

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

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

November 17, 2021

Agency for Toxic Substances and Disease Registry
National Center for Environmental Health

This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by the Agency for Toxic Substances and Disease Registry. It does not represent and should not be construed to represent an agency determination or policy.

This protocol has been updated to reflect changes necessary for the Multi-site Study during the COVID-19 pandemic. Safety precautions, including the use of all appropriate additional personal protective equipment (PPE), will be implemented to keep the Multi-site Study team and participants safe during the study data and sample collection. The Multi-site Study Manual of Procedures (Attachment 12) has been updated and outlines the additional procedures that will be implemented during recruitment, field work and community meetings to ensure that the Multi-site Study continues in compliance with CDC, state, and local requirements.

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

The activities that will be modified include:

- Holding virtual Community Assistance Panel (CAP) meetings. Small group sessions (less than 10 participants) may be held as needed following applicable local, state and CDC guidelines in place at the time of the meeting.
- Ensuring that social distancing and the use of PPE are employed to comply with CDC and state guidelines during door-to-door recruitment.
- Adding information to the recruitment letter and consent documents to reassure potential Multi-site Study participants that all state and CDC guidelines will be followed.
- Asking participants about their and their family's health/COVID-19-status during their appointment reminder phone call and prior to beginning the testing process.
- Monitoring the temperature of Multi-site Study team members (CDC/ATSDR and local institution staff) twice daily and taking participants' temperatures prior to entering the Multi-site Study Office.
- Offering participants, the option to administer the questionnaire over the phone instead of at the study office to reduce exposure time: consent form administration and collection of biological samples will still have to occur at the study office

These changes are included in modified Multi-site Study protocol Attachments including study screening scripts (Att4), appointment reminder script (Att8) and card (Att7a), consent package (Att7b), appointment tracking form (Att9), and study Manual of Procedures (Att12).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

Table of Contents

1. PROJECT OVERVIEW..... 6

 1.1 Summary 6

 1.1.1 Literature Review..... 6

 1.1.2 Health Study Feasibility Assessment..... 7

 1.1.3 Summary of Study Goals..... 8

 1.2 Study Investigators and Roles 10

2. INTRODUCTION..... 12

 2.1 Authority 12

 2.2 Background 12

 2.3 Selection of Sites 13

 2.4 General Approach for Study Recruitment 16

 2.5 Study Objectives and Study Questions 20

 2.5.1 Literature Review 20

 2.5.1.1 Health Effects in Children 21

 2.5.1.2 Health Effects in Adults..... 22

 2.5.2 Hypotheses..... 24

 2.6 Intended Use of Study Findings 26

3.1 Study Design..... 26

3.2 Study Populations and Eligibility..... 27

 3.2.1 Children..... 27

 3.2.2 Adults 28

3.3 Sample Size Considerations 29

1 3.3.1 Children 29

2 3.3.2 Adults 32

3 3.4 Study Roll Out and Communication Plan 36

4 3.5 Recruitment 37

5 3.5.1 Enrollment Procedures 37

6 3.5.1.1. Waiver of documentation of informed consent 38

7 3.6 Data Collection Procedures..... 38

8 3.6.1 Check-in Procedures 39

9 3.6.2 Informed Consent Process 39

10 3.6.2.1 Consent for Specimens and Data 39

11 3.6.2.2 Child Consent 40

12 3.6.2.3 Adult Consent..... 40

13 3.6.2.4 Risks and Benefits 40

14 3.6.3 Update Contact Information and Medication List 41

15 3.6.4 Body and Clinical Measurements..... 41

16 3.6.5 Questionnaire 43

17 3.6.5.1 Children and Parents..... 43

18 3.6.5.1.1 *Child/Parent Neurobehavioral Assessments*..... 44

19 3.6.5.2 Adults 46

20 3.6.6 Exit Procedures 47

21 3.6.6.1 Gift Cards as a Token of Appreciation for Participation 47

22 3.7 Biochemical Analyses 48

23 3.7.1 Children 49

24 3.7.2 Adults 49

25 3.7.3 Quality Control/Quality Assurance 50

26 3.7.4 Reference Values 51

1 3.8 Data Handling..... 51

2 3.8.1 Certificate of Confidentiality 51

3 3.8.3 Impact on Privacy..... 53

4 3.8.3.1 Access Controls and Security 54

5 3.8.4 Data Delivery..... 56

6 3.8.5 Data Ownership and Data Sharing..... 56

7 3.8.6 Storing Residual Blood for Future Use 57

8 3.8.7 Future Exploratory Analyses 58

9 3.9 Exposure Estimation 58

10 3.10 Statistical Analyses..... 61

11 4. RESULTS REPORTING..... 63

12 4.1 Notification of Individual Results 63

13 4.2 Disseminating Results to the Public..... 64

14 5. STRENGTHS AND LIMITATIONS..... 64

15 6. REFERENCES 67

16 7. LIST OF ATTACHMENTS 78

17 8. APPENDICES 79

18

19

1 **1. PROJECT OVERVIEW**

2 **1.1 Summary**

3 **1.1.1 Literature Review**

4 Per- and polyfluoroalkyl substances (PFAS) are a family of chemicals used in industrial applications and
5 consumer products. A number of PFAS chemicals including perfluorooctane sulfonate (PFOS),
6 perfluorooctanoate (PFOA), and perfluorohexane sulfonate (PFHxS) persist in the environment and have
7 long serum half-lives in humans (Wang 2017). PFAS contamination of drinking water is widespread in the
8 U.S. For example, one report indicated that at least six million residents were served by 66 public water
9 supplies that had at least one sample at or above the US EPA Lifetime Health Advisory for PFOA and PFOS
10 (individually or combined) of 70 ng/L (Hu 2016). Industrial facilities that manufacture or use PFAS have
11 contaminated drinking water in surrounding communities in West Virginia, Ohio, New York, Minnesota,
12 Alabama, Vermont, New Hampshire, and New Jersey (Kray 2018). An alternative method of estimating
13 PFAS drinking water contamination put the number of people potentially exposed to PFAS at
14 concentration over 2.5 ng/L at about 110 million (Environmental Working Group 2018). PFOS, PFOA,
15 PFHxS and other PFAS chemicals are constituents in aqueous film-forming foam (AFFF), used to extinguish
16 flammable liquid fires. Since the 1970s, military bases in the U.S. have used AFFF with PFAS constituents
17 for firefighting training as well as to extinguish fires. At some military bases, AFFF use has resulted in the
18 migration of PFAS chemicals through soils to ground water and/or surface water sources of drinking water
19 for the bases and/or surrounding communities (ATSDR 2017a). The Air Force and Navy have identified at
20 least 24 bases with contaminated drinking water in Alaska, California, Colorado, Delaware, Michigan, New
21 Hampshire, New Jersey, New York, Ohio, Pennsylvania, Virginia, and Washington (Kray 2018).

22 A detailed review of epidemiological studies published up through 2016 was included in the ATSDR
23 Feasibility Assessment for Epidemiological Studies at Pease International Tradeport, Portsmouth, New
24 Hampshire (ATSDR 2017a; released Nov 2017). Health effects of PFAS exposure in children were also
25 recently reviewed by Rapazzo (2017). The scientific evidence linking PFAS exposures with adverse health
26 effects is rapidly growing. Epidemiological studies have found associations with changes in lipids
27 (Steenland 2009; Zeng 2015, Mora 2018), levels of uric acid (Steenland 2010), thyroid and sex hormones
28 (Wen 2013; Lopez-Espinosa 2016, Preston 2018), liver (Darrow 2016, Mora 2018), and immune function
29 (Grandjean 2012, 2017), as well as reduced birth weight (Bach 2015, Verner 2015), reproductive effects
30 (Lopez-Espinosa 2011, Bach 2016) and some cancers (; Barry 2013). However, findings across studies have
31 been inconsistent for a variety of reasons, including differences in exposure levels, methods of

1 ascertaining diseases and the exposure and effect biomarkers measured. For some health endpoints, only
2 one or a few studies currently exist.

3 Most studies of the human health effects from PFAS exposures have focused on PFOA and PFOS. These
4 include studies that evaluated data from the National Health and Nutrition Examination Survey (NHANES),
5 occupational studies, and national surveys conducted in other countries where exposures to PFAS were
6 found mostly from consumption of food and beverages in PFAS-contaminated packaging. Studies of West
7 Virginia and Ohio residents and workers exposed to PFOA from a chemical plant (the “C8” studies) have
8 provided extensive and high quality information on PFOA (and to a lesser extent, PFOS), studying a large
9 cohort of highly exposed residents (60,000+) and workers living in the vicinity of the production facility.
10 However, other PFAS such as PFHxS and PFNA were not a primary focus of the C8 studies. Except for the
11 C8 studies, there is scant information on the health effects of exposures to PFAS-contaminated drinking
12 water.

13 ***1.1.2 Health Study Feasibility Assessment***

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

26 In addition, ATSDR determined that cross-sectional epidemiological studies of children and adults at one
27 site (e.g., at the Pease Tradeport) were feasible for some health endpoints (e.g., lipids, kidney function),
28 but the size of the populations would be insufficient for other important health endpoints (e.g., thyroid,
29 liver and immune function, autoimmune diseases). Therefore, the feasibility assessment concluded that:
30 (1) a multi-site PFAS study of children and adults was necessary, (2) the study should be cross-sectional
31 and involve separate evaluations of children (ages 4-17) and adults (ages ≥18), and (3) the study should

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

6 **1.1.3 Summary of Study Goals**

7 The main goal of the cross-sectional multi-site study is to evaluate potential associations between
8 measured and historically reconstructed serum levels of PFAS including PFOA, PFOS, and PFHxS (see
9 **Section 3.10**), and selected health outcomes as described below and detailed in study hypotheses (see
10 **Section 2.5.2**). The study will attempt to recruit at least 2,100 children and 7,000 adults (equally of both
11 sexes for both children and adults) from communities exposed to PFAS-contaminated drinking water. The
12 criteria for selecting study sites are detailed in **Section 2.3** and include:

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

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

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

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

19 Participants will be categorized based on the measured serum concentration of PFAS compounds or on
20 modeled estimated historical serum levels (e.g., referent or low, medium, high). Estimated and measured
21 PFAS serum levels will also be evaluated as continuous variables. At sites with preceding PFAS
22 biomonitoring, the study will evaluate changes in PFAS concentration over time. The study will reconstruct
23 historic serum PFAS concentrations by estimating half-lives and elimination rates as well as water
24 contamination modeling to inform the pharmacokinetic (PK) or physiologically based pharmacokinetic
25 (PBPK) modeling. Historical serum PFAS reconstruction will enable the evaluation of exposure lags and
26 vulnerable periods as well as statistical analyses that can control for confounding and reverse causation
27 due to physiological factors (Dhingra 2017, Weisskopf 2017).

28 In order to restrict this study to drinking water exposures, adults occupationally exposed to PFAS will not
29 be eligible for the study (e.g., ever firefighters or worked in an industry using PFAS chemicals in its
30 manufacturing process). Likewise, children whose birth mothers were occupationally exposed will not be

1 eligible. Eligible females who are pregnant may enroll. The federal regulations do not allow people who
2 are prisoners or under house arrest to take part in this type of study.

3 Based on ATSDR’s literature review of epidemiological studies of PFAS, the study will examine potential
4 associations between PFAS compounds and lipids, renal function and kidney disease, thyroid hormones
5 and disease, liver function and disease, glycemic parameters and diabetes, as well as immune response
6 and function in both children and adults. In addition, the study will investigate differences in sex hormones
7 and sexual maturation, vaccine response, and neurobehavioral outcomes in children as related to PFAS.
8 In adults, additional outcomes of interest include cardiovascular disease, osteoarthritis and osteoporosis,
9 endometriosis, and autoimmune disease.

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

16 **1.2 Study Investigators and Roles**

17 This cooperative research is being conducted under the ATSDR Notice of Funding Opportunity (NOFO) No.
18 CDC-RFA-TS-19-002, titled “Multi-Site Study of the Health Implications of Exposure to PFAS-Contaminated
19 Drinking Water.” The number of research recipients¹ is seven (**Appendix A**). The program is administered
20 by the CDC Extramural Research Program Office (ERPO).

21 Given that the single IRB mandate under the revised 2018 Common Rule will take effect on January 19,
22 2020, this research program shall be managed under the review of a single IRB for cooperative research.
23 See [§46.114](#) (Cooperative Research).

24 Projects that involve the collection or generation of data with federal funds must develop, submit, and
25 comply with a Data Management Plan (DMP) prior to the collection or generation of public health data,

¹A “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).

1 and, to the extent appropriate, provide public access to and archiving/long-term preservation of collected
2 or generated data.²

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

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

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

15 Serum specimens for PFAS analyses will be submitted to the CDC NCEH DLS, Atlanta, GA. Core clinical and
16 research effect biomarkers will be analyzed by a commercial laboratory as specified in the protocol. Urine
17 specimens will be collected and stored for future analysis and study. ATSDR will conduct data analyses of
18 the combined core data from all the study sites with the recipient participation.

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

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

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

1 and delete PII data after the study is completed. **Appendix A** provides a summary of investigators and
2 their site-specific research plans and a copy of each site’s informed consent form.

3

4 **2. INTRODUCTION**

5 **2.1 Authority**

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

9

10 **2.2 Background**

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

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

26 An example of a community drinking water supply contaminated via the use of AFFF at a military base is
27 the Pease International Tradeport, Portsmouth, New Hampshire. In 2014, a drinking water supply well
28 had measured PFOS, PFOA and PFHxS concentrations of 2.5 µg/L, 0.35 µg/L, and 0.96 µg/L, respectively.

1 The source of the contamination was use of AFFF at the former Pease Air Force Base. In 2015, NH DHHS
2 established a Pease biomonitoring program for PFAS. The program obtained blood specimens for PFAS
3 analyses from 1,578 persons (NH DHHS 2016, Daly 2018). The results from the blood-testing program
4 indicated that the exposed population had serum levels of PFOS and PFHxS that were about two to three
5 times higher than the U.S. population based on data from NHANES 2013-4 and from other epidemiological
6 studies in the U.S. In analyses conducted by NH DHHS (Daly 2018), geometric mean PFHxS serum levels
7 were higher for persons who drank ≥ 4 cups of water per day compared to those who drank < 4 cups per
8 day (4.76 $\mu\text{g/L}$ versus 3.77 $\mu\text{g/L}$). NH DHHS measured 8 to 14 PFAS congeners at 3 analytical laboratories.
9 Among PFOA, PFOS, PFHxS and PFNA concentrations, water consumption had the strongest effect on
10 PFHxS serum levels. In particular, water consumption had the highest effect on PFHxS serum levels among
11 persons aged ≤ 19 years ($\beta = 0.31$, $SE = 0.15$, marginal effect = 36.4%). Geometric mean PFOS and PFOA
12 serum levels were also higher among persons who drank ≥ 4 cups of water per day compared with those
13 who drank < 4 cups per day (NH DHHS 2016, Daly 2018). Linear trends were observed for geometric mean
14 serum levels of PFOS, PFOA, and PFHxS and increasing time spent at the Pease Tradeport. The trend was
15 strongest for PFOS and PFHxS (NH DHHS 2016, Daly 2018).

16 **2.3 Selection of Sites**

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

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

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

- 3 1. For public water systems using ground water sources, enumeration of supply wells that provided
4 drinking water to the site. Information on each supply well should include years of operation, well
5 capacity, and daily or monthly pumping rates. This information can be used to determine the
6 monthly proportion of the total water supply provided by each well during the period when PFAS
7 contamination occurred. Information is also necessary about changes to the water system (e.g.,
8 closure of contaminated supply wells) after the contamination was detected.
- 9 2. For a water system supplied by surface water, characteristics of this source.
- 10 3. For a water system purchasing water from another system, characteristics of this source, the
11 period of time purchased, and daily or monthly amount purchased in order to determine the
12 proportion of the total water supply provided by the purchased water.
- 13 4. Characteristics of the drinking water distribution system. For example, for systems using supply
14 wells, it is important to obtain information on whether mixing from the supply wells occurred at
15 the treatment plant before entering the distribution system or if each supply well served a specific
16 area in the system. If water was purchased from another system, then information on the area
17 of the distribution system served by purchased water is necessary. For systems in which PFAS
18 concentrations throughout the distribution system cannot be assumed to be similar (e.g., if all
19 water is not mixed at the treatment plant before distribution), then It may be necessary to obtain
20 sufficient information on the distribution system (e.g., pipe network, elevation and water demand
21 at each node, pipe length and diameter, etc.) so that preliminary modeling using software such
22 as EPANET can be used to estimate PFAS concentrations at various areas in the distribution
23 system.
- 24 5. Description of when and how PFAS samples from monitoring or supply wells (or surface water)
25 were obtained, the location of the wells, and the measured concentrations of PFAS including
26 description of analytical methods used by the laboratory.
- 27 6. If the distribution system was sampled, which PFAS were detected, when, and the measured
28 levels of concentration.
- 29 7. For sites involving private well contamination, the number and locations of the wells, periods of
30 operation, any information on the source of contamination and the PFAS groundwater plume,
31 and the dates of PFAS sampling and the measured concentrations.
- 32 8. Any information on the historical use of AFFF (e.g., amount purchased/used, location and
33 frequency of training exercises, fire incidents, spills, etc.) at the site or in the vicinity of the site

1 (e.g., military base airstrip) which was the source of the drinking water contamination. Any
2 information on the soil and ground water characteristics in the vicinity of AFFF use. Any
3 information on the groundwater PFAS plume.

4 9. If previous human PFAS biomonitoring program was conducted, the PFAS serum results, dates of
5 blood or urine collection, and possible descriptive/predictive factors of the serum concentrations
6 (e.g. volume of water consumed, length of residence at site, differences in age, race, or other
7 population characteristics).

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

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

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

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

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

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

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

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

3 •University of California – Irvine to look at exposures in communities near the UC Irvine Medical Center
4

5 **2.4 General Approach for Study Recruitment**

6 In considering possible study designs, ATSDR focused on the methods used in previous epidemiological
7 research of PFAS exposures (ATSDR 2017a). Adopting study design methods consistent with previous
8 research facilitates the interpretation and synthesis of findings across studies. Most of the epidemiological
9 studies of PFAS exposures were cross-sectional and evaluated serum PFAS measurements. Some studies
10 also evaluated cumulative PFAS serum levels estimated from historical reconstruction models. ATSDR
11 concluded that the multi-site study should be cross-sectional and evaluate measured serum PFAS
12 measurements as well as historically reconstructed estimates of cumulative PFAS serum levels. ATSDR
13 also concluded that methods used to evaluate health-related endpoints in the study should be consistent
14 with methods used in previous epidemiological research of PFAS exposures, given adequate sample size
15 and power. In the future, the follow up to the cross-sectional studies of health-related outcomes proposed
16 to be studied in the longitudinal studies.

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

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

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

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

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

28 In response to Notice of Funding Opportunity and following guidelines of the draft multi-site study
29 protocol, recipients developed detailed recruitment protocols specific for each site. Those were reviewed
30 by external peer review and approved by ATSDR when awarding the cooperative agreement grants. Non-
31 random approaches were made available to site investigators, because association/etiologic studies (as

1 opposed to descriptive studies like NHANES, for which estimation is targeted at individual variables rather
2 than association parameters), selection bias results only when study participation is affected by both the
3 exposure status and disease status (Hernan et al., 2004). The multi-site study is aimed at measuring
4 exposure-disease associations, rather than estimating community-wide disease rates. Thus, non-random
5 participation is only a concern if the two conditions for selection bias are met.

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

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

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

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

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

1 The primary issue in combining data from different sites is the sufficient comparability of the data in
2 respect to conceptual framework and overall objectives of the study (Bangdiwala et al., 2018).
3 Comparability will be ensured in this multi-site study by the implementation of common protocol that
4 requires the same application of: a) eligibility criteria and characteristics; b) computer assisted interviews
5 in study office (RedCap), c) outcomes of interest; d) sample collections/processing/storage procedures; e)
6 timelines of implementation per funding mechanism; f) centralized laboratories for exposure; effect
7 biomarker and clinical tests; g) data quality assurance and management through unified contract
8 mechanism; and h) shared tools for staff training.

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

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

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

1 **2.5 Study Objectives and Study Questions**

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

5 **2.5.1 Literature Review**

6 A literature review was conducted for the Pease feasibility assessment and can be accessed in the final
7 feasibility report (ATSDR 2017a). The literature review from the Pease feasibility assessment concluded
8 that most information on potential health effects concerned exposures to PFOA. In particular, numerous
9 studies have been conducted of West Virginia and Ohio residents and workers exposed to PFOA from a
10 chemical plant via contaminated drinking water and occupationally, respectively (the “C8” studies)
11 (Frisbee 2009). Studies of other workforces also focused primarily on PFOA exposures. The literature
12 review found that less information was available about the potential health effects of PFOS exposures,
13 and little information was available on the potential health effects of exposures to PFHxS. PFHxS and
14 PFOS are often major contaminants in drinking water impacted by AFFF. Except for the C8 studies, there
15 is scant information on the health effects of exposures to PFAS-contaminated drinking water.

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

20 The literature review found that most of the epidemiological studies of PFAS exposures were cross-
21 sectional and evaluated serum PFAS measurements. Some studies also evaluated cumulative PFAS
22 serum levels estimated from modeling methods. ATSDR concluded that studies of populations exposed
23 to the PFAS-contaminated drinking water should be initially be cross-sectional to be comparable with
24 other studies and to establish a baseline for potential follow-up longitudinal studies. Studies should also
25 evaluate measured serum PFAS measurements as well as estimated cumulative PFAS serum levels and
26 use methods for the evaluation of health-related endpoints that are consistent with methods used in
27 previous epidemiological research of PFAS exposures.

1 2.5.1.1 Health Effects in Children

2 There is some evidence that PFAS exposures are associated with decreased birth weight, small birth size
3 for gestational age, measures of intrauterine growth retardation, and preterm birth. In particular, several
4 meta-analyses have found an overall decrease in birthweight associated with PFOA and PFOS (Johnson
5 2014, Negri 2017, Verner 2015; Bach 2015). However, the findings across studies are inconsistent for
6 adverse birth outcomes, and few studies have evaluated PFHxS. Several studies of infants have found that
7 prenatal PFAS exposures affect thyroid function, but only two studies have evaluated thyroid function in
8 older children (Lopez-Espinosa 2012; Lin 2013, Preston 2018).

9 A few studies of children have found elevated uric acid with PFAS exposures, but the possibility of reverse
10 causation exists (Geigere 2013; Kataria 2015; Qin 2016). Positive findings occurred in some of the four
11 studies of PFAS exposures and testosterone and other sex hormones, but the findings were not consistent
12 across studies and further research is necessary (Maisonet 2015; Lopez Espinosa 2016, Zhou 2016).
13 Growing evidence suggests that exposure to per- and polyfluoroalkyl substances (PFASs) may disrupt lipid
14 homeostasis and liver function, but data in children are limited. Indicators of adiposity and glucose
15 metabolism were also linked with PFAS in a large follow up study of children and adolescents (Domazet
16 2016). Recent study (Mora, 2018) suggests that prenatal and mid-childhood PFAS exposure may be
17 associated with modest, but somewhat conflicting changes in the lipid profile and ALT levels in children.

18 There is some evidence from four studies that PFAS exposures might be associated with attention deficit
19 hyperactivity disorder (ADHD), but findings have not been consistent across studies (Stein 2011; Liew
20 2015; Ode 2014; Hoffman 2010). In the Stein (2011) study, the ORs for ADHD and PFOS and PFHxS were
21 1.3 and 1.6, so there was some evidence of an increased risk, although not strong. A study using NHANES
22 data obtained an OR of 1.6 for PFOS and ADHD (Hoffman 2010). Other studies have found conduct and
23 coordination problems associated with PFOS (Fei 2011) and executive function deficits with PFOS and
24 PFHxS (Vuong 2016). Evaluating the evidence for PFAS exposures and neurobehavioral outcomes is
25 difficult for several reasons: 1) the studies used different methods to measure the outcomes, 2) studies
26 are inconsistent in the outcomes evaluated, and 3) too few studies exist. For example, there is little
27 evidence that PFAS affects IQ, primarily because only two studies evaluated it; one in Taiwan, which
28 observed deficits (Lien 2016), and one at C8 which did not (Stein 2011). We believe it is worth evaluating
29 whether the PFAS mixture at individual sites with contamination due to AFFF use is associated with IQ
30 deficits or other neurobehavioral outcomes. A few studies have found associations between PFAS

1 exposures and a decline in antibody response to specific vaccines (Grandjean 2012, 2016), but only two
2 studies evaluated the same vaccine (i.e., rubella; Granum 2013, Stein 2016).

3 In summary, there are considerable data gaps concerning the health effects in children of PFAS exposures.
4 This is because of the small number of studies conducted, inconsistencies in methods and findings across
5 studies, and limited sample sizes in some studies. As for other adverse outcomes, few studies have
6 evaluated the effects on children of PFHxS exposures. A recent systematic review of PFAS studies of
7 children concluded that there was "...generally consistent evidence for PFAS' association with
8 dyslipidemia, immunity including vaccine response and asthma, renal function, and age at menarche"
9 (Rappazzo 2017). The review noted the limited number of studies for any one particular health outcome,
10 the variability in outcome measurement, and the need for longitudinal studies.

11

12 2.5.1.2 Health Effects in Adults

13 Based on its detailed assessment of the epidemiological literature, ATSDR concluded that there was
14 limited information concerning associations with PFAS exposures and most cancers and other adult
15 diseases (ATSDR 2017a).

16 Epidemiologic studies of subjects exposed to PFOA and PFOS at background levels and at occupational
17 settings have reported positive associations with number of health outcomes and conditions. Lipid and
18 cholesterol concentrations were associated with increased PFOA or PFOS (Frisbee 2010; Nelson 2010;
19 Fletcher 2011; Steenland 2015), as were increased uric acid levels (Costa et al., 2009; Steenland 2010;
20 Shankar 2011; Geiger 2013; Gleason 2015), concentrations of thyroid and sex hormones (Olsen and Zobel
21 2007; Knox 2011; Jain 2013; Wen 2013; Winqvist and Steenland 2014), immune parameters
22 (Dalsager 2016), and reproductive effects (Joensen 2013; Kristensen 2013; Crawford 2017).

23 Associations with liver enzymes were found with PFAS in most cross-sectional studies (Olsen 2000;
24 Sakr 2007; Lin 2010; Gallo 2012; Gleason 2015) but were weaker or found no association in the cohort
25 studies of liver enzymes (Sakr 2007b, Darrow 2016). Structural protein cytokeratin 18 (CK-18) and its
26 components have been used as a new non-invasive serum biomarker for non-alcoholic fatty liver disease
27 and suspected steatohepatitis for adults and children (Fieldstein 2013, Shen 2012, Vos 2008). Prevalent
28 coronary heart disease was positively associated in a cross-sectional examination of NHANES (Shankar
29 2012) but not in cohort designs (Winqvist 2014b; Mattsson 2015).

30 Two studies of osteoarthritis show association with PFOA in cross sectional analyses (Innes 2011, Uhl
31 2013) but no association in longitudinal analyses (C8 Science Panel 2012a). Another cross-sectional

1 NHANES study (Khalil 2016) found an association with osteoporosis among women for PFHxS. Two
2 NHANES studies (Lin 2014, Khalil 2016) also found associations with bone mineral density. Although,
3 these studies are cross-sectional, they provide important evidence for a link between PFAS exposures and
4 osteoarthritis and osteoporosis unless there is evidence that confounding or reverse causation can explain
5 these results.

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

10 There is increasing evidence showing associations between PFAS and markers of glucose homeostasis and
11 insulin resistance, and associations with adult type 2 diabetes risk in men and women (Cardenas 2017; He
12 2018; Sun 2018); strengthening the case for adverse metabolic activity of these compounds.

13 Roles of inflammatory cytokines and adipokines have been explored several studies of liver disease such
14 as non-alcoholic fatty liver disease/steatohepatitis and in atherosclerosis (Hennig 2007, Wahlang 2016,
15 Clair 2018). Proinflammatory responses, alteration in leptin signaling, and increases in TNF-alpha and IL-
16 2 were reported in mechanistic studies with various persistent organohalogen pollutants in relation to
17 diabetes and metabolic syndrome (Ferrante 2014; Wieser 2013). These associations have not yet been
18 explored specifically with PFAS compounds.

19 Some positive associations have also been found for cancer outcomes; with C8 studies finding strong
20 associations for liver, kidney, and testicular cancer (Alexander and Olsen 2007; Barry2013; Bonefeld-
21 Jorgensen2014; Hardell2014; Steenland2015).

22 Some studies have found no association between PFAS exposure and health effects such as specific
23 cancers (Alexander and Olsen 2007; Lundin 2009), lipids or metabolic function (Fisher, 2013). Effects of
24 confounding, bias, and chance on observed associations with PFAS compounds were explored in reviews
25 of immune and cancer outcomes (Chang 2014, Chang 2015) and in studies of PFAS and menopause and
26 endometriosis (Dhingra 2017, Ruark 2017, Ngueta 2017).

27 Few studies have evaluated PFHxS exposures and the risk of cancers and other adult diseases. Although
28 epidemiological studies have primarily evaluated PFOA and PFOS, there remain considerable data gaps
29 concerning the health effects of exposures to these chemicals in adults. There have been inconsistencies
30 in findings across studies and limited sample sizes in some studies. For some adverse outcomes, only one

1 or a few studies have been conducted. Finally, except for the C8 studies, there are no published individual-
2 level epidemiological studies in adults that have evaluated the health effects from exposures to PFAS-
3 contaminated drinking water. Therefore, additional research is necessary to determine whether drinking
4 water exposures to PFHxS, PFOS, and PFOA increase the risk of non-cancer diseases. The proposed scope
5 of the funding and sample size estimated for this health study would be too small and insufficient to
6 evaluate cancer health outcomes.

7 **2.5.2 Hypotheses**

8 For children (aged 4-17 years), the Multi-site Study will evaluate the following main hypotheses, following
9 the outline of the biochemical analytical plan (**Attachment 2**):

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

- 11 1. Lipids (higher total cholesterol, low-density lipoprotein, and triglycerides, and higher prevalence
12 of hypercholesterolemia; obesity).
- 13 2. Impaired renal function (a higher level of uric acid, a higher prevalence of hyperuricemia, and a
14 lower estimated glomerular filtration rate (eGFR).
- 15 3. Liver function/damage biomarkers (alanine transaminase (ALT), aspartate aminotransferase
16 (AST), alkaline phosphatase (ALP), γ -glutamyltransferase (GGT), albumin, direct bilirubin,
17 cytokeratin-18 (CK-18)), and non-alcoholic fatty liver disease/steatohepatitis (determined by CK-
18 18 levels).
- 19 4. Glycemic parameters (glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-
20 65 and IA-2), C-peptide, pro-insulin; and diabetes (type 1 and 2).
- 21 5. Measures of thyroid function (differences in thyroid stimulating hormone - TSH, total thyroxin -
22 TT4, free T4, and total triiodothyronine (TT3); thyroglobulin antibody, thyroid peroxidase
23 antibodies (TPO); higher prevalence of hypothyroidism/hyperthyroidism).
- 24 6. Differences in sex hormones, growth and sexual maturation (testosterone, estradiol, and sex
25 hormone-binding globulin (SHBG); insulin-like growth factor - 1 (IGF-1), age at menarche, delayed
26 puberty).
- 27 7. Immune response including prevalence of hypersensitivity-related outcomes (e.g., asthma, atopic
28 dermatitis; higher levels of immunoglobulins (IgG, IgA, IgE, and IgM) and lower antibody
29 responses to rubella, mumps, and diphtheria vaccines).

1 8. Neurodevelopmental outcomes (lower intelligence quotient (full scale IQ), attention-deficit and
2 hyperactivity disorder [ADHD]).

3
4 For adults (aged ≥ 18 years), the Multi-site Study will evaluate the following main hypotheses.

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

- 6 1. Lipids (higher total cholesterol, low-density lipoprotein and triglycerides) and a higher prevalence
7 of hypercholesterolemia).
- 8 2. Higher prevalence of coronary artery disease and hypertension (including hypertensive disorders
9 of pregnancy).
- 10 3. Renal function (higher level of uric acid and a higher prevalence of hyperuricemia, lower
11 estimated glomerular filtration rate (eGFR)) and higher prevalence of kidney disease.
- 12 4. Glycemic parameters (glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-
13 65 and IA-2), C-peptide, pro-insulin) and diabetes (type 1 and 2).
- 14 5. Differences in thyroid hormones (thyroid stimulating hormone (TSH), TT4, free T4, and TT3,
15 thyroglobulin antibody, thyroid peroxidase antibodies (TPO); and higher prevalence of
16 hypothyroidism/hyperthyroidism.
- 17 6. Liver function/damage biomarkers (e.g. alanine transaminase (ALT), aspartate aminotransferase
18 (AST), alkaline phosphatase (ALP), γ -glutamyltransferase (GGT), albumin, direct bilirubin,
19 cytokeratin-18 (CK-18)) and liver disease.
- 20 7. Higher prevalence of osteoarthritis
- 21 8. Higher prevalence of osteoporosis.
- 22 9. Higher prevalence of endometriosis.
- 23 10. Measures of immune response and inflammation (serum levels of IgA, IgE, IgG, IgM, C - reactive
24 protein (CRP), rheumatoid factor, antinuclear antibodies (ANA), inflammatory cytokines and
25 adipokines (interleukin 1- β (IL-1 β), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 8 (IL-8),
26 interleukin 12 (IL-12), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor α (TNF α),
27 leptin, adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1).

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

3
4 **2.6 Intended Use of Study Findings**

5 Given that epidemiological research on the health effects of drinking water exposures to PFAS other than
6 PFOA is at an early stage, the Multi-site Study should make an important contribution to the scientific
7 literature, expand knowledge in this field, and help addressing concerns about past exposure.

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

15

16 **3. METHODS**

17 **3.1 Study Design**

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

- 21 • The recipient will obtain adult consent and parental permission (ages 4-17) and child assent (ages
22 7 -17), to participate in this research study (including consent to be contacted for any future
23 studies).
- 24 • The recipient will administer adult and child questionnaires and seek medical records verification
25 of self-reported diseases and medical histories (including neurobehavioral diseases).
- 26 • The recipient will administer neurobehavioral test batteries to the children and their parents and
27 seek to abstract children’s school records, in particular, special education records.

- 1 • The recipient will obtain blood samples from each participant for analyses of PFAS and a number
2 of effect biomarkers.
- 3 • As part of the current protocol, both children and adults will be asked to provide a urine sample
4 for future analyses of PFAS and relevant effect biomarkers. The recipient will ship the urine
5 samples to CDC biorepository for analysis at a later time when more knowledge is gained about
6 urinary PFAS and effect biomarkers and until the laboratory methods are developed.
- 7 • The recipient will seek consent to store residual blood and urine samples for future analyses of
8 other PFAS and/or relevant effect biomarkers yet to be identified.

9 **3.2 Study Populations and Eligibility**

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

21 Firefighters and others with occupational PFAS exposure from sources other than the drinking water will
22 not be included in the study. In addition, children whose birth mothers had occupational exposures to
23 PFAS from sources other than drinking water will be excluded. The goal is to enroll at least 2,100 children
24 (ages 4-17) and 7,000 adults aged ≥ 18 years with drinking water exposure to PFAS.

25 **3.2.1 Children**

26 The eligibility criteria for children is as follows:

- 27 1. Aged 4 – 17 years at the start of the study,

- 1 2. Resided in areas with documented past or present PFAS drinking water concentrations at the tap,
2 or were exposed in utero or during breastfeeding when the mother consumed the contaminated
3 drinking water,
- 4 3. Drinking water exposure occurred within 15 years of the start of the study.
- 5 4. Children will be excluded if their birth mothers were ever employed as a firefighter, ever
6 participated in fire training exercises using AFFF foam, or were ever employed at industrial
7 facilities that used PFAS chemicals in the manufacturing process.

8 The requirement that the child's last exposure be within 15 years of the start of the study takes into
9 account the half-lives of about 3 years for PFOA and PFOS, and about 5 years for PFHxS, observed in a
10 recent study of drinking water exposures caused by AFFF use at a military facility in Sweden (Li 2017).
11 Slightly longer half-lives for individual PFAS (5 to 8 years) were derived in the draft ATSDR toxicological
12 profile (ATSDR 2018). Based on these half-lives, those last exposed more than 15 years ago will have
13 greatly diminished current serum levels of these PFAS chemicals, making the use of these serum
14 measurements to predict past exposures more problematic.

15 The age range for the child study (4-17 years) was determined by taking into account the age ranges in
16 previous PFAS studies and the age range appropriate for the candidate endpoints. The study will limit
17 inclusion to those ≥ 4 years of age because most of the neurobehavioral tests that will be used in the study
18 are appropriate for children aged ≥ 4 years of age.

19 **3.2.2 Adults**

20 The eligibility criteria for adults is as follows:

- 21 1. Aged ≥ 18 years at the start of the study.
- 22 2. Resided in areas with documented past or present PFAS drinking water concentrations at
23 the tap,
- 24 3. Drinking water exposure occurred within 15 years of the start of the study.
- 25 4. Persons ever employed as a firefighter, ever participated in fire training exercises using AFFF
26 foam, or ever employed at industrial facilities that used PFAS chemicals in the manufacturing
27 process will be excluded.

1 **3.3 Sample Size Considerations**

2 The Pease feasibility assessment included sample size calculations for a wide range of health-related
3 outcomes (ATSDR 2017a). Sample size calculations selected a type 1 (“ α error”) of .05 and type 2 error
4 (“ β error”) of .20. The tables present sample sizes per stratum for specific outcomes for children (Table
5 1) and for adults (Table 2). To determine effect sizes that are reasonable to detect, we selected
6 epidemiological studies using NHANES data. For those outcomes not included in NHANES studies, the C8
7 studies were used. The C8 results were considered more representative of U.S. populations (e.g., in
8 background disease rates and prevalence of non-PFAS risk factors) than studies conducted in other
9 countries, although the PFOS, and especially the PFOA, serum levels in the C8 studies were higher than
10 might occur at other sites. For outcomes not evaluated by NHANES or C8 studies, it was necessary to use
11 studies conducted in other countries. The total sample sizes for children and adults should allow for the
12 categorization of PFAS serum levels (or cumulative PFAS serum levels) into e.g. quartiles of exposure:
13 reference level, low, medium and high.

14 **Attachment 3** includes additional information and assumptions pertinent to selected health outcomes to
15 be studied.

16 **3.3.1 Children**

17 For children, **Table 1** (and **Attachment 3a**) provide the sample size calculations for several health
18 outcomes of interest assuming a type 1 (“ α error”) of .05 and type 2 error (“ β error”) of .20. It was
19 considered important that a study have a total sample size so that exposures could be categorized into
20 tertiles (i.e., reference, medium, and high) or preferably into quartiles (i.e., reference, low, medium and
21 high). Per stratum estimates of needed sample size have been calculated based on different prevalence
22 of outcomes and detected odds ratios or mean difference.

23 The proposed minimum sample size of 2,100 children (equally of both sexes) is large enough to effectively
24 evaluate many of the health outcomes identified in the Pease Feasibility Assessment literature review and
25 the recent systematic review (Rapazzo 2017) as potentially associated with PFAS in children. The health
26 outcomes and biomarkers studied would include mean difference in total cholesterol (ranging from 156
27 to 637 per stratum), uric acid levels (556 per stratum), estimated glomerular filtration rate (eGFR; 275 per
28 stratum), testosterone (about 400 per stratum) and insulin growth factor-1 (IGF-1; 146 per stratum).
29 Based on our estimations, we would also be able to detect differences in risk for obesity and atopic
30 dermatitis. A sample size of 2,100 children would be larger than many of the PFAS studies that evaluated

1 neurobehavioral outcomes such as IQ and ADHD (Wang 2015, Stein 2013, 2014, Fei 2011, Hoffman 2010,
2 Strom 2014).

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

7 In summary, a total sample size of $\geq 2,100$ would be sufficient to evaluate a wide range of biomarkers and
8 outcomes including lipids (and hypercholesterolemia), uric acid (and hyperuricemia), estimated
9 glomerular filtration rate, testosterone, IGF-1, neurobehavioral measures (executive function, attention,
10 IQ) and ADHD, rhinitis, and obesity. Each cooperative agreement recipient will attempt to meet a target
11 recruitment of 300 children.

12 **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)	Frisbee 2010, C8 Study 1,971 boys <12 yrs 2,773 boys 12-18 yrs 1,886 girls <12 yrs 2,520 girls 12-18 yrs	PFOS: 5 th vs 1 st quintile Age: <12 yrs 12-18 Boys: +6.2 +9.3 Girls: +4.6 +9.4	Mean PFOS serum levels were about 20 μ g/L. SD for total cholesterol=29.3 mg/dL	+4.6: 637/stratum +9.3: 156/stratum
High cholesterol		OR = 1.6	Prevalence=34.2%	300/stratum
Thyroid function TT ₄	Lopez-Espinosa 2012, C8 1,078 1-5 yrs 3,132 6-10 yrs 6,447 >10 – 17 yrs	PFOS, 4 th vs 1 quartile: 2.3% change (mean difference = 0.17 μ g/dL)	Mean PFOS serum levels were about 20 μ g/L. SD for TT ₄ as estimated at 1.4. Percent change in TT ₄ was converted to mean difference assuming the	1,080/stratum

Thyroid disease		PFOA: OR=1.44 (PFOS: OR < 1.0)	median TT ₄ was ref. level. Prevalence=0.6% (used PFOA results)	>16,000/stratum
Uric Acid	Kataria 2015, NHANES 1,960; 12-18 yrs	PFOS: 4 th vs 1 st 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: 4 th vs 1 st quartile, OR=1.65	Mean PFOS serum level =16.6. Prevalence=16%	400/stratum
eGFR	Kataria 2015 1,960; 12-18 yrs	PFOA mean serum level =3.5 µg/L. mean difference= -6.6	Standard deviation=27.6	275/stratum
Testosterone	Lopez-Espinosa 2016, C8 1,169 boys; 6-9 yrs 1,123 girls; 6-9 yrs	PFOS (IQR): -5.8% boys (diff=1.9) -6.6% girls (diff=2.45)	Percent change was converted to mean difference assuming median testosterone level was ref. level. SD estimated at 11.85 for girls and 9.63 for boys.	Boys: 404/stratum Girls: 368/stratum
IGF-1 (Insulin-like growth factor – 1)	Lopez-Espinosa 2016, C8	PFHxS (IQR): Boys: -2.5% (diff=17.3) Girls: -2.1%	Percent change was converted to mean difference assuming median IGF-1 in boys as ref. level. SD estimated as 52.6	146/stratum
Delayed Puberty	Lopez-Espinosa 2011. C8 3,072 boys, 8-18 yrs 2,903 girls, 8-18 yrs	PFOS: mean serum level was about 19 µg/L.	OR for delayed puberty and the number of days delayed puberty had narrow CIs	Insufficient information to calculate sample size, but sample sizes in this study were enough for sufficient precision.
ADHD	Stein 2011, C8 10,546; aged 5-18 yrs.	PFHxS mean serum level was 5.2 µg/L. 4 th vs 1 st quartile,	Prevalence:	

		OR=1.5	ADHD Dx: 12.4%	764/stratum
Asthma	Stein 2016, NHANES 640; 12-19 yrs	PFOA mean serum level = 3.6 µg/L. OR=1.2	Prevalence = 11%	2,400/stratum
Atopic dermatitis	Wang 2011 (Taiwan) 244; infants, 2 yrs	PFOS mean serum level=5.5 µg/L., 4 th 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.

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

5 **3.3.2 Adults**

6 For adults, **Table 2** (and **Attachment 3b**) provide the sample size calculations for several health outcomes
7 of interest assuming a type 1 (“α error”) of .05 and type 2 error (“β error) of .20. In this exposure-based
8 study, we assume an appropriate coverage of range of exposures that will enable
9 stratification/categorization to tertiles or quartiles of exposure. Per stratum estimates of needed sample
10 size (e.g. first vs. fourth quartile) have been calculated based on different measures of association such as
11 odds ratios or detected mean difference.

12 The proposed minimum sample size of 7,000 adults (equally of both sexes) is large enough to effectively
13 evaluate many of the health outcomes identified in the Pease Feasibility Assessment literature review.
14 For example, for outcomes like elevated lipids levels (cholesterol) or uric acid, the range of 229 to 660
15 participants per stratum (i.e. quartile) or 200 to 550 per stratum, respectively, given observed differences
16 would be needed. That would translate to overall sample size of about 800 to 2,600 participants being
17 sufficient to detect differences at the specified level of precision and power (Steenland, 2009, 2010; Fisher
18 2013; Shankar 2011). Similar sample sizes would also be required to compare other common health
19 outcomes such as cardiovascular disease (Shankar 2012). Larger samples sizes would be needed for liver
20 function or osteoarthritis, with a total sample in the range of 3,000 to 4,000 subjects (Uhl 2013; Gallo
21 2012; Steenland 2010).

22 For thyroid disease and thyroid function, a total sample size of 7,000 may be sufficient although probably
23 not optimal. However, NHANES studies of thyroid function and thyroid disease obtained statistically

1 significant findings with total sample sizes considerably less than 7,000 (Melzer 2010; Wen 2013).
 2 NHANES studies of liver function also obtained statistically significant findings with total sample sizes
 3 considerably less than 7,000 (Gleason 2015; n=4333). For biomarkers of immune function (e.g.,
 4 immunoglobulins, C-reactive protein and cytokines) and fatty liver disease, there was insufficient
 5 information to calculate sample sizes. However, a total sample size of 7,000 should be sufficient to
 6 evaluate these biomarkers as we assumed similar endpoint differences of those outcomes.

7 For ulcerative colitis, a sample size of 7,000 might be sufficient if the effect size in the C8 study (i.e.,
 8 OR=3.05) was consistent for PFOA serum levels considerably lower than those in the C8 study. For more
 9 modest effect sizes (e.g., ORs < 2.75), a total sample size of 7,000 would not be adequate to evaluate
 10 associations with ulcerative colitis.

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

15 In summary, a total sample size of $\geq 7,000$ in multi-site study should be sufficient to evaluate a broad range
 16 of biomarkers and outcomes such as lipids (and hypercholesterolemia), uric acid (and hyperuricemia),
 17 cardiovascular disease, osteoarthritis, immune biomarkers and biomarkers for fatty liver disease. It also
 18 may be sufficient to evaluate thyroid disease, thyroid function, and liver function. Each cooperative
 19 agreement recipient will attempt to meet a target recruitment of 1,000 adults.

20

21 **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 $\mu\text{g/L}$, 10 th vs 1 st decile: +11 mg/dL	SD=41.9	228/stratum
High cholesterol			Prevalence=15%	660/stratum

		4 th vs 1 st quartile, OR=1.51		
High Cholesterol	Fisher 2013, Canada	PFHxS, mean serum level = 2.2 µg/L, 4 th vs 1 st 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, 4 th vs 1 st 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, 10 th vs 1 st decile: +0.22 mg/dL Hyperuricemia, 5 th vs 1 st quintile: OR=1.26	SD=1.55 Prevalence:24%	780/stratum 1,525/stratum
Uric Acid	Shankar 2011, NHANES 3,883 aged ≥20 yrs	PFOA mean serum level = 3.5 µg/L, 4 th vs 1 st quartile: +0.44 mg/dL Hyperuricemia, 4 th vs 1 st quartile: OR=1.97 PFOS mean serum level = 17.9 µg/L Hyperuricemia, 4 th vs 1 st 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	SD=1.47	1,958/stratum

		µg/L. This would approximately correspond to a mean difference in ALT of +1.8 µIU/mL		
Liver function Elevated ALT	Gleason 2015, NHANES 4,333 aged ≥12 yrs	PFHxS mean serum level = 1.8 µg/L. 4 th vs 1 st 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, 4 th vs 1 st quartile: Thyroid disease ever: Women, OR=1.64 Men, OR=1.58 Thyroid disease with current meds Women, OR=1.86 Men, OR=1.89	Prevalences: 16.18% 3.06% 9.89% 1.88%	410/stratum 2,035/stratum 365/stratum 1,575/stratum
Subclinical hypothyroidism	Wen 2013, NHANES 672 males aged ≥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, 4 th vs 1 st quartile: OR=1.55 PFOS mean serum level = 24.6 µg/L, 4 th vs 1 st 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

1 For rare health outcomes such as ulcerative colitis, other autoimmune diseases, or cancer the sample size of
2 7,000 adults is too small to detect reasonably expected increases in the ORs.

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

8 **Attachment 3** provides further information and details on the derivation of the sample size calculations
9 for Table 2 and also estimates of detectable mean difference and odds ratios for selected clinical tests and
10 health outcomes.

11 **3.4 Study Roll Out and Communication Plan**

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

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

1 **3.5 Recruitment**

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

11 For sites with contaminated private wells, the recipient will request information on the impacted
12 residences and the results of PFAS sampling of their private wells from the state and/or local health and
13 environmental agencies (Attachment 3d). Sampling will target households based on the magnitude of the
14 PFAS concentrations in their private wells – i.e., wells with higher concentrations will be oversampled – in
15 order to ensure a sufficiently wide range of PFAS serum levels to evaluate exposure-response trends
16 effectively.

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

21 Sampled households may have more than one eligible adult and/or child, and some parents may want to
22 enroll in both of the adult and child studies. Trained study staff will use the recruitment tracking form
23 (**Attachment 6**) to track recruitment success and to calculate non-response bias.

24 **3.5.1 Enrollment Procedures**

25 Once potential recruits express interest and are screened for eligibility, study staff will schedule
26 appointments for them at the central study office. The study staff will establish a toll-free telephone line
27 for interested recruits to schedule appointments at their convenience.

1 3.5.1.1. Waiver of documentation of informed consent

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

7 After the eligibility is determined, study staff will mail an Appointment Packet (containing an Appointment
8 Reminder Card (**Attachment 7a**), the Informed Consent materials (**Attachment 7b**), a Study Fact Sheet
9 (**Attachment 7c**) with a description to arrive fasting, and to bring medications and a urine sample to the
10 appointment. Interested recruits will be mailed urine collection supplies. They will be instructed to collect
11 a first-morning voided urine sample on the day of their appointment. An advance copy of the Informed
12 Consent Form will provide an extra opportunity for the interested recruit to read and more fully
13 understand his or her rights in the study and to ask any questions before the scheduled appointment.

14 Study staff will give the interested recruit a reminder telephone call and send a text one to two days
15 before the scheduled appointment (Attachment 8). The study protocol will provide the flexibility to
16 schedule or re-schedule office or questionnaire appointments. Home visits will not be conducted due to
17 COVID-19 precautions. The study staff will make up to five contact attempts to an interested recruit who
18 misses an appointment in order to reschedule the appointment and maximize the number of completed
19 appointments (Attachment 9).

20 **3.6 Data Collection Procedures**

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

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

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

9 **3.6.1 Check-in Procedures**

10 Trained study staff will document the completion of each step from check-in to the provision of gift cards
11 on a hard copy form (**Attachment 9**). This hardcopy form will be stored with the participant's signed
12 Informed Consent Form (**Attachment 7b**) in locked files and in secure rooms. Staff will securely ship all
13 files to ATSDR at the end of data collection. All files and biological samples will be securely stored at the
14 study office prior to shipment.

15 **3.6.2 Informed Consent Process**

16 The informed consent includes a description of study procedures and risks and benefits of participation
17 (**Attachment 7b**), including a Privacy Act Statement (**Attachment 7b1**). A study factsheet will inform the
18 adult participant and the child participant and parent of the chemical tests and clinical outcomes to be
19 measured (**Attachment 7c**). Study staff will emphasize the voluntary nature of participation and will
20 answer any questions the participant, or parent of the child participant, has prior to obtaining signatures.
21 ATSDR has requested CDC IRB waiver of documentation of informed consent for administering the
22 questionnaire by phone (Section 3.5.1.1).

23 **3.6.2.1 Consent for Specimens and Data**

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

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

3 3.6.2.2 Child Consent

4 Before any data collection can begin in the child study, trained study staff will review the hardcopy
5 Parental Permission and Assent Form (**Attachment 7b2**) with the parent who is interested in having the
6 child participate. If the questionnaire portion of the data collection is completed over the phone, verbal
7 consent will be obtained beforehand. The study staff will explain to the parent and child the purpose of
8 the study and request that the parent sign the permission forms. If the child is seven years of age or older,
9 the study staff will request that the child give an assent to participate in the study. The recipient will
10 request that the parent complete a questionnaire about the child and complete a parental
11 neurobehavioral test battery on behalf of the child. The permission form will request that the parent allow
12 the child to donate a fasting blood specimen and store any residual specimens for future analyses. The
13 parental permission form will allow the investigators to administer a neurobehavioral test battery to the
14 child, access the child's medical and school records (including special education records) (**Attachments**
15 **7b2, 7b3 & 7b5**), and to contact the child and parent for possible future studies. Once the parent signs
16 the consent and permission forms (and the child aged ≥ 7 years gives assent to participate), the parent
17 and/or the child become study participants in the future.

18 3.6.2.3 Adult Consent

19 Before any data collection can begin in the adult study, trained study staff will obtain verbal consent for
20 the questionnaire portion, if administered over the phone. They will also review the hardcopy Adult
21 Consent Form for all other components of informed consent with the interested recruit (**Attachment 7b4**).
22 The study staff will explain the purpose of the study and obtain written informed consent for the
23 completion of a questionnaire, the collection of a new fasting blood specimen, the storage of this blood
24 specimen for future analyses, access to medical records (**Attachment 7b5**), and permission to contact the
25 participant in the future for a possible study . After signing the consent form, the adult will become a
26 study participant.

27 3.6.2.4 Risks and Benefits

28 As further described in **Section 3.8.1**, the recipient will inform the participant that his or her participation
29 is protected by a Certificate of Confidentiality under Section 301(d) of the Public Health Service Act as

1 amended by Section 2012 of the 21st Century Cures Act. The recipient will further inform the participant
2 that access to identifiable occupational history, private medical records, and to school records are
3 protected from certain disclosures under Section 301(d) of the PHSA.

4 The risks of participation in this study are minimal (defined in 45 CFR 46.110). In-home urine collections
5 are minimal risk. This study plans for a one-time 30-ml volume of fasting blood collected from the child
6 and a one-time 40-ml volume of fasting blood collected from the adult. These amounts of blood are the
7 minimum necessary to conduct analyses for PFAS and the effect biomarkers (**Attachment 2**). After the
8 blood draw, the participant will be offered a small snack, thereby allowing monitoring of adverse events
9 due to phlebotomy.

10 Participants in this study will not receive any direct benefit from taking part in this research. Their taking
11 part in this research will provide the scientific community and the public a better understanding of how
12 exposures to PFAS-contaminated drinking water may affect human health. Each adult participant and the
13 parent of the child participant will receive the results of the analyses of serum PFAS levels and effect
14 biomarkers. They will receive the results of their urine PFAS and effect biomarker levels, if ATSDR identifies
15 meaningful urinary analyses to perform.

16 ***3.6.3 Update Contact Information and Medication List***

17 The adult participant and the parent of the child participant will be asked to verify and update his or her
18 current contact information for results reporting and potential future contact (**Attachment 10**).

19 The study staff will request that the adult participant and the parent of the child participant bring all
20 current prescription and over the counter medications prior to the study office. This will help the study
21 staff to complete the medications list (**Attachment 11**).

22 ***3.6.4 Body and Clinical Measurements***

23 Trained study staff will perform the body and clinical measurements and specimen collections as
24 described in the Manual of Procedures (**Attachment 12**).

25 *Body Measurements:* Trained study staff will perform body measurements, blood pressure
26 measurements, and blood draws. Three blood pressure (BP) measurements will be taken and averaged.
27 The measured BP level is subject to biological and observer variability; therefore, the study will use three
28 different sizes of the manual cuffs in the measurements; the appropriate cuff size will be selected for each

1 participant and administered 3 times. The purpose of a specific measurement protocol, or training and
2 certifications of technicians and of ongoing quality control is to minimize variability due to known
3 exogenous factors and to reduce imprecision and biases in measurement. Measurement of resting blood
4 pressure, height, weight, and waist and hip circumference can occur in any order, but the BP
5 measurement should occur after the subject has been in the seated position for at least five minutes. BP
6 measurement will occur before venipuncture if the activities are scheduled consecutively. Trained study
7 staff will record the measurements in the Body and Blood Pressure Measures Form (**Attachment 13**).

8 *Fasting Blood Specimen and First Morning Urine Void Collection:* Participants will transport their urine
9 sample to study office for collection. Trained staff will collect and record the urine specimen intake
10 (**Attachment 14**). The blood collection procedure consists of administering and recording responses to a
11 blood draw screening questionnaire for conditions that exclude the participant from the blood draw
12 (hemophilia, skin condition, or chemotherapy in the past four), ask about having diabetes, taking blood
13 thinning medications, participant's weight, pregnancy, and fasting status (**Attachment 14**). Next,
14 phlebotomists will draw 30-ml (about 1.0. ounce or about 6 teaspoons) of blood from the child participant
15 and 40-ml (1.3 ounces or about 8 teaspoons) of blood from the adult participant using standard
16 venipuncture techniques (**Attachment 12**) and record the outcome (**Attachment 14**). If a person is unable
17 to provide the desired volume of blood, a smaller amount can be drawn and documented. Trained study
18 staff will record the phlebotomy and urine collection result on the Blood Draw and Urine Collection Form
19 (**Attachment 14**).

20 Common adverse events from blood draws include bruising, bleeding, and fainting. No serious adverse
21 events are anticipated in drawing these volumes of blood. Fasting diabetic participants who use insulin
22 will receive priority appointments for their blood draw. Light snacks will be provided following blood
23 collection. While each participant will be asked to provide a fasting sample, it is recognized that some
24 may not be able to fast. Variations in lipids levels due to fasting will affect PFAS compounds measurements
25 to a lesser extent as PFAS in serum are bound to proteins not the lipid fraction. In the C8 Science Panel
26 studies, about 25% of participants fasted – but they were not asked to do so (Frisbee 2009).

27 Phlebotomists will extract serum, and label and prepare the serum and urine specimens for secure storage
28 and transport from the study office to the CDC NCEH laboratory in Atlanta, GA (**Attachment 12**).

29 The NCEH laboratory will perform the analyses of serum PFAS according to the biochemical analytical plan
30 (**Attachment 2**) and approved laboratory methods (Kuklenyik 2015). The study staff will aliquot and ship

1 blood and serum specimens to a centralized laboratory for the analyses of the effect biomarkers according
2 to the plan. The recipient will store the urine samples and conduct analyses at a later date when more
3 knowledge is gained about urinary PFAS and effect biomarkers and until the laboratory methods are
4 developed. Residual blood and urines will be archived at CDC Biorepository so that additional PFAS or
5 effect biomarkers can be analyzed as new knowledge and analytical methods become available.

6 **3.6.5 Questionnaire**

7 Each adult participant, and a parent of the child participant, will complete a questionnaire. As a COVID-19
8 precaution, the participants will have an option to schedule questionnaire administration over the phone.
9 Per the original protocol, the questionnaire can also be administered during the appointment for the
10 blood draw.

11 3.6.5.1 Children and Parents

12 Study staff will request that the parents of the child participant complete the questionnaire. The
13 questionnaire will obtain demographic information (e.g., education, primary occupation), residential
14 history, water consumption habits, medical history of the mother and child, the child's medications, the
15 mother's reproductive history (including maternal age at birth of the participating child) and any
16 occupational exposures the mother may have had to PFAS. The questionnaire will be administered in two
17 formats: a form for the child whose parent is not also a participant (**Attachment 15**), and an abbreviated
18 form for the child whose parent is also an adult participant (**Attachment 15a**).

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

25 The questionnaire will request information on the child's height and weight, vaccination history, and
26 whether the child regularly exercises, currently smokes (and the number of cigarettes/day) or consumes
27 alcohol (and the number of drinks/week). The questionnaire will ask when the female child first began to
28 menstruate. The questionnaire will include specific questions addressing health outcomes of interest.
29 For example, for ADHD, the questionnaire will ask, "Has a doctor or health professional ever told your

1 child that your child has/had ADD or ADHD?" If the answer is "yes," a second question will ask for a list of
2 medications the child took for the condition. The questionnaire will ask if the child had learning or
3 behavioral problems, and if so, the type of problem and the treatment used. Questions would be included
4 for the hypersensitivity-related outcomes, asthma, atopic dermatitis (or atopic eczema), and allergies. The
5 study will attempt to confirm diseases and conditions reported in the questionnaire by accessing medical
6 records sending abstraction forms (**Attachments 17&17a**) to the medical care provider identified by the
7 participants on their consent forms (**Attachment 7b5**).

8 *3.6.5.1.1 Child/Parent Neurobehavioral Assessments*

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

10 Trained professionals will administer the following tests to children:

- 11 • The Wechsler Abbreviated Scale of Intelligence – 2nd Edition (WASI – II) test will be administered
12 to measure Full Scale IQ (FSIQ) among children 6-17 years (15 minutes). Intelligence testing of
13 children aged 4 – 5 years will not be conducted.
- 14 • Each child 4-16 years will complete the NEPSY-II selected tests. Except for Theory of Mind, these
15 additional tests are short and useful to assess memory and inhibition. For all the NEPSY – II tests,
16 children 4 years would take about 52 minutes, and children ≥5 years, about 70 minutes.
- 17 • Children aged 4 – 7 years will complete the Connors Kiddie Continuous Performance Test (K-CPT
18 – 2) (8 minutes), and children aged >7 years will complete the Connors CPT – 3 (14 minutes).

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

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

27 A summary of the neurobehavioral test battery is found in **Attachment 18**. Each child will spend an
28 average of 90 minutes to complete the child battery of tests. Each parent will spend an average of 15

- 1 minutes to complete the parent battery of tests. Overall, each parent/child pair will take 105 minutes to
- 2 complete the neurobehavioral test battery (**Attachment 18a**).

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

Neurobehavioral Test	Domain	Age	Administration	Time to Administer	
Wechsler Abbreviated Scale of Intelligence – 2 nd Edition (WASI - II)	Two Subtest Form (FSIQ)	6 – 17*	Child	15 minutes	
A Developmental Neuropsychological Assessment – 2 nd 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	
	Stature (inhibitory control)	4 – 6	Child	3 minutes	
	Word Generation (expressive language, executive control)	4 - 16	Child	4 – 6 minutes	
	Conners Kiddie Continuous Performance Test, 2 nd Edition (Conners K-CPT 2)	Inattentiveness, Impulsivity, Sustained Attention, Vigilance	4-7	Child	8 minutes
	Conners Continuous Performance Test 3 rd 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

1

2 For each child, the recipient will also review and abstract school records, including special education

3 records, to identify learning problems and behavioral problems (**Attachments 18b&18c**). If the parent

4 reports that the child has a developmental disability (e.g., ADHD, autism, or a learning disability), then the

5 recipient shall obtain and abstract the special education records for the child including the individualized

6 education program (IEP), the IEP evaluation report (“Full Individual Evaluation” or “FIE”), and if available,

7 the Independent Educational Evaluation.

8 **3.6.5.2 Adults**

9 Each adult participant will complete a questionnaire requesting demographic information, residential

10 history, water consumption habits, occupational history, medical history and reproductive history

11 (**Attachment 16**). In particular, the questionnaire will ask if the participant ever had kidney disease, liver

12 disease, cardiovascular disease, hypertension, high cholesterol, thyroid disease, diabetes, autoimmune

13 diseases, osteoporosis, osteoarthritis, pregnancy-induced hypertension, infertility, and endometriosis.

14 For each reported disease or condition, the questionnaire will ask about the date of diagnosis, the medical

15 provider who made the diagnosis, and the medications used for treatment the questionnaire will ask the

16 participant about conditions that might affect PFAS serum levels such as date of menopause, menstrual

17 cycle information, blood transfusions, and blood donations. The study will attempt to confirm diseases

18 and conditions reported in the questionnaire by medical records review (**Attachments 17&17a**).

1 **3.6.6 Exit Procedures**

2 At the end of the data collection, study coordinators or staff will review recorded items in the participant's
3 Appointment Tracking Form for completeness (**Attachment 9**).

4 The adult participant or the parent of the child participant will receive a copy of the participant's Body
5 and Blood Pressure Measures Report (**Attachment 19**). These results will be immediately available and
6 will require no further evaluation or interpretation with two exceptions. The adult participant or the
7 parent of the child participant will receive a supplemental notice if the participant has a critical blood
8 pressure measure (diastolic blood pressure > 120 mm Hg, or systolic blood pressure >180 mm Hg). In this
9 case, a Critical Hypertension Notice will be appended to the Body and Blood Pressure Measurements
10 Report along with written and verbal recommendations to obtain immediate medical attention. If the
11 participant does not have a personal physician, the study coordinator will provide a referral. If the
12 participant has an elevated but non-critical blood pressure measure (resting blood pressure > 140/90), an
13 Elevated Hypertension Notice will be appended to the Body and Blood Pressure Measures Report with
14 written and verbal recommendations to obtain clinical follow-up.

15 **3.6.6.1 Gift Cards as a Token of Appreciation for Participation**

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

- 17 • \$25 for body and blood pressure measures, and for blood and urine collection;
18 • \$25 for completed questionnaire; and
19 • \$25 for child/parent completion of the neurobehavioral test battery

20 Trained study staff will document provision of gift cards on the hard copy form (**Attachment 9**). As part of
21 the exit procedures, the participant will sign this form to document receiving the gift card.

22 **3.6.7 Adverse Events**

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

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

6 If a participant loses consciousness, falls, is unable to stand, or experiences chest pain the study staff will
7 decide whether to advise the adult participant or the parent of the child participant to seek immediate
8 medical treatment or to contact emergency medical services. Study staff have identified appropriate local
9 medical care providers that participants may be referred to if clinical results suggest medical attention is
10 needed (**Attachment 12**).

11 **3.7 Biochemical Analyses**

12 *Serum PFAS*: The ATSDR biochemical analytical plan is found in **Attachment 3**. The study will analyze PFAS
13 in fasting serum including linear PFOA, the sum of branched isomers of PFOA, linear PFOS, branched PFOS,
14 and PFHxS (Kato 2018). Other PFAS analyzed will include: 2-(N-methyl-perfluorooctane sulfonamido)
15 acetic acid (Me-PFOSA-AcOH),), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and
16 perfluoroundecanoic acid (PFUnA). For NHANES 2017-8, three PFAS analytes were added: 9-
17 Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid, Adona (ammonium salt of 4,8-dioxa-3H-
18 perfluorononanoic acid, and GenX (ammonium salt of 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-
19 heptafluoropropoxy)-propanoic acid (HFPO-DA), but their reference ranges were all below the limit of
20 detection (CDC 2021) and will not be measured in Pease.

21 *Serum PFAS*: The study's biochemical analytical plan is found in **Attachment 2**. The study will analyze PFAS
22 in fasting serum including linear PFOA, the sum of branched isomers of PFOA, linear PFOS, branched PFOS,
23 and PFHxS (Kato 2018). Other PFAS analyzed will include: 2-(N-methyl-perfluorooctane sulfonamido)
24 acetic acid (Me-PFOSA-AcOH), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and
25 perfluoroundecanoic acid (PFUnA). For NHANES 2017-8, three PFAS analytes were added: 9-
26 Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid, Adona (ammonium salt of 4,8-dioxa-3H-
27 perfluorononanoic acid, and GenX (ammonium salt of 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-
28 heptafluoropropoxy)-propanoic acid (HFPO-DA), but their reference ranges were all below the limit of
29 detection (CDC 2021) and will not be measured in Multi-site study samples.

1 *Urinary PFAS*: The study will store urine samples for potential analyses of PFAS in the future. Urine is an
2 important excretion pathway for human metabolism and PFAS urine elimination may be important
3 influencing serum concentrations (Harada 2005, Zhang 2015). The PFAS compounds measured by current
4 method are listed in **Attachment 2**.

5 **3.7.1 Children**

6 The study will analyze fasting serum for the following biomarkers of lipids, thyroid, glycemic, liver, and
7 kidney function, sex hormones, and immune function (**Attachment 2**):

- 8 • Total cholesterol, low density lipoprotein, high density lipoprotein, total triglycerides,
- 9 • Uric acid, creatinine,
- 10 • Total thyroxine (TT4), free T4, TT3, thyroid stimulating hormone (TSH), thyroglobulin antibodies,
11 thyroid peroxidase antibodies (TPO),
- 12 • Glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide,
13 pro-insulin,
- 14 • Alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -
15 glutamyltransferase (GGT), direct bilirubin, albumin, and cytokeratin-18 (CK-18),
- 16 • Testosterone, estradiol, sex hormone-binding globulin (SHBG), follicle stimulating hormone,
17 insulin-like growth factor,
- 18 • Immunoglobulin G (IgG), IgA, IgE, and IgM; antibodies to measles, mumps, rubella, tetanus, and
19 diphtheria.

20 The child study will use the cut points of 50 ng/dL of total testosterone and 20 pg/mL of estradiol to
21 identify sexual maturation in boys and girls, respectively (Lopez-Espinosa 2011). The child study will
22 measure IgG antibodies for measles, rubella, and diphtheria to determine vaccine responses. It will
23 analyze allergen specific IgE (mold, dust mites, dog, cat, cow's milk, peanut, hen's egg, and birch). The
24 study will analyze serum levels of thyroid stimulating hormone (TSH) and total/free T4 separately and use
25 these measurements to determine clinical and subclinical hypothyroidism and hyperthyroidism. The
26 study will measure uric acid, total cholesterol, low-density and high-density lipoprotein, and triglycerides.
27 We also propose to measure liver enzymes and CK-18 (Feldstein 2013, Mora 2018, and Santoro 2013).

28 **3.7.2 Adults**

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

- 1 • Total cholesterol, low density lipoprotein, high density lipoprotein, total triglycerides,
- 2 • Uric acid, creatinine,
- 3 • Total thyroxine (TT4), free T4, TT3, thyroid stimulating hormone (TSH), thyroglobulin antibody,
- 4 thyroid peroxidase antibodies (TPO),
- 5 • Glucose, insulin, glycosylated hemoglobin (HbA1c), auto-antibodies (GAD-65 and IA-2), C-peptide,
- 6 pro-insulin,
- 7 • Alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -
- 8 glutamyltransferase (GGT), direct bilirubin, albumin, and cytokeratin-18 (CK-18),
- 9 • Immunoglobulin G (IgG), IgA, IgE, and IgM; C reactive protein, rheumatoid factor, and antinuclear
- 10 antibodies (ANA),
- 11 • Cytokines and adipokines (e.g. IL-1 β , IL-4, IL-6, IL-8, IL-12, MCP-1, TNF α , leptin, adiponectin,
- 12 resistin, PAI-1).

13 **3.7.3 Quality Control/Quality Assurance**

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

17 The NCEH laboratory analyzing PFAS serum levels, and the other participating laboratory analyzing the
18 effect biomarkers, will fulfill quality assurance/quality control criteria (QA/QC) including a documented
19 quality assurance plan and adherence to required quality control procedures specified in an approved
20 method. The laboratories will ensure that the analytical data are scientifically valid, defensible, and of
21 known and acceptable precision and accuracy. QA/QC procedures, including appropriate calibration of
22 instruments, running standards and blanks, reporting limits of detection, and other parameters will be in
23 place before specimens are tested. Specimen collection, storage, and transportation techniques are
24 specified in the Manual of Procedures to ensure the integrity of the specimens (**Attachment 12**).
25 Specimens will be stored at the proper temperature and isolated from potential sources of contamination.

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

1 **3.7.4 Reference Values**

2 The participating laboratory will provide reference values and action levels for the effect biomarkers
3 which will be reported in **Attachments 20&21**. The recipient will report the participant’s PFAS results using
4 reference values from the most recent NHANES report (**Attachment 22**). Currently, the 2013-14 report is
5 available and provides reference values for children. **Section 4** provides additional descriptions of the
6 procedures for advance and final results reporting.

7 **3.8 Data Handling**

8 **3.8.1 Certificate of Confidentiality**

9 ATSDR requests to issue a Certificate of Confidentiality (CoC) under Section 301(d) of the Public Health
10 Service (PHS) Act, as amended by Section 2012 of the 21st Century Cures Act, P.L. 114-255 (42 U.S.C.
11 241(d)), states that the Secretary shall issue CoCs to persons engaged in biomedical, behavioral, clinical,
12 or other research activities in which identifiable, sensitive information is collected. In furtherance of this
13 provision, CDC research commenced or ongoing after December 13, 2016 and in which identifiable,
14 sensitive information is collected, as defined by Section 301(d), is deemed issued a CoC and therefore
15 researchers are required to protect the privacy of individuals who are subjects of such research in
16 accordance with Section 301(d) of the PHSA.

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

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

27 Since the answer to any one of Questions 2-5 is YES, ATSDR determined that a CoC will apply to the
28 research; therefore, in accordance with subsection 301(d) of the Public Health Service Act, ATSDR and
29 any of its cooperative agreement recipients shall not:

- 1 • Disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or
2 other proceeding, the name of such individual or any such information, document, or
3 biospecimen that contains identifiable, sensitive information about the individual and that was
4 created or compiled for purposes of the research, unless such disclosure or use is made with the
5 consent of the individual to whom the information, document, or biospecimen pertains; or
- 6 • Disclose or provide to any other person not connected with the research the name of such an
7 individual or any information, document, or biospecimen that contains identifiable, sensitive
8 information about such an individual and that was created or compiled for purposes of the
9 research.

10 Disclosure is permitted only when:

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

21 ATSDR and its cooperative agreement recipients conducting this research are required to establish and
22 maintain effective internal controls (e.g., policies and procedures) that provide reasonable assurance
23 that the research contract is managed in compliance with Federal statutes, regulations, and the terms
24 and conditions of the award (**Attachment 12**). Recipients are also required to ensure: 1) that any
25 investigator or institution not funded by CDC/ATSDR who receives a copy of identifiable, sensitive
26 information protected by this CoC, understands that it is also subject to the requirements of subsection
27 301(d) of the PHS Act; and 2) that any subrecipient that receives funds to carry out part of this CDC
28 award involving a copy of identifiable, sensitive information protected by a Certificate understands that
29 it is subject to subsection 301(d) of the PHS Act.

30 For studies in which informed consent is sought, ATSDR and its cooperative agreement recipients shall
31 inform research participants of the protections and the limits to protections provided by this CoC

1 (Attachment 7b). Therefore, all study staff will receive training on the importance of protecting the
2 confidentiality of human research subjects and of personal information acquired, including the collection
3 of biological specimens. The study will minimize the risk of loss of confidentiality and privacy through
4 careful attention to procedures for such protections in the collection, handling, and reporting of
5 individually identifiable and sensitive data (Attachment 12).

6 **3.8.2 Data Management and Security**

7 Data management for this study described below includes guidance on:

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

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

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

23 **3.8.3 Impact on Privacy**

24 Because the study staff will collect, store, manage, and maintain IIF on an already established record
25 system, there would be a likely effect on the participant’s privacy if a breach of data security occurred.
26 Therefore, its established record system has stringent safeguards in place as described in the following
27 section. Research datasets will include only coded information that might be sensitive, such as questions
28 on reproductive outcomes, fertility, or fecundability. These files will not have associated information that

1 might directly identify these participants. IIF will be stored in a separate master key dataset, which will
2 enable ATSDR investigators to link the participant's research data with his or her IIF via a study-generated
3 ID. Maintaining this contact information is necessary to provide results of the tests or re-contact them in
4 the future for a longitudinal study. Therefore, stringent data security measures will be in place, including
5 administrative, physical, and technical controls as described below.

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

9 3.8.3.1 Access Controls and Security

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

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

24 Once collected from the participant, all hardcopy informed consents and data collection forms will be
25 stored in locked files in locked rooms in the study office and at ATSDR. Informed consent will also be
26 scanned into electronic form and transferred to ATSDR to provide backup in the case of incidental damage
27 to the paper forms.

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

4 Data security measures at ATSDR will comply with the *CDC/ATSDR Protection of Information Resources*
5 *Policy* and the *CDC/ATSDR IT Security Program Implementation Standards*. These policies apply to all
6 authorized ATSDR employees. All incidents involving a suspected or confirmed breach of IIF must be
7 reported to OCISO according to the policy titled *OCISO/CDC Standard for Responding to Breaches of*
8 *Personally Identifiable Information (PII)*.

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

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

16 *Administrative Controls* –Authorized recipient researchers and CDC/ATSDR employees are required to:

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

26 All study records are subject to the ATSDR Comprehensive Record Control Schedule (CRCS), B-371, which
27 contains authorized disposition instructions for ATSDR's administrative and program records. ATSDR is
28 legally required to maintain its program-related records in accordance with disposition instructions
29 contained in this comprehensive records control schedule. These retention periods have a direct impact

1 on completing Freedom of Information Act (FOIA) requests and in applying the requirements of the
2 Privacy Act. The current schedule requires ATSDR to retain and archive program records for a period of
3 75 years after the end of the study activities.

4 **3.8.4 Data Delivery**

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

8 Study staff will prepare draft datasets to record questionnaire responses and medical record/school
9 record data to send to ATSDR for review and approval. ATSDR will work with the study staff to resolve
10 missing values and other data issues. The study staff will also keep and deliver a shipping log of blood
11 specimens sent to the NCEH laboratory in Microsoft Excel format (**Attachment 12**). The log will include
12 the include vial type, volume, ID code, date, and carrier details. ATSDR will receive lab results from the
13 participating laboratories. The lab dataset will be merged by study ID with the questionnaire data to create
14 a combined questionnaire and lab dataset.

15 All dataset formats will be transformed to SAS datasets (SAS 9.3, Cary NC). All final data management will
16 be performed on this platform. Site investigators may also use other CDC approved statistical software
17 before converting to SAS. Final datasets will be sent to ATSDR using encrypted, password coded
18 spreadsheets through a password protected data sharing facility. The contractor will deliver to ATSDR the
19 code and the master key dataset by which the response data are potentially relinkable to PII.

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

24 **3.8.5 Data Ownership and Data Sharing**

25 Coded research datasets will be available to all ATSDR study investigators listed in **Attachment 1**. We will
26 produce coded datasets by removing the following: name, SSN, date of birth, address, former address
27 (es), phone number, and date of completion of the blood draw and questionnaire. SSN will be collected
28 at enrollment for linkage to medical records and school records. Once the linkage has occurred, the SSN

1 will be kept with other PII in a separate access restricted secure database. Age will replace date of birth
2 in the data analysis file because it is the necessary variable in exposure and health outcome analyses.

3 Release of de-identified multi-site combined data to outside investigators including recipients must be
4 approved by ATSDR. A data use agreement (DUA) will be prepared, detailing the condition of use of the
5 data and proposed analyses for each outside project. The DUA condition of use will specify that ATSDR
6 will not release the link between the study IDs and the participants' PII to the outside researchers. The
7 DUA will also specify that:

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

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

19 ***3.8.6 Storing Residual Blood for Future Use***

20 After performing the chemical and clinical tests, there may be some residual blood. In the consent form,
21 we will ask participant's permission to save this residual blood for additional future analyses of PFAS and
22 possibly additional effect biomarkers. We will only store blood of those participants who will consent to
23 have their blood archived for additional PFAS and effect biomarker analyses (**Attachment 7b**).

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

1 We will consent participants at enrollment and not recontact them for the additional analyses of stored
2 biospecimens related to this PFAS research: Because new scientific knowledge, tests, or methods may
3 arise, we would like to save this leftover biospecimens for additional analyses on exposures or health
4 conditions related to PFAS. ATSDR is also committed to investigate the possible confounding between
5 lead and other heavy metal exposures and associations between PFAS and neurobehavioral outcomes in
6 children. Residual from the blood obtained from the child will be stored and available for future analysis.
7 In addition, ATSDR or recipients may release de-identified research datasets or de-identified biospecimens
8 for future studies related to PFAS to outside investigators under a data use agreement that will prohibit
9 any attempt to identify you or your child as a research subject. In this case, your individual test results will
10 not be reported to you.

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

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

18 ***3.8.7 Future Exploratory Analyses***

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

21 **3.9 Exposure Estimation**

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

- 24 • PFAS serum measurements obtained in the study,
- 25 • Historical reconstruction of PFAS concentrations in the drinking water consumed by the
26 participant,
- 27 • Questionnaire data on the participant's consumption of PFAS-contaminated drinking water and
28 factors that might affect PFAS serum levels,

- 1 • Age-, sex-, and calendar year-specific “background” PFAS serum levels from NHANES, and
- 2 • Physiologically based pharmacokinetic (PBPK) models.

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

6 The C8 studies used PBPK modeling to estimate cumulative serum levels of PFOA and PFOS (Shin 2011).
7 The model incorporated information from the historical reconstruction of PFAS concentrations in the
8 drinking water serving the C8 areas, questionnaire data on each participant’s water consumption, and the
9 serum levels of PFOA and PFOS obtained from study participants. A recent effort to reconstruct historical
10 exposures worked well for PFOA and PFOS; but less well for PFHxS (Gomis 2017). Low environmental
11 concentrations, lack of decline in older population, possible ongoing exposure in children/younger adults,
12 and scarcity of time-trend data in consumer products were cited as reason for poor prediction
13 characteristics of PFHxS models (Gomis 2017). However, if there are high correlations in serum levels
14 between PFHxS and PFOS and/or PFOA, then it may be possible to estimate cumulative PFHxS serum levels
15 based on the historical estimates for serum PFOS and/or PFOA.

16 Recently, an online serum PFOA calculator for adults became available using a modified one-compartment
17 exponential decay model to estimate PFOA serum levels from PFOA concentrations in drinking water
18 (Bartell 2017). Developing a similar calculation for serum PFOS is possible. The studies of children and
19 adults by ATSDR and recipients will explore this approach to estimate serum PFOA, PFOS, PFHxS and PFNA
20 levels and make comparisons with serum levels from the blood specimens obtained in this study (and if
21 available, previous PFAS serum measurements). The recipient may consider the use of a one-
22 compartment PBPK model similar to one used by Shin (2011) and Avanas (2016), and also used as the
23 basis for a recent PFOA serum calculator (Bartell SM 2017).

24 A number of improvements in PBPK modeling approaches, especially as related to multi-compartment
25 models, have been developed recently and the recipient should take those into consideration (Loccisano
26 2013, Fabrega 2014, 2016; Verner 2015, 2016).

27 The recipient should attempt to integrate a broad range of information on individuals’ sociodemographics
28 (birth year, age, sex, ethnicity), PFAS pharmacokinetics (e.g. tissue partitioning and distribution volumes,
29 elimination rates), as well as exposure sources as pertain for the general population (e.g. breastfeeding,
30 water consumption, blood transfusion) and secretion routes (e.g. parity, breastfeeding history, and

1 menstruation in women; donating blood) which will be collecting in the adult and child questionnaire.
2 Questionnaires also includes detailed information on menstruation cycles for women (regular/irregular,
3 length, heavy/light flow, last menstruation before blood draw; Wong 2015, Verner and Longnecker 2015).
4 The recipient can assume the contributions from dietary intake, cookware, cleaning supplies, etc. to be
5 similar to the background US population (Domingo 2012, Christensen 2017). The recipient can also
6 assume that NHANES calendar year-, age- and sex-specific PFAS serum concentrations reflect these
7 background exposures (Calafat 2007, Ye 2017).

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

12 In order to estimate historical concentrations of PFAS in the drinking water and historical PFAS serum
13 levels, each recipient will follow a general approach to information gathering and modeling. Each
14 recipient should obtain as much information as possible on the source of the PFAS contamination. If the
15 source is environmental emissions from an industrial facility, then the recipient should request
16 information from the facility about these emissions (e.g., periods, locations, frequencies and amounts of
17 emissions, and whether the emissions are to surface water, ground water and/or air). If the source is
18 AFFF use at a military base, airport or fire training area, then the recipient should seek information on the
19 period and location of use, the annual amount of AFFF used, and any accidental or non-routine use (e.g.,
20 to extinguish a major fire, or a major spill) and the date, location and amount used.

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

28 If the contaminated drinking water is from a municipal system, then the characteristics of the distribution
29 system will be obtained from the water purveyor. If supply wells are used, then the recipient will request
30 historical and current information on these wells including monthly or daily production logs and dates of

1 operation. If a surface water source is used or if water is purchased from another purveyor, then the
2 recipient will request information about this source.

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

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

11 **3.10 Statistical Analyses**

12 ATSDR staff will perform statistical analyses with the participation of the recipients using SAS, R and STATA
13 on the combined multi-site study dataset. ATSDR staff may also use SPSS for data management. ATSDR
14 staff will calculate descriptive statistics (including means, geometric means, medians, standard deviations,
15 and percentiles) to identify the presence and distribution of PFAS and effect biomarker analytes. Statistical
16 methods will include multiple linear regression of continuous (untransformed and natural log
17 transformed) effect biomarkers on continuous (untransformed and natural log transformed) PFAS serum
18 levels and categorized PFAS serum levels, and logistic regression of categorized effect biomarkers (e.g.,
19 hypercholesterolemia) or disease prevalence on continuous (untransformed and natural log transformed)
20 and categorical PFAS serum levels. ATSDR staff will use restricted cubic spline methods (or generalized
21 additive models using cubic regression splines) for linear and logistic regression to obtain flexible,
22 smoothed exposure-response curves.

23 To identify risk factors that may act as confounders for a particular health outcome, the analysis will
24 implement a “10% change in the estimate” rule (Maldonado 1993). It must be remembered that for any
25 appreciable confounding to occur, the factor must be a strong risk factor for the outcome under
26 consideration and must also be strongly correlated with the PFAS exposure under evaluation. For
27 unmeasured risk factors, ATSDR proposed the use of negative controls and quantitative bias analyses (see
28 below). These are all standard approaches for evaluating confounding by any risk factor including “co-
29 exposures” by other environmental contaminants.

1 For example, evaluation of the confounding effects of smoking in occupational studies evaluating a
2 chemical exposure and lung cancer typically observe only moderate confounding (e.g., between 20% and
3 30%, Blair et al. 2007). This is so even though smoking is an extremely strong risk factor for lung cancer
4 and, at least in earlier occupational studies, typically was at least moderately associated with the chemical
5 exposure or the exposed workforce. None of the diseases and clinical measures or neurobehavioral tests
6 under evaluation in the Multi-site Study have a risk factor remotely as strong as smoking is for lung cancer.
7 Although there are likely to be at least moderate correlations among the PFAS chemicals, confounding of
8 one PFAS chemical by another PFAS chemical should be minor because it is not known that any are strong
9 risk factors for any of the diseases or clinical measures or neurobehavioral tests under the study.
10 (Nevertheless, we will evaluate whether a PFAS chemical confounds an association between another PFAS
11 chemical and a disease or clinical measure by the 10% change-in-the-estimate rule mentioned above.)
12 Moreover, it is very unlikely that any other (i.e., non-PFAS) chemicals or metals will be highly or even
13 moderately correlated with PFAS chemicals. For example, correlations (Pearson correlation coefficient, R)
14 between mercury and PFOA, PFOS, PFHxS and PFNA are consistently <0.20 among children in the NHANES
15 data. In addition, lead and mercury are not very strong risk factors for any disease or clinical measure or
16 neurobehavioral test – i.e., they are considerably weaker risk factors for health outcomes than smoking
17 is for lung cancer.

18 Primary analyses will focus on estimated cumulative PFAS serum levels. Supplemental analyses will
19 evaluate PFAS serum levels in the blood specimens obtained in the study as well as estimated maximum
20 and average PFAS serum levels. The primary analyses will evaluate each PFAS chemical separately; sum
21 of PFAS measures may also be considered. Statistical analyses using prevalent cases in a cohort design
22 which takes into consideration the times of diagnosis will also be conducted. ATSDR will explore the use
23 of methods for evaluating multi-pollutant mixtures, such as the hierarchical Bayesian model, to analyze
24 the effects of exposures to the PFAS mixtures. There are several caveats and recommendations in
25 conducting analyses of mixtures to determine the optimal method that avoids amplifying bias due to
26 confounding (Weisskopf et al 2018).

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

1 In summary, to gauge the potential and magnitude of possible selection bias and information biases, as
2 well as confounding bias due to unmeasured risk factors, two approaches will be taken. First, quantitative
3 methods described in Lash et al (2009) will be used to estimate the possible magnitude of selection and
4 informational biases. Second, “negative control” diseases will be used to also estimate the potential and
5 magnitude of these biases (Lipsitch et al 2010). Negative control diseases are those diseases not known
6 to be associated with the exposures of interest. In the multi-site study, the exposures of interest are PFAS
7 serum levels. The negative control diseases for children included in the questionnaire are celiac disease,
8 scleroderma, lupus, and Crohn’s disease. In addition to these diseases, negative control diseases for adults
9 include Parkinson disease, emphysema, chronic bronchitis, multiple sclerosis, and fibromyalgia.

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

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

19 **4. RESULTS REPORTING**

20 **4.1 Notification of Individual Results**

21 Some of the clinical tests may include results that indicate disease or serious medical condition. Due to
22 the scheduled timespan between blood specimen collection and the actual laboratory analyses, we are
23 unable to report study results in a short period. Study staff will report to the participant the result of a
24 clinical test that clearly indicates the potential for a serious health consequence immediately after
25 receiving the result from the laboratory. An advance notification phone call from the study investigators
26 (**Attachment 20**) with a subsequent letter of clinical tests results will be sent to the participant when the
27 abnormal results are identified, processed, and checked for accuracy (**Attachment 20a-b**). Study staff will
28 advise the participants to consult his/her physician, or to contact the physician associated with the study
29 for explanation of clinical findings.

1 Participants will also receive results of their effect biomarker tests after the study is completed. Contract
2 labs will provide their clinical reference abnormal or 'high' levels, if available, for interpretation of clinical
3 test results (**Attachment 21**). Participants will receive their PFAS test results. The recipient will provide to
4 the 50th and 95th percentiles from NHANES for comparison to the U.S. population (CDC, 2018). Study staff
5 will advise participants to consult ATSDR with questions about their results if they wish to do so.

6 **4.2 Disseminating Results to the Public**

7 The recipient will consult with the local and/or state health agency, local community groups, and the
8 National PFAS Contamination Coalition to determine the most effective method of disseminating the
9 results to the participants and the public. If the recipient establishes a community assistance panel (CAP)
10 in the study area, then the CAP will participate in study community outreach and recruitment activities as
11 well as provide advice on effective methods of results dissemination.

12 The recipient may consider using a user-centered digital interface developed by the Silent Spring Institute
13 for reporting results to each participant. The recipient will present study results to the community in public
14 meetings, printed community handout materials, participating in local radio programs and in informal
15 activities. The recipient also will provide a study website with information about the study findings and
16 general information about any future follow up studies.

17 Generally, ATSDR will publish study results only as group data analyses in peer-reviewed scientific journals
18 or government reports. If individual data are presented, those will not be linked to participants' identities.
19 In the event that some other exceptional characteristics would enable personal identification, those
20 would be masked or modified as needed to protect individual privacy. ATSDR will use manuscripts
21 published in peer-reviewed scientific journals and presentations at major scientific meetings to inform the
22 scientific community about the results of the Multi-site studies.

23 **5. STRENGTHS AND LIMITATIONS**

24 Cross-sectional studies are especially suitable for assessing effect biomarkers and the prevalences of
25 nonfatal diseases, in particular, diseases with no clear point of onset (Checkoway 2004). However, if the
26 cross-sectional study concurrently measures the exposure and the outcome (i.e., the disease or effect
27 biomarker), it might be difficult to determine whether the exposure caused the outcome or whether the
28 outcome influenced the measured exposure level (Flanders 1992, 2016). For example, as discussed above,
29 the concurrent measurement of serum PFAS levels and kidney function biomarkers might raise the

1 question of “reverse causation” because kidney function can affect the levels of PFAS in serum. One
2 approach to minimize the problem of reverse causation or possible confounding due to health outcomes
3 that affect PFAS serum levels is by estimating exposures based on the historical reconstruction modeling
4 of serum PFAS levels. In addition, it might be possible to estimate exposures during critical vulnerable
5 periods (e.g., in utero exposure) through the modeling of historical serum PFAS levels. However, the
6 modeling of historical PFAS serum levels is subject to uncertainties and data limitations, and published
7 methods currently are available only to model serum levels of PFOA and PFOS.

8 ATSDR will establish working groups to oversee thorough technical evaluation and quality assurance and
9 quality control (QA/QC) for all methods and models in the historical reconstruction of groundwater
10 resources and distribution of drinking water and for all PK/PBPK models used for historical serum
11 reconstruction. These groups will serve multiple functions such as sharing information with ATSDR and
12 across sites and overseeing quality control. Site visits, and if needed audits of modeling data at each site
13 will be part of those efforts.

14 The recipients are required to estimate historical PFAS concentrations for both drinking water and serum.
15 The required level of precision will be agreed upon by the site investigators as well as discussion of
16 measurement variability, limits of detections etc. and the criteria for determining the precision of the
17 serum concentration estimates without using the drinking water data. The recipients’ model approaches
18 for the multi-site study will be externally peer-reviewed per the CERCLA mandate and the Information
19 Quality Bulletin. Other issues concerning cross-sectional study designs are similar to those that confront
20 other observational study designs, such as cohort studies. These issues include: 1) the ability to clearly
21 define, enumerate and recruit (without introducing selection bias) the exposed and comparison
22 populations, 2) the comparability of the exposed and comparison populations on risk factors other than
23 the PFAS exposures, 3) accurate exposure assessment, and 4) accurate measurement of effect biomarkers
24 and ascertainment of diseases. In addition, a bias similar to the “healthy worker survival effect” bias could
25 occur in a cross-sectional study because the study population consists of those who remained in the study
26 area (and, for example, did not leave the study area due to health problems caused by exposure to the
27 PFAS contaminated drinking water). While the resulting cohort is a ‘survivor cohort’, the studies have
28 shown that the only if survival after incidence differs by exposure level can results be biased (Barr 2015)
29 for the non-fatal and even in the case of fatal disease.

30 All epidemiological studies of environmental exposures and health outcomes have limitations and
31 uncertainties. Whether a study will find an association between an environmental exposure and health

1 effects is unknown prior to conducting the study. No single study will provide definitive answers to the
2 community about whether their exposures to the PFAS-contaminated drinking water caused their health
3 problems. The ability of the multi-site study to provide useful information will depend largely on the
4 success of recruiting a sufficient number of study participants and obtaining sufficient information on the
5 PFAS contamination to estimate historical PFAS serum levels with reasonable accuracy.

1 6. REFERENCES

- 2 Agency for Toxic Substances and Disease Registry (ATSDR). Feasibility Assessment for Epidemiological
3 Studies at Pease International Tradeport. Portsmouth, New Hampshire. November 2017a. Available at:
4 [https://www.atsdr.cdc.gov/sites/pease/documents/Pease_Feasibility_Assessment_November-](https://www.atsdr.cdc.gov/sites/pease/documents/Pease_Feasibility_Assessment_November-2017_508.pdf)
5 [2017_508.pdf](https://www.atsdr.cdc.gov/sites/pease/documents/Pease_Feasibility_Assessment_November-2017_508.pdf)
- 6 Agency for Toxic Substances and Disease Registry (ATSDR). ATSDR's PFAS Exposure Assessment
7 Technical Tools (PEATT). Atlanta, Georgia. November 2017b. Available at: on request ATSDR/DCAI
- 8 Alexander BH, Olsen GW. Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers.
9 *Ann Epidemiol.* 2007;17(6):471-8.
- 10 Avanasì R, Shin HM, Vieira VM, Savitz DA, Bartell SM. Impact of Exposure Uncertainty on the Association
11 between Perfluorooctanoate and Preeclampsia in the C8 Health Project Population. *Environ Health*
12 *Perspect.* 2016;124(1):126-32
- 13 Bach CC, Bech BH, Brix N, Nohr EA, Bonde JP, Henriksen TB. Perfluoroalkyl and polyfluoroalkyl
14 substances and human fetal growth: a systematic review. *Crit Rev Toxicol* 2015;45(1):53-67.
- 15 Barry V, Winqvist A, Steenland K. Perfluorooctanoic Acid (PFOA) Exposures and Incident Cancers among
16 Adults Living Near a Chemical Plant. *Environ Health Perspect* 2013; 121:1313–1318.
- 17 Bartell SM. Online serum PFOA calculator for adults. *Environ Health Perspect* 2017; 125:104502.
- 18 Bangdiwala SI, Bhargava A, O'Connor DP, Robinson TN, Michie S, Murray DM, Stevens J, Belle SH,
19 Templin TN, Pratt CA. Statistical methodologies to pool across multiple intervention studies. *Transl*
20 *Behav Med.* 2016; 6(2):228-35.
- 21 Basagaña X, Pedersen M, Barrera-Gómez J, Gehring U, Giorgis-Allemand L, Hoek G, Stafoggia M,
22 Nieuwenhuijsen MJ, Brunekreef B, Slama R; ESCAPE Birth Outcomes working group. Analysis of
23 multicentre epidemiological studies: contrasting fixed or random effects modelling and meta-analysis.
24 *Int J Epidemiol.* 2018; 47(4):1343-1354.
- 25 Blair A, Stewart P, Lubin JH, Forastiere F. Methodological issues regarding confounding and exposure
26 misclassification in epidemiological studies of occupational exposures. *Am J Ind Med.* 2007
27 Mar;50(3):199-207.
- 28 Bonfeld-Jørgensen EC, Long M, Fredslund SO, Bossi R, Olsen J. Breast cancer risk after exposure to
29 perfluorinated compounds in Danish women: a case-control study nested in the Danish National Birth
30 Cohort. *Cancer Causes Control.* 2014;25(11):1439-48.
- 31 C8 Science Panel 2012a.
32 http://c8sciencepanel.org/pdfs/Probable_Link_C8_Osteoarthritis_29Oct2012v2.pdf.
- 33 C8 Science Panel 2012b. http://c8sciencepanel.org/pdfs/Probable_Link_C8_Kidney_29Oct2012.pdf.
- 34 Cardenas et al. Plasma concentrations of per- and polyfluoroalkyl substances at baseline and
35 associations with glycemic indicators and diabetes incidence among high-risk adults in the diabetes
36 prevention program trial. *Environ Health Perspect* 2017;125(10):107001.

- 1 Calafat AM, Wong LY, Kuklennyik Z, Reidy JA, Needham LL. Polyfluoroalkyl chemicals in the U.S.
2 population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and
3 comparisons with NHANES 1999-2000. *Environ Health Perspect.* 2007;115(11):1596-602.
- 4 Centers for Disease Control and Prevention (CDC). Fourth Report on Human Exposure to Environmental
5 Chemicals, Updated Tables, (January 2017). Atlanta, GA: U.S. Department of Health and Human Services,
6 Centers for Disease Control and Prevention. <https://www.cdc.gov/exposurereport/>
- 7 Centers for Disease Control and Prevention. Fourth Report on Human Exposure to Environmental
8 Chemicals, Updated Tables, (March 2018). Atlanta, GA: U.S. Department of Health and Human Services,
9 Centers for Disease Control and Prevention. <https://www.cdc.gov/exposurereport/>.
- 10 Chang ET, Adami HO, Boffetta P, Wedner HJ, Mandel JS. A critical review of perfluorooctanoate and
11 perfluorooctanesulfonate exposure and immunological health conditions in humans. *Crit Rev Toxicol.*
12 2016;46(4):279-331.
- 13 Chang ET, Adami HO, Boffetta P, Cole P, Starr TB, Mandel JS. A critical review of perfluorooctanoate and
14 perfluorooctanesulfonate exposure and cancer risk in humans. *Crit Rev Toxicol.* 2014;44 Suppl 1:1-81.
- 15 Checkoway H, Pearce N, Kriebel D. *Research Methods in Occupational Epidemiology*, Second Edition.
16 Oxford U. Press 2004.
- 17 Christensen et al. Perfluoroalkyl substances and fish consumption. *Environ Res* 2017; 154:145-151.
- 18 Clair HB, Pinkston C, Pavuk M, Dutton ND, Brock G, Prough RA, Falkner CK, Wahlang B, McClaine CJ, and
19 Cave MC. High Prevalence of Environmental Liver Disease and Suspected Toxicant Associated
20 Steatohepatitis in a Large United States Residential Cohort with High Polychlorinated Biphenyl
21 Exposures. *Toxicol Sci.* 2018; 164(1):39-49.
- 22 Crawford NM, Fenton SE, Strynar M, Hines EP, Pritchard DA, Steiner AZ. Effects of perfluorinated
23 chemicals on thyroid function, markers of ovarian reserve, and natural fertility. *Reprod Toxicol.*
24 2017;69:53-59.
- 25 Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Høst A, Grandjean P, Jensen TK. Association
26 between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years
27 among 359 children in the Odense Child Cohort. *Environ Int.* 2016 Nov;96:58-64.
- 28 Daly ER, Chan BP, Talbot EA et al. Per- and polyfluoroalkyl substance (PFAS) exposure assessment in a
29 community exposed to contaminated drinking water, New Hampshire, 2015. *Int J Hyg Environ Health*
30 2018;221:569-577.
- 31 Darrow LA, Groth AC, Winquist A, Shin HM, Bartell SM, Steenland K. Modeled perfluorooctanoic acid
32 (PFOA) exposure and liver function in a Mid-Ohio Valley Community. *Environ Health Perspect*
33 2016;124:1227-1233.
- 34 Dhingra R, Winquist A, Darrow LA, Klein M, Steenland K. A Study of Reverse Causation: Examining the
35 Associations of Perfluorooctanoic Acid Serum Levels with Two Outcomes. *Environ Health Perspect.*
36 2017;125(3):416-421.

- 1 Domazet SL, Grøntved A, Timmermann AG, Nielsen F, Jensen TK. Longitudinal associations of exposure
2 to perfluoroalkylated substances in childhood and adolescence and indicators of adiposity and glycose
3 metabolism 6 and 12 years later: The European Youth Heart Study. *Diabetes Care* 2016;39(10):1745-51.
- 4 Domingo et al. Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. *Food Chemistry*
5 2012;135:1575-1582.
- 6 Emmett, EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM. Community Exposure to
7 Perfluorooctanoate: Relationships Between Serum Concentrations and Exposure Sources. *J Occ Environ*
8 *Med* 2006; 48(8):759-770.
- 9 Emmett, EA, Shofer FS, Freeman D, Desai C, Shaw LM. Community Exposure to Perfluorooctanoate:
10 Relationships Between Serum Levels and Certain Health Parameters. *J Occ Environ Med* 2006;48(8):771-
11 779.
- 12 Environmental Working Group. [https://www.ewg.org/research/report-110-million-americans-could-](https://www.ewg.org/research/report-110-million-americans-could-have-pfas-contaminated-drinking-water)
13 [have-pfas-contaminated-drinking-water](https://www.ewg.org/research/report-110-million-americans-could-have-pfas-contaminated-drinking-water). Accessed Nov 28, 2018.
- 14 Fàbrega F, Kumar V, Schuhmacher M, Domingo JL, Nadal M. PBPK modeling for PFOS and PFOA:
15 validation with human experimental data. *Toxicol Lett.* 2014 ;230(2):244-51.
- 16 Fàbrega F, Nadal M, Schuhmacher M, Domingo JL, Kumar V. Influence of the uncertainty in the
17 validation of PBPK models: A case-study for PFOS and PFOA. *Regul Toxicol Pharmacol.* 2016;77:230-9.
- 18 Fei C, Olsen J. Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at
19 age 7 years. *Environ Health Perspect.* 2011;119:573-578.
- 20 Feldstein AE, Alkhoury N, De Vito R, Alisi A, Lopez R, Nobili V. Serum cytokeratin-18 fragment levels are
21 useful biomarkers for nonalcoholic steatohepatitis in children. *Am J Gastroenterol.* 2013;108(9):1526-31.
- 22 Ferrante MC, Amero P, Santoro A, Monnolo A, Simeoli R, Di Guida F, Mattace Raso G, Meli R.
23 Polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) alter leptin signaling and lipid metabolism in
24 differentiated 3T3-L1 adipocytes. *Toxicol Appl Pharmacol.* 2014; 279(3):401-8.
- 25 Fisher M, Arbuckle TE, Wade M, Haines DA. Do perfluoroalkyl substances affect metabolic function and
26 plasma lipids?—Analysis of the 2007–2009, Canadian Health Measures Survey (CHMS) Cycle1. *Environ*
27 *Res* 2013;121:95-103.
- 28 Fitz-Simon N, Fletcher T, Luster MI, Steenland K, Calafat AM, Kato K, Armstrong B. Reductions in serum
29 lipids with a 4-year decline in serum perfluorooctanoic acid and perfluorooctanesulfonic acid.
30 *Epidemiology* 2013;24:569-76.
- 31 Flanders WD, Lin L, Pirkle JL, Caudill SP. Assessing the direction of causality in cross-sectional studies. *Am*
32 *J Epidemiol* 1992;135:926-935.
- 33 Flanders WD, Klein M, Mirabelli MC. Conditions for valid estimation of causal effects on prevalence in
34 cross-sectional and other studies. *Ann Epidemiol* 2016;26:389-394.
- 35 Fleisch AF, Rifas-Shiman SL, Mora AM, Calafat AM, Ye X, Luttmann-Gibson H, Gillman MW, Oken E, Sagiv
36 SK. Early-Life Exposure to Perfluoroalkyl Substances and Childhood Metabolic Function. *Environ Health*
37 *Perspect.* 2017;125(3):481-487.

- 1 Friedenreich CM. Methods for pooled analyses of epidemiologic studies. *Epidemiology*. 1993;4(4):295-
2 302.
- 3 Frisbee SJ, Brooks Jr AP, Maher A, et al. The C8 health project: design, methods, and participants.
4 *Environ Health Perspect* 2009;117:1873-1882.
- 5 Frisbee SJ, Shankar A, Knox SS, Steenland K, Savitz DA, Fletcher T, Ducatman AM. Perfluorooctanoic acid,
6 perfluorooctanesulfonate, and serum lipids in children and adolescents: Results from the C8 Health
7 Project. *Arch Pediatr Adolesc Med* 2010;164:860-869.
- 8 Fu Y, Wang T, Fu Q, Wang P, Lu Y. Associations between serum concentrations of perfluoroalkyl acids
9 and serum lipid levels in a Chinese population. *Ecotoxicol Environ Saf* 2014;106:246-52.
- 10 Gallo V, Leonardi G, Genser B, Lopez-Espinosa MJ, Frisbee SJ, Karlsson L, Ducatman AM, Fletcher T.
11 Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver
12 function biomarkers in a population with elevated PFOA exposure. *Environ Health Perspect*
13 2012;120:655–660.
- 14 Geiger SD, Xiao J, Shankar A. Positive association between perfluoroalkyl chemicals and hyperuricemia in
15 children. *Am J Epidemiol* 2013;177:1255-1262.
- 16 Gleason JA, Post GB, Fagliano JA. Associations of perfluorinated chemical serum concentrations and
17 biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. *Environ Res*.
18 2015;136:8-14 Gomis MI, Vestergren R, MacLeod M, Mueller JF, Cousins IT. Historical human exposure to
19 perfluoroalkyl acids in the United States and Australia reconstructed from biomonitoring data using
20 population-based pharmacokinetic modelling. *Environ Int* 2017;108:92-102.
- 21 Gomis MI, Vestergren R, MacLeod M, Mueller JF, Cousins IT. Historical human exposure to perfluoroalkyl
22 acids in the United States and Australia reconstructed from biomonitoring data using population-based
23 pharmacokinetic modelling. *Environ Int*. 2017;108:92-102.
- 24 Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, Heilmann C. Serum
25 Vaccine Antibody Concentrations in Children Exposed to Perfluorinated Compounds. *JAMA* 2012;307:
26 391–397.
- 27 Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Budtz-Jørgensen E. Serum vaccine antibody
28 concentrations in adolescents exposed to perfluorinated compounds. *Environ Health Perspect*
29 2017;125(7):077018.
- 30 Granum, Haug LS, Namork E, Stølevik SB, Thomsen C, Aaberge IS, van Loveren H, Løvik M, Nygaard UC.
31 Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels
32 and immune-related health outcomes in early childhood. *J Immunotoxicol* 2013; 10:4, 373-379.
- 33 Harada K, Inoue K, Morikawa A, Yoshinaga T, Saito N, Koizumi A. Renal clearance of perfluorooctane
34 sulfonate and perfluorooctanoate in humans and their species-specific excretion. *Environ Res* 2005;
35 99:253–261
- 36 He X, Liu Y, Xu B, Gu L, Tang W. PFOA is associated with diabetes and metabolic alteration in US men:
37 National Health and Nutrition Examination Survey 2003-2012. *Sci Total Environ* 2018;625:566-74.

- 1 Hennig, B., Oesterling, E., & Toborek, M. Environmental toxicity, nutrition, and gene interactions in the
2 development of atherosclerosis. *Nutr Metab Cardiovasc Dis.* 2007; 17(2):162-169.
- 3 Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. *Epidemiology.*
4 2004;15(5):615-25.
- 5 Hoffman K, Webster TF, Weisskopf MG, Weinberg J, Vieira VM. Exposure to polyfluoroalkyl chemicals
6 and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. *Environ Health Perspect*
7 2010;118(12):1762-7.
- 8 Innes KE, Ducatman AM, Luster MI, Shankar A. Association of osteoarthritis with serum levels of the
9 environmental contaminants perfluorooctanoate and perfluorooctane sulfonate in a large Appalachian
10 population. *Am J Epidemiol.* 2011;174(4):440-50.
- 11 Jain RB. Association between thyroid profile and perfluoroalkyl acids: data from NHANES 2007-2008.
12 *Environ Res.* 2013 Oct;126:51-9.
- 13 Ji K, Kim S, Kho Y, Paek D, Sakong J, Ha J, Kim S, Choi K. Serum concentrations of major perfluorinated
14 compounds among the general population in Korea: Dietary sources and potential impact on thyroid
15 hormones. *Environ Int* 2012;45:78-85.
- 16 Joensen UN, Veyrand B, Antignac JP, Blomberg Jensen M, Petersen JH, Marchand P, Skakkebaek NE,
17 Andersson AM, Le Bizec B, Jørgensen N. PFOS (perfluorooctanesulfonate) in serum is negatively
18 associated with testosterone levels, but not with semen quality, in healthy men. *Hum Reprod.*
19 2013;28(3):599-608.
- 20 Karlsen M, Grandjean P, Weihe P, Steuerwald U, Oulhote Y, Valvi D. Early-life exposures to persistent
21 organic pollutants in relation to overweight in preschool children. *Reprod Toxicol* 2017;68:145-153.
- 22 Kataria A, Trachtman H, Malaga-Dieguez L, Trasande L. Association between perfluoroalkyl acids and
23 kidney function in a cross-sectional study of adolescents. *Environ Health* 2015;14:89.
- 24 Kato K, Kalathil AA, Patel AM, Ye X, Calafat AM. Per- and polyfluoroalkyl substances and fluorinated
25 alternatives in urine and serum by on-line solid phase extraction-liquid chromatography-tandem mass
26 spectrometry. *Chemosphere.* 2018;209:338-345.
- 27 Khalil N, Chen A, Lee M, Czerwinski SA, Ebert JR, DeWitt JC, Kannan K. Association of Perfluoroalkyl
28 Substances, Bone Mineral Density, and Osteoporosis in the U.S. Population in NHANES 2009-2010.
29 *Environ Health Perspect.* 2016;124(1):81-7.
- 30 Knox SS, Jackson T, Frisbee SJ, Javins B, Ducatman AM. Perfluorocarbon exposure, gender and thyroid
31 function in the C8 Health Project. *J Toxicol Sci.* 2011;36(4):403-10.
- 32 Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, Halldorsson TI, Becher G, Haug
33 LS, Toft G. Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction.
34 *Hum Reprod.* 2013;28(12):3337-48.
- 35 Kray JB, Wightman SJ. Contaminants of emerging concern: A new frontier for hazardous waste and
36 drinking water regulation. *Natural Resources & Environment* 2018;32:36-40.
- 37 Lash TL, Fox MP, Fink AK. *Applying Quantitative Bias Analysis to Epidemiologic Data.* Springer (NY, 2009).

- 1 Lavrakas PJ ed. Encyclopedia of Survey Research Methods. Sage Publication, 2008.
- 2 Lien GW, Huang CC, Shiu JS, Chen MH, Hsieh WS, Guo YL, Chen PC. Perfluoroalkyl substances in cord
3 blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere.
4 2016;156:118-27.
- 5 Liew Z, Ritz B, von Ehrenstein OS, Bech BH, Nohr EA, Fei C, Bossi R, Henriksen TB, Bonefeld-Jørgensen EC,
6 Olsen J. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal
7 exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort.
8 Environ Health Perspect 2015;123(4):367-73.
- 9 Lin CY, Wen LL, Lin LY, Wen TW, Lien GW, Hsu SH, Chien KL, Liao CC, Sung FC, Chen PC, Su TC. The
10 associations between serum perfluorinated chemicals and thyroid function in adolescents and young
11 adults. J Hazard Mater 2013;244-245:637-644.
- 12 Lipsitch M, Tchetgen E, Cohen T. Negative controls: A tool for detecting confounding and bias in
13 observational studies. Epidemiol 2010;21:383-388.
- 14 Loccisano AE, Longnecker MP, Campbell JL Jr, Andersen ME, Clewell HJ 3rd. Development of PBPK
15 models for PFOA and PFOS for human pregnancy and lactation life stages. J Toxicol Environ Health A.
16 2013;76(1):25-57
- 17 Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, Fletcher T. Influenza Vaccine
18 Response in Adults Exposed to Perfluorooctanoate and Perfluorooctanesulfonate. Toxicol Sci 2014;
19 138(1): 76–88.
- 20 Lopez-Espinosa MJ, Fletcher T, Armstrong B, Genser B, Dhatariya K, Mondal D, Ducatman A, Leonardi G.
21 Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty
22 among children living near a chemical plant. Environ Sci Technol 2011;45:8160-8166.
- 23 Lopez-Espinosa MJ, Mondal D, Armstrong B, Bloom MS, Fletcher T. Thyroid function and perfluoroalkyl
24 acids in children living near a chemical plant. Environ Health Perspect 2012;120:1036-1041.
- 25 Lopez-Espinosa MJ, Mondal D, Armstrong BG, Eskenazi B, Fletcher T. Perfluoroalkyl substances, sex
26 hormones, and insulin-like growth factor-1 at 6–9 years of age: a cross-sectional analysis within the C8
27 Health Project. Environ Health Perspect 2016;124:1269-1275.
- 28 Maisonet M, Näyhä S, Lawlor DA, Marcus M. Prenatal exposures to perfluoroalkyl acids and serum lipids
29 at ages 7 and 15 in females. Environ Int 2015a;82:49-60.
- 30 Maisonet M, Calafat AM, Marcus M, Jaakkola JJ, Lashen H. Prenatal exposure to perfluoroalkyl acids and
31 serum testosterone concentrations at 15 years of age in female ALSPAC study participants. Environ
32 Health Perspect 2015b;123:1325-1330.
- 33 Maldonado G, Greenland S. Simulation Study of Confounder-Selection Strategies. Am J Epidemiol
34 1993;138: 923–36.
- 35 Mattsson K, Rignell-Hydbom A, Holmberg S, Thelin A, Jönsson BA, Lindh CH1, Sehlstedt A, Rylander L.
36 Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based
37 longitudinal study. Environ Res 2015;142:148-54.

1 Melzer D, Rice N, Depledge MH, Henley WE, Galloway TS. Association between serum perfluorooctanoic
2 acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. *Environ*
3 *Health Perspect* 2010;118:686–692.

4 Mora AM, Fleisch AF, Rifas-Shiman SL, Woo Baidal JA, Pardo L, Webster TF, Calafat AM, Ye X, Oken E,
5 Sagiv SK. Early life exposure to per- and polyfluoroalkyl substances and mid-childhood lipid and alanine
6 aminotransferase levels. *Environ Int*. 2018;111:1-13.

7 Nelson JW, Hatch EE, Webster TF. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight,
8 and insulin resistance in the general U.S. population. *Environ Health Perspect* 2010;118:197-202.

9 New Hampshire Division of Public Health Services, Department of Health and Human Services (NH
10 DHHS). Pease PFC Blood-testing Program: April 2015 – October 2015. Pease Tradeport, Portsmouth, NH,
11 June 2016. [https://www.dhhs.nh.gov/sites/g/files/ehbemt476/files/documents/2021-11/pease-pfc-
12 blood-testing.pdf](https://www.dhhs.nh.gov/sites/g/files/ehbemt476/files/documents/2021-11/pease-pfc-blood-testing.pdf)

13 Ngueta G, Longnecker MP, Yoon M, Ruark CD, Clewell HJ Rd, Andersen ME, Verner MA. Quantitative
14 bias analysis of a reported association between perfluoroalkyl substances (PFAS) and endometriosis:
15 The influence of oral contraceptive use. *Environ Int*. 2017;104:118-121.

16 Ode A, Kallen K, Gustafsson P, Rylander L, Jonsson BA, Olofsson P, Ivarsson SA, Lindh CH, Rignell-
17 Hydbom A. Fetal Exposure to Perfluorinated Compounds and Attention Deficit Hyperactivity Disorder in
18 Childhood. *PLoS ONE* 2014; 9(4): e95891.

19 Olsen GW, Zobel LR. Assessment of lipid, hepatic, and thyroid parameters with serum
20 perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. *Int Arch Occup*
21 *Environ Health*. 2007;81(2):231-46. Epub 2007 Jun 29.

22 Oulhote Y, Steuerwald U, Debes F, et al. Behavioral difficulties in 7-year old children in relation to
23 developmental exposure to perfluorinated alkyl substances. *Environ Int* 2016;97:237-245.

24 Porta M. ed. *A Dictionary of Epidemiology, Sixth Edition*, Edited for the International Epidemiological
25 Association. Oxford University Press (2014, NY) pp. 200, 246.

26 Qin XD, Qian Z, Vaughn MG, Huang J, Ward P, Zeng XW, Zhou Y, Zhu Y, Yuan P, Li M, Bai Z, Paul G, Hao
27 YT, Chen W, Chen PC, Dong GH, Lee YL. Positive associations of serum perfluoroalkyl substances with
28 uric acid and hyperuricemia in children from Taiwan. *Environ Pollution* 2016;212:519-524.

29 Rappazzo KM, Coffman E, Hines EP. Exposure to Perfluorinated Alkyl Substances and Health Outcomes
30 in Children: A Systematic Review of the Epidemiologic Literature. *Int. J. Environ. Res. Public Health* 2017,
31 14, 691.

32 Roetzheim RG, Freund KM, Corle DK, et al. Analysis of combined data from heterogeneous study
33 designs. *Clin Trials*. 2012; 9(2):176-87.

34 Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*, 3rd Edition. Walters Kluwer/ Lippincott
35 Williams & Wilkins, Philadelphia, PA. 2008.

36 Rothman KJ. Curbing type I and type II errors. *Eur J Epidemiol* 2010;25:223-224.

Rothman KJ. Six persistent research misconceptions. *J Gen Intern Med*. 2014;29:1060-4.

- 1 Ruark CD, Song G, Yoon M, Verner MA, Andersen ME, Clewell HJ 3rd, Longnecker MP. Quantitative bias
2 analysis for epidemiological associations of perfluoroalkyl substance serum concentrations and early
3 onset of menopause. *Environ Int.* 2017;99:245-254.
- 4 Sakr CJ, Kreckmann KH, Green JW, Gillies PJ, Reynolds JL, Leonard RC. Cross-sectional study of lipids and
5 liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as
6 part of a general health survey in a cohort of occupationally exposed workers. *J Occup Environ Med.*
7 2007a;49(10):1086-96.
- 8 Sakr CJ, Leonard RC, Kreckmann KH, Slade MD, Cullen MR. Longitudinal study of serum lipids and liver
9 enzymes in workers with occupational exposure to ammonium perfluorooctanoate. *J Occup Environ*
10 *Med.* 2007b;49(8):872-9
- 11 Santoro N, Feldstein AE, Enoksson E, Pierpont B, Kursawe R, Kim G, Caprio S. The association between
12 hepatic fat content and liver injury in obese children and adolescents: effects of ethnicity, insulin
13 resistance, and common gene variants. *Diabetes Care.* 2013;36(5):1353-60.
- 14 Shankar A, Xiao J, Ducatman A. Perfluoroalkyl chemicals and elevated serum uric acid in US adults. *Clin*
15 *Epidemiol* 2011;3:251-258.
- 16 Shankar A, Xiao J, Ducatman A. Perfluorooctanoic acid and cardiovascular disease in US adults. *Arch*
17 *Intern Med* 2012;172:1397-403.
- 18 Shen J, Chan HL, Wong GL, Choi PC, Chan AW, Chan HY, Chim AM, Yeung DK, Chan FK, Woo J, Yu J, Chu
19 WC, Wong VW. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers.
20 *J Hepatol.* 2012;56(6):1363-70.
- 21 Shin HM, Vieira VM, Ryan PB et al. Retrospective exposure estimation and predicted versus observed
22 serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. *Environ Health*
23 *Perspect* 2011;119:1760-1765.
- 24 Stang A, Poole C, Kuss O: The ongoing tyranny of statistical significance testing in biomedical research.
25 *Eur J Epidemiol* 2010, 25:225-30.
- 26 Steenland K, Tinker S, Frisbee S, Ducatman A, Vaccarino V. Association of perfluorooctanoic acid and
27 perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. *Am J Epidemiol*
28 2009;170:1268-1278.
- 29 Steenland K Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with
30 uric acid among adults with elevated community exposure to PFOA. *Environ Health Perspect*
31 2010;118:229–233.
- 32 Steenland K, Zhao L, Winqvist A, Parks C. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly
33 exposed population of community residents and workers in the mid-Ohio valley. *Environ Health*
34 *Perspect.* 2013;121(8):900-5.
- 35 Steenland K, Zhao L, Winqvist A. A cohort incidence study of workers exposed to perfluorooctanoic acid
36 (PFOA). *Occup Environ Med.* 2015;72(5):373-80.
- 37 Stein CR, Savitz DA. Serum perfluorinated compound concentration and attention deficit/hyperactivity
38 disorder in children 5-18 years of age. *Environ Health Perspect* 2011;119:1466-1471.

- 1 Stein CR, Savitz DA, Bellinger DC. Perfluorooctanoate and neuropsychological outcomes in children.
2 *Epidemiology* 2013;24:590-599.
- 3 Stein CR, Savitz DA, Elston B, Thorpe PG, Gilboa SM. Perfluorooctanoate exposure and major birth
4 defects. *Reprod Toxicol.* 2014a;47:15-20.
- 5 Stein CR, Savitz DA, Bellinger DC. Perfluorooctanoate exposure in a highly exposed community and
6 parent and teacher reports of behavior in 6-12-year-old children. *Paediatr Perinat Epidemiol*
7 2014b;28:146-156. Stein CR, McGovern KJ, Pajak AM, Maglione PJ, Wolff MS. Perfluoroalkyl and
8 Polyfluoroalkyl Substances and Indicators of Immune function in children aged 12–19 y: National Health
9 and Nutrition Examination Survey. *Pediatr Res.* 2016a; 79(2):348-57.
- 10 Stein CR, Ge Y, Wolff MS et al. Perfluoroalkyl substance serum concentrations and immune response to
11 FluMist vaccination among healthy adults. *Environ Res* 2016b;149:171-178.
- 12 Sun Q, Zong G, Valvi D, Nielsen F, Coull B, Grandjean P. Plasma concentrations of perfluoroalkyl
13 substances and risk of type 2 diabetes: A prospective investigation among US Women. *Environ Health*
14 *Perspect* 2018;126(3):037001.
- 15 Trochim WMK. *Research Methods Knowledge Base.* 2006.
16 <https://socialresearchmethods.net/kb/sampron.php>.
- 17 Tyrer S, Heyman B. Sampling in epidemiological research: issues, hazards and pitfalls. *BJPsych Bull.*
18 2016;40(2):57-60.
- 19 Uhl SA, James-Todd J, Bell ML. Association of Osteoarthritis with Perfluorooctanoate and
20 Perfluorooctane Sulfonate in NHANES 2003–2008. *Environ Health Perspect* 2013;121:447-452.
- 21 U.S. Environmental Protection Agency (EPA). Provisional Health Advisories for Perfluorooctanoic Acid
22 (PFOA) and Perfluorooctyl Sulfonate (PFOS); 2009. Available from
23 http://water.epa.gov/action/advisories/drinking/upload/2009_01_15_criteria_drinking_pfoa_pfos.pdf.
24
- 25 U.S. Environmental Protection Agency (EPA). Fact Sheet – PFOS & PFOA Drinking Water Health
26 Advisories. Ground Water and Drinking Water; 2016a. Available from:
27 [https://www.epa.gov/sites/production/files/2016-
28 06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.16.pdf](https://www.epa.gov/sites/production/files/2016-06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.16.pdf).
- 29 U.S. Environmental Protection Agency (EPA). Third Unregulated Contaminant Monitoring Rule, July
30 2016b. Available from: [https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant
31 monitoring-rule#3](https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule#3).
- 32 Verner MA, Longnecker MP. Comment on "enhanced elimination of perfluorooctanesulfonic Acid by
33 menstruating women: evidence from population-based pharmacokinetic modeling". *Environ Sci Technol.*
34 2015;49(9):5836-7.
- 35 Verner MA, Luccisano AE, Morken NH, Yoon M, Wu H, McDougall R, Maisonet M, Marcus M, Kishi R,
36 Miyashita C, Chen MH, Hsieh WS, Andersen ME, Clewell HJ 3rd, Longnecker MP. Associations of
37 perfluoroalkyl substances (PFAS) with lower birth weight: an evaluation of potential confounding by
38 glomerular filtration rate using a physiologically based pharmacokinetic model (PBPK). *Environ Health*
39 *Perspect* 2015;123(12):1317-24.

- 1 Verner MA, Ngueta G, Jensen ET, Fromme H, Völkel W, Nygaard UC, Granum B, Longnecker MP. A
2 Simple Pharmacokinetic Model of Prenatal and Postnatal Exposure to Perfluoroalkyl Substances (PFASs).
3 *Environ Sci Technol*. 2016; 19;50(2):978-86.
- 4 Vos MB, Barve S, Joshi-Barve S, Carew JD, Whittington PF, McClain CJ. Cytokeratin 18, a marker of cell
5 death, is increased in children with suspected nonalcoholic fatty liver disease. *J Pediatr Gastroenterol*
6 *Nutr*. 2008 Oct;47(4):481-5.
- 7 Vuong AM, Yolton K, Webster GM, Sjödin A, Calafat AM, Braun JM, Dietrich KN, Lanphear BP, Chen A.
8 Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and executive function
9 in school-age children. *Environ Res*. 2016;147:556-64.
- 10 Wahlang B, Prough RA, Falkner KC, Hardesty JE, Song M, Clair HB, Clark BJ, States JC, Arteel GE, Cave MC.
11 Polychlorinated Biphenyl-Xenobiotic Nuclear Receptor Interactions Regulate Energy Metabolism,
12 Behavior, and Inflammation in Non-Alcoholic-Steatohepatitis. *Toxicol Sci*. 2016;149(2):396-410.
- 13 Wang JJ, Hsieh WS, Chen CY, Fletcher T, Lien GW, Chiang HL, Chiang CF, Wu TN, Chen PC. The effect of
14 prenatal perfluorinated chemicals exposures on pediatric atopy. *Environ Res* 2011; 111:785–791.
- 15 Wang Z, DeWitt JC, Higgins CP, Cousins IT. A never-ending story of per- and polyfluoroalkyl substances
16 (PFASs)? *Environ Sci Technol* 2017;51:2508-2518.
- 17 Weisskopf MG, Webster TF. Trade-offs of personal versus more proxy exposure measures in
18 environmental epidemiology. *Epidemiol* 2017;28:635-643.
- 19 Weisskopf MG, Seals RM, Webster TF. Bias Amplification in Epidemiologic Analysis of Exposure to
20 Mixtures. *Environ Health Perspect* 2018;126:047003.
- 21 Wen LL, Lin LY, Su TC, Chen PC, Lin CY. Association between serum perfluorinated chemicals and thyroid
22 function in U.S. adults: The National Health and Nutrition Examination Survey 2007–2010. *J Clin*
23 *Endocrinol Metab* 2013;98:E1456-E1464.
- 24 Winquist A, Steenland K. Perfluorooctanoic acid exposure and thyroid disease in community and worker
25 cohorts. *Epidemiology* 2014a;25:255-264.
- 26 Winquist A, Steenland K. Modeled PFOA exposure and coronary artery disease, hypertension, and high
27 cholesterol in community and worker cohorts. *Environ Health Perspect* 2014b; 122:1299-1305.
- 28 Wieser V, Moschen AR, Tilg H. Inflammation, cytokines and insulin resistance: a clinical perspective. *Arch*
29 *Immunol Ther Exp (Warsz)*. 2013; 61(2):119-25.
- 30 Wong F, MacLeod M, Mueller JF, Cousins IT. Enhanced elimination of perfluorooctane sulfonic acid by
31 menstruating women: evidence from population-based pharmacokinetic modeling. *Environ Sci Technol*.
32 2014;48(15):8807-14.
- 33 Worley RR, Yang X, Fisher J. Physiologically based pharmacokinetic modeling of human exposure to
34 perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting
35 current serum concentrations. *Toxicol Appl Pharmacol*. 2017a;330:9-21.

- 1 Worley RR, Moore, SM, Tierney BC, Ye X, Calafat AM, Campbell S, Woudneh MB, Fisher J. Per- and
2 polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community.
3 Environ Int. 2017b;106:135-143.
- 4 Ye X, Kato K, Wong LY, Jia T, Kalathil A, Latremouille J, Calafat AM. Per- and Polyfluoroalkyl Substances in
5 Sera from Children 3 to 11 Years of Age Participating in the National Health and Nutrition Examination
6 Survey 2013–2014. Int J Hyg Environ Health 2017;Sep29: S1438-4639(17)30588-6 [Epub ahead of print]
- 7 Zeng XW, Qian Z, Emo B, Vaughn M, Bao J, Qin XD, Zhu Y, Li J, Lee YL, Dong GH. Association of
8 polyfluoroalkyl chemical exposure with serum lipids in children. Sc Total Environ 2015;512-513;364-370.
- 9 Zhang T, Sun H, Qin X, Gan Z, Kannan K. PFOS and PFOA in paired urine and blood from general adults
10 and pregnant women: assessment of urinary elimination. Environ Sci Pollut Res Int. 2015;22(7):5572-9.
- 11 Zhou Y, Hu LW, Qian ZM, Chang JJ, King C, Paul G, Lin S, Chen PC, Lee YL, Dong GH. Association of
12 perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status.
13 Environ International 2016;94:189-195.
14
15

1	7. LIST OF ATTACHMENTS
2	
3	Attachment 1. Investigators and Key Study Personnel
4	Attachment 2. Biochemical Analytical Plan in Children and Adults
5	Attachment 3. Justification for Sample Size Calculations
6	Attachment 3a. Sample Size for Child Study
7	Attachment 3b. Sample Size for Adult Study
8	Attachment 4. Eligibility Screening Script
9	Attachment 5 –Recruitment Materials
10	Attachment 6 – Recruitment Tracking Form
11	Attachment 7 – Appointment Packet
12	Attachment 7a – Appointment Reminder Card
13	Attachment 7b – Informed Consent Packet
14	Attachment 7b1 – Privacy Act Statement
15	Attachment 7b2 – Parental Permission and Child Assent Forms
16	Attachment 7b3 – Parental Consent to Release Student Information
17	Attachment 7b4 – Adult Consent Form
18	Attachment 7b5 – Parent/Child/Adult Permission for Medical Record Abstraction
19	Attachment 7c – Study Fact Sheet
20	Attachment 8 – Appointment Reminder Telephone Script
21	Attachment 9 – Appointment Tracking Form
22	Attachment 10 – Update Contact Information Hardcopy Form
23	Attachment 11 – Medication List
24	Attachment 12 – Manual of Procedures
25	Attachment 13 – Body and Blood Pressure Measures Form
26	Attachment 14 – Blood Draw and Urine Collection Form
27	Attachment 15 – Child Questionnaire – Long Form
28	Attachment 15a – Child Questionnaire – Short Form
29	Attachment 16 – Adult Questionnaire
30	Attachment 17 – Request for Medical Record Abstraction
31	Attachment 17a – Medical Record Abstraction Form
32	Attachment 17b – Medical Record Abstraction Form
33	Attachment 18 – Child/Parent Neurobehavioral Test Battery
34	Attachment 18a – NBT Time Estimation Table, by Age in Years
35	Attachment 18b – Request for Child School Record Abstraction
36	Attachment 18c – Child School Record Abstraction Form
37	Attachment 19 – Body and Blood Pressure Measurements Report
38	Attachment 20 – Advance Reporting Script for Clinical Tests
39	Attachment 20a – Advance Clinical Test Report Tracking Form
40	Attachment 20b – Letter Report of Critical Values
41	<i>Attachment 21 – Clinical Test Results Report</i>
42	Attachment 22 – PFAS Results Report
43	Attachment 22a – ATSDR PFAS Factsheet
44	

1 **8. APPENDICES**

2 Appendix A – Summary of Site Investigators, Research Plans, and Informed Consent

3 Appendix B- Communications Toolkit