

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

(The Multi-site Study)

New Information Collection Request

Supporting Statement Part B –

Collections of Information Employing Statistical Methods

Principal Investigator: Marian Pavuk, MD, PhD

Title: Senior Epidemiologist, Environmental Epidemiology Branch

Email: [fsh8@cdc.gov](mailto:fsh8@cdc.gov)

Phone: 770-488-3671

Fax: 770-488-1541

Date: December 12, 2019

# Table of Contents

---

B.1. Respondent Universe and Sampling Methods.....	3
B.2. Procedures for the Collection of Information.....	5
B.3. Methods to Maximize Response Rates and Deal with Non-response.....	6
B.4. Test of Procedures or Methods to be Undertaken.....	8
B.5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data.....	9
References.....	9
List of Appendices.....	10
Multi-site Study Protocol and Attachments.....	10

## Part B. Collections of Information Employing Statistical Methods

---

### B.1. Respondent Universe and Sampling Methods

Statistical methods will be used to recruit participants for the Multi-site Study where applicable. If information is not available to use statistically based methods, non-probabilistic sampling methods (e.g., “snowballing” sampling or respondent-driven sampling), may be used. The respondent universe and the rationale for using statistically based or non-probabilistic sampling methods are described in the **Multi-site Study Protocol**. In general, the recruitment strategies are to be “exposure-driven” in order to achieve a wide distribution of exposure levels among study participants. The main goals of the research study are to examine associations between health outcomes and measured and historically reconstructed serum levels of PFAS.

Respondent Universe: In summary, recipients and ATSDR will enroll approximately 9,100 participants (7,000 adults and 2,100 children and their parents). Each cooperative agreement recipient will attempt to meet a target recruitment of 300 children and 1,000 adults.

The study populations and eligibility are discussed in the **Multi-site Protocol Section 3.2**. Statistical justification for desired sample sizes is provided in **Multi-site Study Protocol Section 3.3** and in more detail in **Attachment 3**.

Adults will be 18 years or older, and children will be 4-17 years of age at enrollment. Ideally, the parent should be the mother, who can best answer some survey questions about the child’s exposures and about the mother’s pregnancy and breastfeeding history. A parent can enroll with more than one child. In this case, the recipients (ATSDR awardees) will enroll each child separately along with his or her parent. Parents, if eligible, may also enroll in the adult study.

To restrict this study to drinking water exposures, any adult occupationally exposed to PFAS will not be eligible for the study (i.e. ever firefighters or in chemical manufacture). Likewise, children whose birth mothers were occupationally exposed will not be eligible. This restriction applies to both the exposure and the referent group.

The cooperative agreement recipients’ sites include communities whose drinking water was impacted by AFFF use at military bases or by industrial PFAS releases. Site selection considerations included the levels of PFAS drinking water concentrations at a site, the size of the population exposed, the experience of the researchers in conducting drinking water epidemiological studies, and geographic coverage. A key aim was to select sites so that a wide range in PFAS exposures levels were included in the study in order to enable the evaluation of

exposure-response trends including effects at the lower and upper ranges of exposures. A ground water contaminant fate and transport model and a water system distribution model may be necessary to identify the areas with contaminated drinking water, determine the period when the drinking water was contaminated, and historically reconstruct PFAS drinking water concentrations.

Based on ATSDR's literature review of epidemiological studies of PFAS (ATSDR 2017), the study will examine associations between PFAS compounds and lipids, renal function and kidney disease, thyroid hormones and disease, liver function and disease, glycemic parameters and diabetes, as well as immune response and function in both children and adults. In addition, the study will investigate PFAS differences in sex hormones and sexual maturation, vaccine response, and neurobehavioral outcomes in children. In adults, additional outcomes of interest include cardiovascular disease, osteoarthritis and osteoporosis, endometriosis, and autoimmune disease.

Sampling Methods: The Multi-site Study will be cross-sectional in design. If feasible, the recipient shall identify and enumerate all households served by the contaminated drinking water supply in the selected community in order to recruit potential participants to meet the sample size requirements for children and adults. If enumeration of all households is not feasible, then the recipient should consider non-probabilistic sampling approaches.

Recruitment methods are described in the **Multi-site Study Protocol Section 3.5**. See further discussion in **Section A.2 of Supporting Statement A – Justification**.

Trained study staff will recruit, screen for eligibility, and enroll participants (**Attachment 4**). ATSDR assumes that 5 percent of the people who volunteer will not meet eligibility requirements. For purposes of annualized time and cost estimation, ATSDR assumes a 40 percent response rate across all sites.

Statistical sampling methods (e.g., a two stage cluster sample) will be used for recruitment of study participants if all the affected households can be enumerated.

- If the PFAS drinking water concentrations vary widely across the community, then the recipient should consider using targeted sampling approaches including oversampling of areas with higher PFAS concentrations in order to ensure a sufficiently wide distribution of exposure levels among study participants to evaluate exposure-response trends.
- If enumeration of all households is not feasible, or if participation rates are expected to be low, then the recipient should consider non-probabilistic sampling approaches such as “judgement” and “snowball” sampling approaches (Tyrer 2016).

Steps in screening are:

- Administer the eligibility screening scripts and schedule appointments (**Attachment 4**).
- Begin tracking the recruitment process (**Attachment 6**).
- Mail out appointment packets (**Attachment 7**), which will contain the following documents to keep and read before their appointments:
  - Appointment reminder cards (**Attachment 7a**), with instructions on how to prepare for the appointment
  - Informed consent packets (**Attachment 7b**),
    - Privacy Act Statement (**Attachment 7b1**)
    - Parental Permission and Child Assent Forms (**Attachment 7b2**)
    - Parental Consent to Release Student Information (**Attachment 7b3**)
    - Adult Consent Form (**Attachment 7b4**)
    - Parent/Child/Adult Permission for Medical Record Abstraction (**Attachment 7b5**)
  - Study Fact Sheet (**Attachment 7c**)
- Encourage participation with appointment reminder calls (**Attachments 8&9**).

## B.2. Procedures for the Collection of Information

At the appointment, enrollment and data collection procedures are described in the **Multi-site Study Protocol Section 3.5.3, Section 3.6**, and in the Manual of Procedures for staff and contractor training (**Attachment 12**). Steps in enrollment are:

- Administration of informed consent, parental permission, and child assent (**Attachment 7b**).
- Update participant contact information, if needed (**Attachment 10**).
- Record participant medication list (**Attachment 11**).
- Take body and blood pressure measures (**Attachment 13**).
- Collect blood and urine biospecimens (**Attachment 14**).
- Administer questionnaire (**Attachment 15, 15a, 16**).
- (For children and parents) Administer the neurobehavioral test battery (**Attachment 18**).

After the appointment, ATSDR recipients will seek:

- Approval for medical record abstraction from medical office administrators (**Attachment 17**).

- Medical record verification for self-reported conditions noted in the questionnaire (**Attachment 17a-b**).
- Approval for education record abstraction from school administrators (**Attachment 18b**).
- Education record verification to compare to the results of the children's neurobehavioral assessments and their parents' assessments of their children (**Attachment 18c**).

### B.3. Methods to Maximize Response Rates and Deal with Non-response

The **Multi-site Study Protocol Section 3.3.1** describes the estimated number of eligible children and assumptions about participation rates needed to achieve statistical goals:

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

The proposed sample size of 2,000 children is large enough to effectively evaluate many of the health outcomes identified in the Pease Feasibility Assessment literature review and the recent systematic review (Rapazzo 2017) as potentially associated with PFAS in children. The health outcomes and biomarkers studied would include mean difference in total cholesterol (ranging from 156 to 637 per stratum), uric acid levels (556 per stratum), estimated glomerular filtration rate (eGFR; 275 per stratum), testosterone (about 400 per stratum) and insulin growth factor-1 (IGF-1; 146 per stratum). Based on our estimations, we would also be able to detect differences in risk for obesity and atopic dermatitis. A sample size of 2,000 children would be larger than many of the PFAS studies that evaluated neurobehavioral outcomes such as IQ and ADHD (Wang 2015, Stein 2013, 2014, Fei 2011, Hoffman 2010, Strom 2014).

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

In summary, a total sample size of  $\geq 2,000$  would be sufficient to evaluate a wide range of biomarkers and outcomes including lipids (and hypercholesterolemia), uric acid (and hyperuricemia), estimated glomerular filtration rate, testosterone, IGF-1, neurobehavioral measures (executive function, attention, IQ) and ADHD, rhinitis, and obesity.”

The **Multi-site Study Protocol Section 3.3.2** and Attachment 3 describe the estimated number of eligible adults and assumptions about participation rates needed to achieve statistical goals:

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

The proposed sample size of 6,000 adults is large enough to effectively evaluate many of the health outcomes identified in the Pease Feasibility Assessment literature review. For example, for outcomes like elevated lipids levels (cholesterol) or uric acid, the range of 229 to 660 participants per stratum (i.e. quartile) or 200 to 550 per stratum, respectively, given observed differences would be needed. That would translate to overall sample size of about 800 to 2,600 participants being sufficient to detect differences at the specified level of precision and power (Steenland, 2009, 2010; Fisher 2013; Shankar 2011). Similar sample sizes would also be required to compare other common health outcomes such as cardiovascular disease (Shankar 2012). Larger samples sizes would be needed for liver function or osteoarthritis, with a total sample in the range of 3,000 to 4,000 subjects (Uhl 2013; Gallo 2012; Steenland 2010).

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

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

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

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

for fatty liver disease. It also may be sufficient to evaluate thyroid disease, thyroid function and liver function.”

In order to maximize participation in the Multi-site Study, ATSDR recipients will have the flexibility to schedule or re-schedule office or home visits within the study period (**Multi-site Study Protocol Section 3.5.3**).

- Interested recruits who are unable or unwilling to come to the study office, will be offered an in-home appointment by trained study staff to complete the study. Interested recruits who request or require a home interview, blood draw, and urine collection must reside within a one-hour drive from the study office.
- Study staff will give the interested recruit a reminder telephone call one to two days before the scheduled appointment (**Attachment 6**).
  - The study staff will make up to five contact attempts to an interested recruit who misses an appointment in order to reschedule the appointment and maximize the number of completed appointments (**Attachment 9**).

## B.4. Test of Procedures or Methods to be Undertaken

The **Multi-site Study Protocol** builds on activities undertaken in preparation of data collection for the ATSDR proof of concept research study currently being conducted of the Pease International Tradeport population (Portsmouth, NH) exposed to PFAS-contaminated drinking water (OMB Control No. 0923-0061). Several modifications to the Multi-Site Study protocol and questionnaire were made based on these activities including:

- modification of the childhood neurobehavioral test battery to minimize burden,
- adjustment of the volume of blood to be collected from adults and children to ensure sufficient quantities for the clinical biomarker tests,
- modification of the childhood questionnaire,
- refinements to the medical records abstraction forms, and
- adaptation of the Pease data management system and community engagement strategy.

In addition, the protocol includes additional, quantitative bias analyses based on peer reviewer and OMB comments to the Pease Study protocol.

Some of the proposed data collection instruments for the Multi-Site Study have also been based on those successfully used in the “Anniston Community Health Survey: Follow up and



Dioxin Analyses (ACHS-II)” (OMB Control No. 0923-0049; discontinued 11/12/2015)  
**(Attachments 10, 11, 13, 14).**

In the Pease Study, exposures to PFAS-contaminated drinking water occurred primarily at workplaces and day care centers at the Pease International Tradeport. Therefore, the Pease Study questionnaire has been modified for the Multi-Site Study to account for a likely different exposure scenario, i.e., drinking water exposures occurring primarily at the residence **(Attachments 15, 15a, 16)**. The eligibility screeners **(Attachment 4)**, medical records abstraction forms **(Attachments 17a, 17b)**, and the school records abstraction form **(Attachment 18c)** are the same forms as those currently being used in the Pease Study.

## B.5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

Table B.5.1. Personnel Consulted on Statistical Design

Name	Title	Affiliation	Phone	Email
<i>FEDERAL AGENCY</i>				
Marian Pavuk, MD, PhD	Senior Epidemiologist, PI Multi-site Study	ATSDR	(770) 488-3671	<a href="mailto:fsh8@cdc.gov">fsh8@cdc.gov</a>
Frank Bove, ScD, MS	Senior Epidemiologist, PI Multi-site Study	ATSDR	(770) 488-3809	<a href="mailto:fjb0@cdc.gov">fjb0@cdc.gov</a>

Table B.5.2. Personnel Responsible for Collection and Analysis of Information

Name	Title	Affiliation	Phone	Email
Marian Pavuk, MD, PhD	Senior Epidemiologist, PI Multi-site Study	ATSDR	(770) 488-3671	<a href="mailto:fsh8@cdc.gov">fsh8@cdc.gov</a>
Frank Bove, ScD, MS	Senior Epidemiologist, PI Multi-site Study	ATSDR	(770) 488-3809	<a href="mailto:fjb0@cdc.gov">fjb0@cdc.gov</a>
Michael Lewin, MS	Mathematical Statistician	ATSDR	(770) 488-3812	<a href="mailto:mdl0@cdc.gov">mdl0@cdc.gov</a>
Contractor Name7, PhD	Title7	Company F	(xxx) xxx-xxxx	<a href="mailto:name7@companyf.com">name7@companyf.com</a>
Contractor Name8, PhD	Ttitle8	Company F	(xxx) xxx-xxxx	<a href="mailto:name8@companyf.com">name8@companyf.com</a>
Contractor Name9, PhD	Ttitle9	Company F	(xxx) xxx-xxxx	<a href="mailto:name9@companyf.com">name9@companyf.com</a>

## References

Agency for Toxic Substances and Disease Registry (ATSDR). Feasibility Assessment for Epidemiological Studies at Pease International Tradeport. Portsmouth, New Hampshire. November 2017. Available at: [https://www.atsdr.cdc.gov/sites/pease/documents/Pease\\_Feasibility\\_Assessment\\_November-2017\\_508.pdf](https://www.atsdr.cdc.gov/sites/pease/documents/Pease_Feasibility_Assessment_November-2017_508.pdf)

Tyrer S, Heyman B. Sampling in epidemiological research: issues, hazards and pitfalls. *BJPsych Bull.* 2016;40(2):57-60.

## List of Appendices

- Appendix A. Authorizing Legislation
- Appendix B. 60-day Federal Register Notice
  - Appendix B1. Public Comments and Program Responses
- Appendix C. Notice of Funding Opportunity (NOFO)
- Appendix D. ATSDR Pease Feasibility Assessment
- Appendix E. Privacy Impact Assessment
- Appendix F. IRB Approval Memo
- Appendix G. Data Sharing and Disclosure Review

## Multi-site Study Protocol and Attachments

- Attachment 1. Investigators and Key Study Personnel
- Attachment 2. Biochemical Analytical Plan in Children and Adults
- Attachment 3. Justification for Sample Size Calculations
  - Attachment 3a. Sample Size for Child Study
  - Attachment 3b. Sample Size for Adult Study
- Attachment 4. Eligibility Screening Script
- Attachment 5. Recruitment Materials
- Attachment 6. Recruitment Tracking Form
- Attachment 7. Appointment Packet
  - Attachment 7a. Appointment Reminder Card
  - Attachment 7b. Informed Consent Packet
    - Attachment 7b1. Privacy Act Statement
    - Attachment 7b2. Parental Permission and Child Assent Forms
    - Attachment 7b3. Parental Consent to Release Student Information
    - Attachment 7b4. Adult Consent Form
    - Attachment 7b5. Parent/Child/Adult Permission for Medical Record Abstraction
  - Attachment 7c. Study Fact Sheet
- Attachment 8. Appointment Reminder Telephone Script
- Attachment 9. Appointment Tracking Form
- Attachment 10. Update Contact Information Hardcopy Form
- Attachment 11. Medication List
- Attachment 12. Manual of Procedures
- Attachment 13. Body and Blood Pressure Measures Form
- Attachment 14. Blood Draw and Urine Collection Form

Attachment 15. Child Questionnaire – Long Form  
Attachment 15a. Child Questionnaire – Short Form  
Attachment 16. Adult Questionnaire  
Attachment 17. Request for Medical Record Abstraction  
Attachment 17a. Medical Record Abstraction Form - Adult  
Attachment 17b. Medical Record Abstraction Form - Child  
Attachment 18. Child/Parent Neurobehavioral Test Battery  
Attachment 18a. NBT Time Estimation Table, by Age in Years  
Attachment 18b. Request for Child School Record Abstraction  
Attachment 18c. Child School Record Abstraction Form  
Attachment 19. Body and Blood Pressure Measurements Report  
Attachment 20. Advance Reporting Script for Clinical Tests  
Attachment 20a. Advance Clinical Test Report Tracking Form  
Attachment 20b. Letter Report of Critical Values  
Attachment 21. Clinical Test Results Report  
Attachment 22. PFAS Results Report  
Attachment 22a. ATSDR PFAS Factsheet