomarkers according to the plan. The recipient will store the urine samples and conduct analyses at a later date when more knowledge is gained about urinary PFAS and effect biomarkers and until the laboratory methods are developed. Residual blood and urines will be archived at NCEH so that additional PFAS or effect biomarkers can be analyzed as new knowledge and analytical methods become available.

3.6.5 Questionnaire

Each adult participant, and a parent of the child participant, will complete a questionnaire 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), residential

history, water consumption habits, 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 15**), and an abbreviated form for the child whose parent is also an adult participant (**Attachment 15a**).

The questionnaire will obtain the mother's and child's residential history in the study area, and the dates and length of time of the pregnancy and breastfeeding of the child. The questionnaire will also obtain information on the water consumption habits (including use of water for formula, juices, etc., bottled water use) of the mother and child when they resided in the study area. Information on the mother's workplaces in the study area (location and dates) and the child's daycare and schools in the study area (location and dates) will be obtained.

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 sending abstraction forms (**Attachments 17&17a**) to the medical care provider identified by the participants on their consent forms (**Attachment 7b5**).

3.6.5.1.1 Child/Parent Neurobehavioral Assessments

Table 3 provides the neurobehavioral test battery for children enrolled in the Multi-site Study.

Trained professionals will administer the following tests to children:

- The Wechsler Abbreviated Scale of Intelligence – 2nd 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 18**. 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 18a**).

Table 3. Neurobehavioral Test Battery for Children

Neurobehavioral Test	Domain	Age	Administration	Time to Administer
Wechsler Abbreviated Scale of Intelligence – 2nd Edition (WASI - II)	Two Subtest Form (FSIQ)	6 – 17*	Child	15 minutes
A Developmental Neuropsychological Assessment – 2nd 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, 2nd Edition (Conners K-CPT 2)	Inattentiveness, Impulsivity, Sustained Attention, Vigilance	4-7	Child	8 minutes
Conners Continuous Performance Test 3rd edition (CPT 3)	Inattentiveness, Impulsivity, Sustained Attention, Vigilance	8-17	Child	14 minutes
Strengths and Difficulties Questionnaire© (SDQ©)	Double-sided form with impact supplement (behavioral problems)	4 – 17	Parent about Child	5 minutes
Behavior Rating Inventory of Executive Function® (BRIEF®)	Executive Function	6-17	Parent about Child	10 minutes
Behavior Rating Inventory of Executive Function® – Preschool Version (BRIEF®-P)	Executive Function - Preschool	4-5	Parent about Child	10 minutes

For each child, the recipient will also review and abstract school records, including special education records, to identify learning problems and behavioral problems (**Attachments 18b&18c**). If the parent reports that the child has a developmental disability (e.g., ADHD, autism, or a learning disability), then the recipient 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, residential history, water consumption habits, occupational history, medical history and reproductive history (**Attachment 16**). In particular, the questionnaire will ask if the participant ever had kidney disease, liver disease, cardiovascular disease, hypertension, high cholesterol, thyroid disease, diabetes, autoimmune diseases, osteoporosis, osteoarthritis, pregnancy-induced hypertension, infertility, 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 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 17&17a**).

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 9**).

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 19**). 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, the recipient 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 9**). 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 12**).

3.7 Biochemical Analyses

Serum PFAS: The study's biochemical analytical plan is found in **Attachment 2**. The study will analyze 12 PFAS in fasting serum including PFOA (linear and the sum of branched isomers of PFOA), PFOS (linear and the sum of perfluoromethylheptane sulfonate isomers, and PFHxS (Kuklenyik 2015). Other PFAS

analyzed will include: perfluorooctane sulfonamide (PFOSA), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), perfluorobutane sulfonic acid (PFBuS), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUA), and perfluorododecanoic acid (PFDoA).

{Note: the study may include measurement of additional PFAS if methods become available by the start of the study. Addition of new analytes will be submitted to the CDC IRB for approval of amendments}

Urinary PFAS: The study will also analyze PFAS compounds in first morning void urines at later time on stored urine samples. Urine is an important excretion pathway for human metabolism and PFAS urine elimination may be important influencing serum concentrations (Harada 2005, Zhang 2015). The PFAS compounds to be measured in the future are listed in **Attachment 2**.

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 2):**

- Total cholesterol, low density lipoprotein, high density lipoprotein, total triglycerides,
- Uric acid, creatinine,
- Total thyroxine (TT4), free T4, TT3, thyroid stimulating hormone (TSH), thyroglobulin antibodies, thyroid peroxidase antibodies (TPO),
- Glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide, pro-insulin,
- Alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), direct bilirubin, albumin, and cytokeratin-18 (CK-18),
- Testosterone, estradiol, sex hormone-binding globulin (SHBG), follicle stimulating hormone, insulin-like growth factor,
- Immunoglobulin G (IgG), IgA, IgE, 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/free 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, and Santoro 2013).

3.7.2 Adults

The study will analyze the following biomarkers in the adult fasting serum (**Attachment 2**)**:**

- Total cholesterol, low density lipoprotein, high density lipoprotein, total triglycerides,
- Uric acid, creatinine,
- Total thyroxine (TT4), free T4, TT3, thyroid stimulating hormone (TSH), thyroglobulin antibody, thyroid peroxidase antibodies (TPO),
- Glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide, pro-insulin,
- Alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), direct bilirubin, albumin, and cytokeratin-18 (CK-18),
- Immunoglobulin G (IgG), IgA, IgE, and IgM; C reactive protein, rheumatoid factor, and antinuclear antibodies (ANA),
- Cytokines and adipokines (e.g. IL-1β, IL-4, IL-6, IL-8, IL-12, MCP-1, TNFα, 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 serum, blood, and urine specimens will be securely stored at the study office until shipped to the NCEH laboratory.

The NCEH laboratory analyzing PFAS serum levels, and the other participating laboratory analyzing the effect biomarkers, 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 12**). Specimens will be stored at the proper temperature and isolated from potential sources of contamination.

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

3.7.4 Reference Values

The participating laboratory will provide reference values and action levels for the effect biomarkers which will be reported in **Attachments 20&21**. The recipient will report the participant's PFAS results using reference values from the most recent NHANES report (**Attachment 22**). 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

ATSDR requests to issue a Certificate of Confidentiality (CoC) under 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

Since the answer to any one of Questions 2-5 is YES, ATSDR determined that a CoC will apply to the research; therefore, in accordance with subsection 301(d) of the Public Health Service Act, ATSDR and any of its cooperative agreement recipients 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 cooperative agreement recipients 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 12**). Recipients 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 cooperative agreement recipients shall inform research participants of the protections and the limits to protections provided by this CoC (**Attachment 7b**). 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 12**).

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 for a longitudinal study. Therefore, stringent data security measures will be in place, including administrative, physical, and technical controls as described below.

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

3.8.3.1 Access Controls and Security

The recipient 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 the recipient 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 to the CDC security office.

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. Informed consent will also be scanned into electronic form and transferred to ATSDR to provide backup in the case of incidental damage to the paper forms.

Upon completion of the project and once the ATSDR has received all approved study related paper documents, the recipient 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 recipient researchers and 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 12**). 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. Site investigators may also use other CDC approved statistical software before converting to SAS. 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; scanned electronic copy will be sent to CDC. 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 multi-site combined data to outside investigators including recipients 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 (**Attachment 7b**).

The 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 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. ATSDR is also committed to investigate the possible confounding between lead and other heavy metal exposures and associations between PFAS and neurobehavioral outcomes in children. Residual from the blood obtained from the child will be stored and available for future analysis. 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 you.

After we complete this study ATSDR or recipients 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,
- 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.

If previous PFAS serum measurements are available for some of the participants (e.g., from a biomonitoring program), then these results will be used to validate the modeled historical PFAS serum estimates.

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; but less well for PFHxS (Gomis 2017). 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 by ATSDR and recipients will explore this approach to estimate serum PFOA, PFOS, PFHxS and PFNA levels and make comparisons with serum levels from the blood specimens obtained in this study (and if available, previous PFAS serum measurements). The recipient may consider the use of a one-compartment PBPK model similar to one used by Shin (2011) and Avanasi (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 the recipient should take those into consideration (Loccisano 2013, Fabrega 2014, 2016; Verner 2015, 2016).

The recipient should attempt to integrate a 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). The recipient can assume the contributions from dietary intake, cookware, cleaning supplies, etc. to be similar to the background US population (Domingo 2012, Christensen 2017). The recipient can also assume that NHANES calendar year-, age- and sex-specific PFAS serum concentrations reflect these background exposures (Calafat 2007, Ye 2017).

All PK models used to estimate historical serum PFAS concentrations will undergo peer review by PBPK modeling and PFAS experts to ensure their applicability to human serum reconstruction. This applies to models that have already been published in the scientific literature, and models produced in-house by the recipients and/or ATSDR.

In order to estimate historical concentrations of PFAS in the drinking water and historical PFAS serum levels, each recipient will follow a general approach to information gathering and modeling. Each recipient should obtain as much information as possible on the source of the PFAS contamination. If the source is environmental emissions from an industrial facility, then the recipient should request information from the facility about these emissions (e.g., periods, locations, frequencies and amounts of emissions, and whether the emissions are to surface water, ground water and/or air). If the source is

AFFF use at a military base, airport or fire training area, then the recipient should seek information on the period and location of use, the annual amount of AFFF used, and any accidental or non-routine use (e.g., to extinguish a major fire, or a major spill) and the date, location and amount used.

Once information on the source is obtained, the recipient should seek information on how the PFAS contamination migrated from the source to the drinking water supply. For example, the recipient should request information on the soil, ground water and/or surface water characteristics in the vicinity of the industrial emissions or AFFF use, as well as the location of drinking water intakes, supply wells (and nearby monitoring wells), and/or private wells serving the study area. If the PFAS contamination migrated from the source via ground water, then the recipient should seek information on the extent of the contamination plume from the state environmental agency, EPA, and/or the industrial facility.

If the contaminated drinking water is from a municipal system, then the characteristics of the distribution system will be obtained from the water purveyor. If supply wells are used, then the recipient will request historical and current information on these wells including monthly or daily production logs and dates of operation. If a surface water source is used or if water is purchased from another purveyor, then the recipient will request information about this source.

The recipient will also request the results of all relevant PFAS sampling: in the surface water near the drinking water intakes, in the distribution system, in the supply wells and nearby monitoring wells, in purchased water from other water purveyors, and in the private wells in the study area.

The recipients will use standard modeling software (e.g. MODFLOW and MT3DMS for groundwater flow, and groundwater fate and transport; and EPANET for distribution system modeling). Each recipient will prepare a report on the historical reconstruction that will be peer reviewed by water modeling and PFAS experts in a process established by the ATSDR/NCEH Office of Science following the CERCLA mandate and the Information Quality Bulletin.

3.10 Statistical Analyses

ATSDR staff will perform statistical analyses with the participation of the recipients using SAS, R and STATA on the combined multi-site study dataset. 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. 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). It must be remembered that for any appreciable confounding to occur, the factor must be a strong risk factor for the outcome under consideration and must also be strongly correlated with the PFAS exposure under evaluation. For unmeasured risk factors, ATSDR proposed the use of negative controls and quantitative bias analyses (see below). These are all standard approaches for evaluating confounding by any risk factor including "co-exposures" by other environmental contaminants.

For example, evaluation of the confounding effects of smoking in occupational studies evaluating a chemical exposure and lung cancer typically observe only moderate confounding (e.g., between 20% and 30%, Blair et al. 2007). This is so even though smoking is an extremely strong risk factor for lung cancer and, at least in earlier occupational studies, typically was at least moderately associated with the chemical exposure or the exposed workforce. None of the diseases and clinical measures or neurobehavioral tests under evaluation in the Multi-site Study have a risk factor remotely as strong as smoking is for lung cancer. Although there are likely to be at least moderate correlations among the PFAS chemicals, confounding of one PFAS chemical by another PFAS chemical should be minor because it is not known that any are strong risk factors for any of the diseases or clinical measures or neurobehavioral tests under the study. (Nevertheless, we will evaluate whether a PFAS chemical confounds an association between another PFAS chemical and a disease or clinical measure by the 10% change-in-the-estimate rule mentioned above.) Moreover, it is very unlikely that any other (i.e., non-PFAS) chemicals or metals will be highly or even moderately correlated with PFAS chemicals. For example, correlations (Pearson correlation coefficient, R) between mercury and PFOA, PFOS, PFHxS and PFNA are consistently <0.20 among children in the NHANES data. In addition, lead and mercury are not very strong risk factors for any disease or clinical measure or neurobehavioral test – i.e., they are considerably weaker risk factors for health outcomes than smoking is for lung cancer.

Primary analyses will focus on estimated cumulative PFAS serum levels. Supplemental analyses will evaluate PFAS serum levels in the 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; sum of PFAS measures may also be considered. Statistical analyses using prevalent cases in a cohort design which takes into consideration the times of diagnosis will also be conducted. 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. 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).

ATSDR will use quantitative methods to assess the impact of possible selection and information bias, as well as possible confounding due to unmeasured risk factors (Lash 2009). In addition, ATSDR will also identify "negative control" diseases with no known association with PFAS exposures to assess the impact of these potential biases (Lipsitch 2010). ATSDR conducted a literature search to identify these negative control diseases and included them in the questionnaire.

In summary, to gauge the potential and magnitude of possible selection bias and information biases, as well as confounding bias due to unmeasured risk factors, 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 multi-site 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 results are 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. The recipient will provide to the 50^{th} and 95^{th} percentiles from NHANES for comparison to the U.S. population (CDC, 2018). 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 recipient will consult with the local and/or state health agency, local community groups, and the National PFAS Contamination Coalition to determine the most effective method of disseminating the results to the participants and the public. If the recipient establishes a community assistance panel (CAP) in the study area, then the CAP will participate in study community outreach and recruitment activities as well as provide advice on effective methods of results dissemination.

The recipient may consider using a user-centered digital interface developed by the Silent Spring Institute for reporting results to each participant. The recipient will present study results to the community in public meetings, printed community handout materials, participating in local radio programs and in informal activities. The recipient also will provide a study website 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 Multi-site 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.

ATSDR will establish working groups to oversee thorough technical evaluation and quality assurance and quality control (QA/QC) for all methods and models in the historical reconstruction of groundwater resources and distribution of drinking water and for all PK/PBPK models used for historical serum reconstruction. These groups will serve multiple functions such as sharing information with ATSDR and

across sites and overseeing quality control. Site visits, and if needed audits of modeling data at each site will be part of those efforts.

The recipients are required to estimate historical PFAS concentrations for both drinking water and serum. The required level of precision will be agreed upon by the site investigators as well as discussion of measurement variability, limits of detections etc. and the criteria for determining the precision of the serum concentration estimates without using the drinking water data. The reciepients' model approaches for the multi-site study will be externally peer-reviewed per the CERCLA mandate and the Information Quality Bulletin.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). While the resulting cohort is a 'survivor cohort', the studies have shown that the only if survival after incidence differs by exposure level can results be biased (Barr 2015) for the non-fatal and even in the case of fatal disease.

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 will provide definitive answers to the community about whether their exposures to the PFAS-contaminated drinking water caused their health problems. The ability of the multi-site study to provide useful information will depend largely on the success of recruiting a sufficient number of study participants and obtaining sufficient information on the PFAS contamination to estimate historical PFAS serum levels with reasonable accuracy.

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7. LIST OF ATTACHMENTS

Attachment 1. Investigators and Key Study Personnel
Attachment 2. Biochemical Analytical Plan in Children and Adults
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