Research Protocol: Evaluating the Association between Serum Concentrations of Per- and Polyfluoroalkyl Substances (PFAS) and Symptoms and Diagnoses of Selected Acute Viral Illnesses

Funded and Sponsored By:

Agency for Toxic Substances and Disease Registry (ATSDR) and the Centers for Disease Control and Prevention's (CDC) National Center for Environmental Health (NCEH)
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Authority

ATSDR and CDC are authorized to conduct this study under the 1980 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), as amended by the 1986 Superfund Amendments and Reauthorization Act (SARA) (42 U.S.C. 9601, 9604), and the Public Health Service Act Section 301 (42 U.S.C. 241) and Section 311 (42 U.S.C. 243), respectively.

Potential Conflicts of Interest

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

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Abbreviations and Acronyms

ATSDR	Agency for Toxic Substances and Disease Registry
COVID-19	Coronavirus Disease 2019
CSV	Comma-Separated Values
EA	Exposure Assessment
EPA	Environmental Protection Agency
MeFOSAA	N-methyl perfluorooctane sulfonamido acetic acid
NCEH	National Center for Environmental Health
NHANES	National Health and Nutrition Examination Survey
PEATT	PFAS Exposure Assessment Technical Tools
PFAS	Per- and polyfluoroalkyl substances
PFDA	perfluorodecanoic acid
PFDoA	perfluorododecanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFUnA	perfluoroundecanoic acid
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

Title

"PFAS and Viral Infections Study" formally titled *Evaluating the Association between Serum Concentrations of Per- and Polyfluoroalkyl Substances (PFAS) and Symptoms and Diagnoses of Selected Acute Viral Illnesses*

Protocol Summary

In 2019 and 2020, the Agency for Toxic Substances and Disease Registry (ATSDR) conducted statistically based biomonitoring PFAS exposure assessments (EAs) in eight communities that had documented exposures to PFAS in drinking water. ATSDR, in partnership with the Association of State and Territorial Health Officials and the New York State Department of Health and Pennsylvania Department of Health, also supported two EAs that were designed to test the PFAS Exposure Assessment Technical Tools (PEATT). PFAS concentrations were measured in serum collected from EA and PEATT assessment participants, and a questionnaire was administered to gather information to characterize each individual's exposure. During the same period, ATSDR initiated a health study at the Pease International Tradeport that included measurement of participants' serum-PFAS concentrations and collection of information about individual exposures.

This protocol describes a follow-up longitudinal study that will recruit participants from the existing EA, PEATT assessment, and Pease Study cohort members who have existing serum-PFAS measurements and who have given prior consent for additional contact from CDC/ATSDR.

The National Toxicology Program concluded that there is evidence from human and animal studies that PFAS exposure reduces antibody responses to vaccines and infectious disease resistance (NTP, 2016). However, the results of previous epidemiologic studies of PFAS exposure and susceptibility to viral infection among adults are somewhat inconsistent and existing studies on PFAS and COVID-19 are sparse. CDC/ATSDR will invite participants to complete a new series of surveys to determine whether PFAS exposure increases susceptibility to viral infections including, but not limited to, COVID-19. Surveys will be administered in 5 rounds spaced by 3 months, over the course of one calendar year. The initial survey will be delivered in hardcopy by mail, with follow-up surveys delivered through email links to the secure web-based CDC REDCap platform, or through optional mailed hardcopies. In total, each participant will be asked to participate in the study for 12-14 months and will spend a total of about 2.5 hours responding to 5 total surveys (i.e., each survey should take about 30 minutes to complete, and this will be done quarterly).

On the initial survey (Appendix A and B):

- Participants will be asked about height, weight, influenza vaccination history, source of drinking water, smoking and alcohol consumption history, and presence of underlying medical conditions relevant to COVID-19 or other viral illness severity (e.g., hypertension, heart disease, diabetes, renal disease, asthma, other chronic lung disease, cancer, an immunosuppressive condition).
- Participants will be asked about characteristics of their household and situations related to work, school, and commuting, that may increase a person's risk of exposure to viruses through close contact with others.

- Participants will be asked questions specifically related to COVID-19, including potential contact with infected persons, suspected illness, medical care sought, testing performed, and COVID-19 vaccination status (all since January 2020).

On the follow-up surveys (Appendix C, C1, D, and D1):

- Participants will be asked about changes since administration of the previous survey. This includes changes in chronic medical conditions, influenza and COVID-19 vaccination status, and situations that may increase exposure to viral infections.
- Participants will be asked about symptoms they experienced since administration of the previous survey (e.g., cough, fever/chills, shortness of breath, myalgia, diarrhea, nausea/vomiting, sore throat, headache, nasal congestion), approximate date of onset, and number of days the symptoms lasted.
- Participants who report symptoms will be asked whether they sought healthcare, the type of care setting (e.g., physician's office, emergency department, hospital admission, telehealth visit), what testing was performed, and results of the testing.
- Participants will be asked questions specifically related to COVID-19, similarly to the initial survey.

Additionally, participants will be provided with a symptom diary (Appendix E) that will help them keep track of any symptoms they may experience between surveys. The symptom diary will be sent to participants with the initial survey, and with subsequent surveys. The diary will include questions that mimic what will be asked in follow-up surveys to facilitate easy entry of this information when follow-up surveys are sent. The symptom diaries will be provided for the participants' own use in tracking symptoms and will not be collected from participants.

Survival analysis will be used to assess associations between the previously collected serum-PFAS concentrations and incident viral infections for outcomes that are expected to occur only once during the follow-up period (e.g., diagnosis of COVID-19). Recurrent-event survival analysis, using the counting process approach, will be used for outcomes that are expected to occur multiple times during the follow-up period (e.g., upper respiratory illness). Models will control for potential confounders (e.g., age, gender, race/ethnicity) and account for clustering within households (in both types of analyses) and within individuals (in the recurrent events analysis). The analysis will be a within-community analysis to control for the local COVID-19 transmission level, combining information across communities.

Participating Agencies

Institution	Role
Agency for Toxic Substances and Disease Registry	Sponsoring Agency, Funding Agency
Centers for Disease Control and Prevention/National Centers for Environmental Health	Collaborating Agency

Introduction

Background

Per- and polyfluoroalkyl substances (PFAS) are a large, diverse group of thousands of chemicals (Buck et al., 2011). They have been used extensively in a wide range of industrial and consumer applications

(Fromme, Tittlemier, Völkel, Wilhelm, & Twardella, 2009). Perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) have been well-studied, but there is little toxicity data available for other PFAS (DeWitt, 2015).

PFAS contain strong carbon-fluorine bonds. PFAS are resistant to degradation and are highly persistent in the environment. Humans are exposed through contaminated drinking water and diet, as well as through dust and use of consumer products (Fromme et al., 2007; Halldorsson et al., 2008; Jain, 2014). Infants may also be exposed *in utero*, through consumption of breastmilk from mothers exposed to PFAS, and through formula reconstituted with water contaminated with PFAS. Many PFAS have long half-lives (several years for many types of PFAS) once they enter the human body (Olsen et al., 2007; Zhang et al., 2013; Worley et al., 2017).

Epidemiological studies have evaluated the associations between PFAS exposure and health effects in humans. The evidence comes from studies in occupationally exposed populations, studies in residential populations exposed to higher levels of PFAS in drinking water, and studies in the general population. The available studies suggest associations (but not necessarily cause-and-effect relationships) between PFAS and several health outcomes:

- Increased cholesterol levels (Fitz-Simon et al., 2013; Olsen & Zobel, 2007; Sakr, Kreckmann, et al., 2007; Sakr, Leonard, Kreckmann, Slade, & Cullen, 2007; Steenland, Tinker, Frisbee, Ducatman, & Vaccarino, 2009; Winquist & Steenland, 2014)
- Changes in liver enzymes (Gallo et al., 2012; Olsen, Burris, Burlew, & Mandel, 2000; Olsen & Zobel, 2007; Sakr, Kreckmann, et al., 2007; Sakr, Leonard, et al., 2007)
- Small decreases in infant birth weight (Bach et al., 2015; Darrow, Stein, & Steenland, 2013; Marks et al., 2019; Stein, Savitz, & Dougan, 2009; Wikström, Lin, Lindh, Shu, & Bornehag, 2020)
- Decreased vaccine response in children (Grandjean et al., 2012; Grandjean et al., 2017; Granum et al., 2013; Looker et al., 2014; Stein, Ge, et al., 2016)
- Increased risk of high blood pressure or pre-eclampsia in pregnant women (Darrow et al., 2013; Stein et al., 2009)
- Increased risk of kidney or testicular cancer (Barry, Winquist, & Steenland, 2013; IARC, 2017; Shearer et al., 2020)

More research is needed to fully understand how exposure to PFAS may affect human health, including the effect of exposure to mixtures of PFAS.

The impact of exposure to PFAS on the immune system is of particular concern. The National Toxicology Program concluded that there is evidence from human and animal studies that PFAS exposure reduces antibody responses to vaccines and infectious disease resistance (NTP, 2016), and the European Food Safety Authority recently concluded that PFOS and PFOA are associated with reduced antibody response to vaccination, based on evidence from several epidemiological studies (European Food Safety Authority, 2020). Several studies have looked at the association between PFAS exposure and infectious disease outcomes. Studies in children have investigated associations between PFAS exposure and increased risk for hospitalization due to infectious disease (Dalsager et al., 2021; Fei, McLaughlin, Lipworth, & Olsen, 2010), increased risk of respiratory tract infections and associated symptoms (Ait Bamai et al., 2020; Dalsager et al., 2016; Goudarzi et al., 2017; Granum et al., 2013; Huang et al., 2020; Impinen et al., 2019; Kvalem et al., 2020; Manzano-Salgado et al., 2019; Okada et al., 2012), and decreased vaccine response (Abraham et al., 2020; Grandjean et al., 2012; Grandjean et al., 2017;

Granum et al., 2013; Mogensen et al., 2015; Pilkerton, Hobbs, Lilly, & Knox, 2018; Stein, Ge, et al., 2016; Stein, McGovern, Pajak, Maglione, & Wolff, 2016; Timmermann et al., 2020; Zeng et al., 2019). Fewer studies have been conducted in adults; these studies looked at PFAS exposure in relation to vaccine response (Kielsen et al., 2016; Looker et al., 2014; Shih et al., 2021; Stein, Ge, et al., 2016; Zeng et al., 2020) as well as respiratory infections and associated symptoms (Looker et al., 2014). A general population study in adolescents (12-19 years) and adults (20-49 years) found increased total pathogen burden associated with serum-PFAS (Bulka, Avula, & Fry, 2021). Detailed summaries of these studies, including the specific PFAS species that were found to be associated with the outcomes of interest, are presented in Appendix F.

Justification for Study

Exposure to PFAS is nearly ubiquitous in the United States. Evidence from epidemiological studies suggests that PFAS exposure may impact susceptibility to viral infections; however, the results of studies on PFAS exposure and infectious disease are somewhat inconsistent. Moreover, there is a dearth of studies on the association between PFAS exposure and susceptibility to viral infections in adults. The coronavirus disease 2019 (COVID-19) pandemic presents a unique concern and opportunity. If PFAS affect the immune system, it is possible that they could affect susceptibility to infection with SARS-CoV-2 (the virus that causes COVID-19) or severity of COVID-19.

This study will recruit participants from existing ATSDR cohorts – ATSDR PFAS EA Participants, PEATT assessment participants, and Pease Study Participants (potential total n = 3,170) – to investigate possible associations between PFAS and respiratory viral infection in general, including but not limited to COVID-19. The cohorts from which participants will be recruited had a substantial number of participants with high PFAS exposure, as well as a sufficient range of serum-PFAS concentrations to allow examination of associations between the outcomes and serum-PFAS concentrations across a wide range of exposures. Table 1 below provides an overview of the serum-PFAS quantiles from the EAs as an example of the range of PFAS levels among the potential participants for this study. Recruiting participants from these existing cohorts helps to minimize logistical challenges and takes advantage of existing serum-PFAS measurements. Because many types of PFAS have long half-lives, the recently conducted serum-PFAS measurements can be used as a marker of longer-term PFAS exposure. This is important, as no further biological samples will be collected in this study. The long half-lives of PFAS gives confidence that using serum measures collected 2-3 years ago can serve as a marker for long-term exposure as well as for recent exposures.

Table 1. Quantiles of serum-PFAS levels (ng/µL) among EA participants (n=2,034)*

PFAS	Min	25th %	Median	75th %	Max
MeFOSAA	<LOD	<LOD	0.1	0.2	4.6
PFDA	<LOD	0.1	0.2	0.2	3
PFHxS	<LOD	3.2	9.2	30.1	1210
PFNA	<LOD	0.2	0.4	0.8	8.2
PFOA	0.14	1.37	2.37	4.87	172.6
PFOS	0.14	4.1	8.15	20.4	963.7
PFUnA	<LOD	<LOD	0.1	0.2	1.8

LOD: Limit of Detection; MeFOSAA: N-methyl perfluorooctane sulfonamido acetic acid; PFDA: perfluorodecanoic acid; PFHxS:

perfluorohexane sulfonic acid; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonic acid; PFUnA: perfluoroundecanoic acid
*To provide a general idea of the distribution of concentrations, these quantiles were calculated across the various EA cohorts and do not account for clustering within households or communities.

This study will collect survey information quarterly over a period of 12-14 months. This frequency of data collection was selected to enable collection of information about participants' experience of symptoms throughout an entire year, to include the seasons for various types of respiratory infections. Moreover, the frequent surveys will make it easier for participants to recall their symptoms and other relevant information and decrease the potential for recall bias of symptoms associated with viral infections.

This study will examine associations between PFAS exposure and susceptibility to a range of respiratory viral infections (e.g., flu), which would include COVID-19, as well as gastrointestinal viral infections (e.g., stomach flu). A previous study (Grandjean et al., 2020) provided preliminary evidence that elevated plasma perfluorobutanoic acid (PFBA) concentrations are associated with increased risk of more severe COVID-19 infection; however, this study was conducted in a population with low, background-level PFAS exposures. Additional research on the topic is needed in populations with a broader range of PFAS exposures.

Objectives

The study is a longitudinal study, primarily examining outcomes that occur after the time of the exposure assessment, and after the time of enrollment in this study. However, participants will be asked for information on some outcomes that occurred prior to the time of their enrollment in this study, some of which may have occurred prior to their exposure assessment. The proposed study will assess the association between serum-PFAS concentrations and the self-reported frequency of various groups of symptoms of viral infection (as a marker for susceptibility to viral infection). The overall objectives of this study are the following:

1) Examine the association between serum-PFAS collected through the EAs, PEATT assessments, and Pease Study and the frequency of occurrence of selected syndromes (combinations of self-reported symptoms), which will be used as a proxy for viral infections; and
2) Examine the association between serum-PFAS collected through the EAs, PEATT assessments, and Pease Study and self-reported positive test results indicating specific viral infections.

These associations will be assessed through hazard ratios and 95% confidence intervals, comparing serum-PFAS concentrations in quartiles 2-4 with quartile 1 (with quartiles defined across the whole study population).

Procedures and Methods

Overview of Existing Cohorts

This study will recruit participants from existing cohorts, specifically inviting individuals for whom CDC/ATSDR already has measured serum-PFAS concentrations. These cohorts include the PFAS Exposure Assessments, the PEATT Assessments and the Pease study, which are described below. These cohorts have been selected because all of them involved measurement of serum-PFAS concentrations among

members of specific communities, thus no further biological specimens will need to be collected for this study. The cohorts include both adults (18 years and older) and children (ages 4-17 years). All participants will be enrolled individually; in other words, parents and children will represent individual participants, not as part of a parent-child dyad. However, the analysis will account for the fact that individual participants from the same household will have correlated exposures and outcomes (clustering by household). CDC/ATSDR will only recruit participants who gave written informed consent to be contacted for additional studies in the future, and for their serum-PFAS concentration data to be used in future studies. Based on preliminary review of the informed consent forms, nearly all (approximately 99%) PFAS EA participants gave consent for additional contact. We will use the estimated percentage of participants consenting to additional contact in the PFAS EAs as a proxy for the percentage consenting to additional contact for the Pease and PEATT cohorts (for which this information is not yet available) in order to estimate potential sample sizes.

PFAS Exposure Assessments and PEATT Assessments

In 2019 and 2020, ATSDR conducted PFAS EAs in eight communities across the United States. These EAs were conducted in communities near current or former military bases and that are known to have had PFAS in their drinking water. The primary goal of these EAs was to provide information to communities about the levels of PFAS in their bodies.

Two additional EAs were funded by ATSDR and conducted by the New York State and Pennsylvania health departments to assess the PEATT (a tool-kit developed to assist health departments in conducting PFAS EAs). These two assessments are referred to as the PEATT assessments.

The EAs used a one-stage cluster sample, in which each household in the area receiving impacted water was a cluster, and all individuals in a selected household were included in the sample. Where possible, clusters (households) were randomly selected from the sampling frame. At some sites the impacted area included an insufficient number of households to allow for random selection while still achieving recruitment targets. For these sites, all eligible households in the impacted area were invited to participate. This yielded a representative sample of the population in the area potentially impacted by PFAS contamination of drinking water. Each individual in the selected households was invited to participate. Each person enrolled was treated as an individual in the study (i.e., parents and children were enrolled individually, not as parent-child dyads). Written informed consent (including Privacy Act Statement, consent, assent, and parental permission forms) was obtained from participants upon sample collection; these forms are securely archived at ATSDR. The ATSDR PFAS EA Protocol is provided here. The PEATT assessments were conducted by state health departments using a similar sampling design.

Table 2 provides details of each of the PFAS EA and PEATT assessment cohorts.

Table 2. PFAS EA and PEATT Assessment Cohorts

EA or PEAT Assessment Location	Blood Sample Collection Dates	Number of Participants		Serum-PFAS Concentrations (geometric mean, µg/L)
		Adults	Children (aged 4-17 years)	
Hampden County, MA	Sept 2019	409	52	PFOS – 5.9; PFOA – 1.9;

(EA)				PFNA – 0.4; PFHxS – 4.7
Berkeley County, WV (EA)	Sept/Oct 2019	251	30	PFOS – 5.1; PFOA – 1.5; PFNA – 0.4; PFHxS – 2.9
New Castle County, DE (EA)	Oct 2019	202	12	PFOS – 21.5; PFOA – 5.0; PFNA – 1.0; PFHxS – 20.1
Spokane County, WA (EA)	Nov 2019	302	47	PFOS – 42.4; PFOA – 9.7; PFNA – 0.7; PFHxS – 72.9
Lubbock County, TX (EA)	Feb 2020	196	24	PFOS – 4.2; PFOA – 2.2; PFNA – 0.2; PFHxS – 6.0
Fairbanks North Star Borough, AK (EA)	Aug 2020	79	10	Not available as of 4/2021
El Paso County, CO (EA)	Sept 2020	325	34	Not available as of 4/2021
Orange County, NY (EA)	Oct 2020	59	2	Not available as of 4/2021
Westhampton, NY (PEATT)	April – Oct 2018	143	18*	PFOS – 6.56; PFOA – 1.54; PFNA – 0.64; PFHxS – 3.03
Bucks and Montgomery Counties, PA (PEATT)	May – Sept 2018	169	19*	PFOS – 10.24; PFOA – 3.13; PFNA – 0.74; PFHxS – 6.64
TOTAL		2135	248	

* PEATT assessments defined children as ≤ 19 years
*To provide a general idea of the distribution of concentrations, these geometric means were calculated across the various cohorts and do not account for clustering within households.

The Pease Study

The Pease Study (CDC Protocol No. 7161) is the first site of the national Multi-site Study (CDC Protocol No. 7207), which is looking at the human health effects of PFAS exposure through drinking contaminated water. Approximately 8,000 people work at or frequent the Pease International Tradeport in Portsmouth, NH. There are also two daycare centers located on the site. In May 2014, drinking water wells that supply the Pease International Tradeport were tested for PFAS. The Haven Well, one of three wells that serves the Pease International Tradeport and the New Hampshire Air National Guard base at Pease, showed elevated levels of PFOS. Because the level of PFOS exceeded the "provisional health advisory" set by the U.S. Environmental Protection Agency (EPA), the well was shut down by the City of Portsmouth on May 12, 2014, and since that time it has been physically disconnected from the system.

The cross-sectional Pease Study aims to enroll a convenience sample of participants who were eligible for a previous 2015-2017 Pease biomonitoring program (Daly et al., 2018; DHHS, 2016) and a small number of referents from other areas of New Hampshire. This study will expand the scientific understanding of PFAS by looking at the association between health outcomes and PFAS exposure from drinking water. Blood samples from participants in the Pease Study will be analyzed for PFAS. As part of the Pease study, ATSDR will also evaluate health-related blood biomarkers like cholesterol levels, markers of immune function, and markers of thyroid function; and will collect information about medical history. Urine samples will be collected and archived for future studies. The Pease Study aims to recruit 1,100 adults and 525 children. Written informed consent (including Privacy Act Statement, consent, assent, and parental permission forms) is obtained from participants; these forms are being securely archived at ATSDR.

Recruitment for the Pease Study was completed on December 31, 2021, with 781 adults and 178 children enrolled in the study. Only the serum-PFAS concentration data and selected survey data (see section on data collection procedures below) for Pease Study participants will be used for participants in the PFAS and viral infections study, not data on other health-related biomarkers.

Recruitment

As part of a recruitment packet, the present study will mail invitation letters (Appendix G) to all participants from the studies discussed above (anticipated total across sites of approximately 3,170; 2,800 adults and 370 children, aged 4-17 years), who agreed to be contacted for follow-up studies. Cohort members will be asked if they want to participate in this follow-up study on viral infections including, but not limited to, COVID-19. This sample may include multiple individuals from the same household, but each person will be treated as an individual and the analysis will account for clustering by household (see details in the statistical analysis section).

Potential participants will be offered gift cards as an incentive to participate in the study, with incentives of $10 provided for each completed survey, up to $50 in total for the surveys. If participants complete all 5 surveys, they will receive an additional $25; therefore, participants who complete all 5 surveys will receive a total incentive of $75. Monetary incentives, including those as low as $1, are associated with higher response rates compared with nonmonetary incentives (Cho, Johnson, & Vangeest, 2013).

To improve response rates, we will ask participants how they prefer to complete their follow-up surveys – through paper-based or web-based surveys. Cohort members will indicate their preference when they enroll by consenting to be a part of the study. Additionally, participants who initially select paper-based surveys will be asked on each subsequent paper-based survey if they would like to switch to web-based surveys for the rest of the study. The initial packets (including recruitment materials, consent forms and initial surveys) will all be paper-based and sent by mail, because mailed invitation letters are associated with higher responses rates for both paper- and web-based surveys (Converse, Wolfe, Huang, & Oswald, 2008; Freedman, McGonagle, & Couper, 2018).

Recruitment Packet

For study recruitment of adults, a letter of invitation, a study fact sheet, two copies of the consent form, Privacy Act Statement, initial survey, symptom diary, and pre-paid return envelope will be mailed in one package to each individual from the cohorts discussed in previous sections. For children, the package will be addressed to the parent or guardian of the child and will include parental permission and child assent forms. Each individual will receive their own recruitment packet even if they are members of the same household, as each person will be enrolled individually in the study.

Recruitment packages for PEATT participants will be sent from New York or Pennsylvania State Health Departments and will include an additional cover-letter (Appendix G1).

To increase response rate, CDC/ATSDR has developed recruitment communications materials, including a recruitment postcard and a fact sheet that will be sent to participants (Appendix G2). The fact sheet will be included in the recruitment packet, while the postcard will be sent two weeks prior to the packets going out to make potential participants aware of the upcoming study.

Letter of Invitation

A letter of invitation (Appendix G) will be mailed as part of the recruitment packet. The letter will explain the study purpose and how the study will be conducted over the study period. Potential participants will be provided a telephone number and email contact information for contacting study personnel and will have the opportunity to ask questions about this study prior to enrollment.

Consent Form

The hardcopy consent/permission/assent forms will be included at the beginning of the initial survey(s) (Appendix A, B). An extra copy of the adult consent form (Appendix H), parental permission form (Appendix I), and assent form for children under the age of 18 years (Appendix J) will be included for participants to keep. The consent/permission/assent form will explain the authority and purpose of the study, the procedure for completing the surveys, and benefits and risks of participation in the study. The consent/permission/assent form will explain privacy and confidentiality protection of personal information, with whom data will be shared, and how data will be stored and used. People who agree to be a part of this study will be able to indicate on the consent form how they would like to receive follow-up surveys (by mail or REDCap) and how they would like to receive reminder notifications.

The completed and signed consent/permission/assent form and completed initial survey (Appendix A, B) will be returned by mail in the pre-paid envelope that will be provided. The study team will make two attempts to follow up with individuals from whom an incomplete package is received (only consent/permission/assent form received or only survey received). The consent/permission/assent form(s) must be completed and signed to participate in this study. If a completed survey is received without a signed consent form and the study team is not able to obtain a consent/permission/assent form after two follow up attempts, the survey will be destroyed.

If there is no response within 4 weeks, as indicated by a returned consent/permission/assent form and initial survey, the study team will follow up with the non-responding potential participant via telephone call (Appendix L). If there is no response to the telephone follow-up within 2 weeks, one additional attempt to contact non-responding potential participants will be made via telephone call. If there is still no response, no further attempts will be made.

Data Collection Procedures

Participants who return the signed consent and assent/parental permission forms (described below), as well as the initial survey, will be enrolled in the study. For those for whom consent is obtained, information on serum-PFAS concentrations will be obtained from the original study in which they participated (EA, PEATT or Pease study). No further serum samples or other biological specimens will be collected from participants as part of this study.

For ascertainment of the outcomes of interest, as well as information about some covariates, four follow-up surveys will be sent, one every 3 months for a period of one year, to all enrolled participants. The follow-up surveys will be made available via REDCap or mailed out as hardcopies approximately 3 months apart. Please see sections below for detailed descriptions of the follow-up surveys. The duration and timing of this study was chosen to collect information throughout an entire year, including one complete influenza season.

When designing the surveys for this study, the study team first reviewed the adult and child surveys used for the EA, PEATT assessment, and Pease studies to assess the uniformity of the questions across

studies. This review was conducted to identify the information relevant to this study that has already been gathered. That review allowed us to minimize repetition of data collection, and to identify the additional baseline information that is needed to evaluate the relationship between PFAS exposure and susceptibility to viral infection. This additional baseline information will be collected in the initial survey, explained below.

Information collected from the EA, Pease, and PEATT assessment participants that is relevant to this study included the following:

- Name (last name, first name)
- Date of birth (month/day/year)
- Sex (Male of Female)
- Race/Ethnicity
- History of kidney disease
- Occupational history
- Previous blood test for PFAS (including date and result)

As part of the process of obtaining informed consent, participants will be informed that this study will use their previously measured serum-PFAS concentrations and previous survey data. They will be informed that this study will not collect any further blood or other biological samples because the survey data collected in this study will be linked back to their serum-PFAS levels that were analyzed previously

Surveys

Participants in this study will be asked to complete a series of surveys and the information will be used to examine the relationship between PFAS exposure and susceptibility to acute respiratory and gastrointestinal viral illness including, but not limited to, influenza and COVID-19. The initial paper survey (Appendix A, B), will be mailed to participants, as described above. Participants will choose to complete the follow-up surveys using the secure, web-based platform, REDCap (Appendix C1, D1), or via mailed paper copy (Appendix C, D). Participants who choose to complete their surveys via REDCap will receive a link to the survey every 3 months for the duration of the study via an email address that they provide. If participants choose instead to complete follow-up surveys by mail, they will receive the survey by mail every 3 months with a pre-paid return envelope. Participants who choose to complete the follow-up surveys by mail will be given the option to switch to REDCap for each subsequent survey.

Participants will be encouraged to keep a symptom diary (Appendix E) to improve recall between surveys. The participant will be encouraged to use the diary to complete the follow-up surveys. Additionally, participants can request digital copies of the diaries if they find that easier to keep track of. Participants will **not** be asked to submit the diary. The surveys and diary are described separately below. Information obtained from the surveys will not be used for diagnostic purposes, administration of medical advice, or providing treatment.

Initial Survey

The initial survey for adults can be found in Appendix A and for children (aged 4-17 years) in Appendix B. Parents and children will each receive their own surveys. Parents will be encouraged to help their children fill out the child surveys, as needed.

In Section 1 of the Initial Survey, participants will be provided with instructions for completion and submission of the survey by mail.

In Section 2, participants will be asked about demographic information including year of birth, height and weight; height and weight are needed to calculate BMI, as BMI is a documented risk factor for COVID-19 severity (Tartof et al., 2020). They will also be asked about influenza vaccination status, source of drinking water, smoking and alcohol consumption history (adults only), and the presence of underlying chronic medical conditions that may increase a person's susceptibility to viral infections, including SARS-CoV-2, the virus that causes COVID-19 (Guan et al., 2020; Liguoro et al., 2020; Pathak, Salemi, Sobers, Menard, & Hambleton, 2020; Shane et al., 2020; Stokes et al., 2020).

Participants will be asked about whether their drinking water in their house is supplied by a private well with known PFAS-contamination. For those using private wells with PFAS contamination, we will also ask about the timing of the first change that was made to the drinking water supply in response to PFAS exposure. Since we are not collecting new blood samples for this study, the inclusion of these questions will allow us to potentially understand if people could have had additional exposure to PFAS in drinking water after PFAS levels in their blood were measured. For individuals on municipal water, the timings of the municipal water supply changes are known to the investigators and took place prior to PFAS blood level sampling.

There is strong evidence that having certain underlying medical conditions can increase the severity of SARS-CoV-2 infection and risk of mortality ("CDC Science Brief: Evidence used to update the list of underlying medical conditions that increase a person's risk of severe illness from COVID-19," 2021; Harrison, Fazio-Eynullayeva, Lane, Underhill, & Lip, 2020; Rosenthal, Cao, Gundrum, Sianis, & Safo, 2020; Williamson et al., 2020). When examining the intersection of PFAS exposure and susceptibility to or severity of infection, it is important to control for conditions that may be confounders.

Previously collected demographic information (see list above), including race/ethnicity and sex, will not be collected again. That information will be obtained from previous survey data that will be linked for each individual participant.

In Section 3, participants will be asked questions relating to household characteristics and situations (e.g., work environment, in-person school attendance, travel activities) that can increase a person's contact with others, potentially leading to an increased risk of exposure to viruses, including SARS-CoV-2. For the initial survey, we will ask about the two-week period prior to receiving the initial survey.

In Section 4, participants will be asked questions specific to COVID-19, including if they have had other types of COVID-19 testing, and if they have received a SARS-CoV-2 vaccine and when the vaccine(s) was administered.

At the end of the survey, they will be asked to take a moment to look at the symptom diary that is in the packet with the survey (see details about the diary below). They will also be asked to write the date of survey completion at the top of that symptom diary to help them remember when they completed the survey.

Follow-up Surveys

The follow-up paper and REDCap surveys for adults can be found in Appendix C and C1 and for children (aged 4-17 years) in Appendix D and D1, respectively. Parents and children will each receive their own surveys. Parents will be encouraged to help their children fill out the child surveys, as needed.

In Section 1 of the Follow-up Survey, participants will be given instructions for completion and submission of the survey. Participants will be asked to refer to the date of last survey completion as the beginning of the time period covered in each follow-up. These initial dates will be auto populated for participants based on when their last survey was completed.

In Section 2, participants will be asked if they have received an influenza or COVID-19 vaccination since the previous survey and if they have been diagnosed with any new chronic medical conditions since completion of the previous survey.

In section 3, participants will be asked if they have experienced any changes in activities or situations that increase close contact with other people since the previous survey.

In Section 4, participants will be asked about symptoms (e.g., cough, fever/chills, shortness of breath, myalgia, diarrhea, nausea/vomiting, sore throat, headache, nasal congestion) since they completed the last survey, including approximate date of onset and duration of symptoms. The symptom list includes those that are known to be associated with viral infections (Arruda, Pitkäranta, Witek, Doyle, & Hayden, 1997; Bialek, 2020; Eccles, 2005; Shane et al., 2020; Solomon, Sherman, & Kanjilal, 2020; Song et al., 2020; Stokes et al., 2020; Thompson et al., 2013; Turner, 1997). Participants will be asked about travel activity in the 14 days prior to onset of symptoms by a mode of travel that would likely increase contact time with other people. 2-14 days is a typical incubation period for most viral infections, though some incubation periods can be longer. Participants who report symptoms will be asked if they sought medical care and the type of care setting (e.g., physician's office, emergency department, urgent care, hospital admission, telehealth visit). Participants who sought medical care will be asked what type of diagnostic testing was performed and the results of the testing.

In Section 5, participants will be asked questions specific to COVID-19, including if they have been in close contact with someone with a laboratory-confirmed case or suspected case and if they have had various types of COVID-19 testing.

Symptom Diary

Participants will also be encouraged to keep a Symptom Diary (Appendix E) over the course of the study to improve recall between surveys. A paper version of the diary (in two different formats) will be mailed with the first package of materials and will be for the participant to use in completing the periodic follow-up surveys. Additionally, participants can request electronic versions of the symptom diaries. Participants will be asked to look at the diary at the end of the initial survey and to enter the date of initial survey completion at the top of the diary. There is information provided at the beginning of the follow-up surveys to remind participants to look back at their symptom diaries to help them more easily complete the survey. Additionally, there is text at the end of each survey to remind participants to start a new diary and use the symptom diary until they receive their next follow-up survey. Participants will **not** be asked to submit the diary.

Tracing and Follow-up Procedures

If recruitment packages are returned undelivered, minimal tracing procedures will be undertaken due to resource limitations. If packages are returned with a forwarding address, a new package will be sent to the new address. Because individuals who are being recruited for this study recently participated in other ATSDR-funded investigations, contact information for these individuals has been recently verified and successfully used. Therefore, there is limited concern about the addresses returning undeliverable mail. If this does happen, we will work with the investigators from the previous studies to ensure that we have the correct contact information. No further tracing attempts will be made for the initial recruitment packages.

For the initial recruitment package, if there is no response within four weeks, as indicated by a returned consent/permission/assent form and initial survey, the study team will follow up with the non-responding potential participant via telephone call (Appendix L). If there is no response to the telephone follow-up within 2 weeks, one additional attempt to contact non-responding potential participants will be made via telephone call. If there is still no response, no further attempts will be made.

If the follow-up survey has not been completed within 2 weeks for REDCap surveys or 4 weeks for paper-based surveys, we will send a reminder to the participant by the preferred method chosen on their consent form (see Appendix L for reminder messages). If there is no response to the reminder within 2 weeks, one additional reminder to complete the follow-up survey will be sent to the participant. If there is still no response within an additional 2 weeks, no further reminders will be sent for that round of surveys. However, the participant will still be asked to complete the survey in any future rounds. Each follow-up survey will cover a specific 3-month time period for which participants will be asked to provide information.

All participant forms will include an email address and phone number to inform participants how to reach out with any questions/concerns. If participants reach out to indicate difficulties with understanding consent materials or survey questions, the research team will address these concerns on a case-by-case basis.

Data Handling and Analysis

Data Protection

After enrollment, each individual will be assigned a unique identifier that will be used throughout the remainder of the study. This ensures an additional layer of confidentiality. Only one member of the study staff will have access to personal identifiers. Survey data will be collected through mailed paper surveys (for the baseline survey and as an option for the follow-up surveys) and through the CDC REDCap system (as an option for the follow-up surveys). Data collected through paper surveys will be entered into REDCap by study personnel. REDCap data will be directly downloaded to an encrypted CDC network drive with restricted access controls and routine backup/restore services to ensure data integrity. The tool allows for data to be downloaded in various formats (e.g., comma-separated values csv).

Strict role-based access rules will be in place to limit data access to the study team on a need-to-know basis. All requests for data access must be approved by the study principal investigator, and the PFAS Data Repository team will ensure all access permissions are granted appropriately. Signing a non-

disclosure agreement and Rules of Behavior for Data Access will be required before access to the data sets is granted. All data will be stored on the CDC network drive and will not be permitted to be kept (other than temporary copies used by analytic software) on any un-authorized locations, such as on personal computers or laptops.

Records will be retained and disposed of in accordance with the CDC/ATSDR Scientific and Research Records Control Schedule. Physical copies of study materials and reports will be maintained at ATSDR until no longer needed by program officials and will be kept in accordance with the corresponding retention schedules. Computer documents will be disposed of when no longer needed by program officials and will also be kept in accordance with the corresponding retention schedules. Disposal methods will include erasing computer files, shredding paper materials, or transferring records to the Federal Records Center when no longer needed for evaluation and analysis. Records are retained for 20 years after the retirement of the record system.

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

Consistent with applicable federal laws, regulations, and policies, CDC intends to use its best efforts and the procedures set out in this data management and protection plan to

- protect the privacy and confidentiality of any potentially identifiable information,
- protect any proprietary or commercial information provided to it by a data steward or other national source, and
- protect, to the extent allowable, any other data exempted from disclosure under the Freedom of Information Act (5 U.S.C. Sec. 552) or other applicable federal laws or privileges.

Certificate of Confidentiality

Consistent with Section 301(d) of the Public Health Service Act, a Certificate of Confidentiality (CoC) would apply to this research because this research is funded, conducted, or supported by CDC and the following are true:

1) The activity constitutes research;
2) The research involves Human Subjects as defined by 45 CFR Part 46;
3) The research involves information about an individual for which there is at least a very small risk, that some combination of the information, a request for the information, and other available data sources could be used to deduce the identity of an individual.

Therefore, CDC and any of its collaborators, contractors, grantees, investigators or collaborating institutions that receive “identifiable, sensitive Information” as defined by subsection 301(d) of the Public Health Service Act shall not:

- Disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was

created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains; or

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

Disclosure is permitted only when:

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

CDC/ATSDR staff conducting this research will establish and maintain effective internal controls (e.g., policies and procedures) that provide reasonable assurance that the research is managed in compliance with subsection 301(d) of the Public Health Service Act. CDC will ensure: 1) that any investigator or institution not funded by CDC who receives a copy of identifiable, sensitive information protected by this Certificate, understands that it is also subject to the requirements of the Certificate; and 2) that any subrecipient that receives CDC funds to carry out part of this research involving a copy of identifiable, sensitive information protected by a Certificate understands that it is subject to subsection 301(d) of the PHS Act. Therefore, all study staff will receive training on the importance of protecting the confidentiality of human research subjects and of personal information acquired.

The Certificate of Confidentiality is addressed in the consent, permission, and assent forms (Appendix H, I, and J, respectively).

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

Data Linkage

Each participant's survey data will be linked to their previously collected serum, demographic data, and other survey data (as listed above) using name and date of birth. This identifying linking information will be collected on the signed consent form which is attached to the initial paper survey. This will ensure that the appropriate previously collected data are linked to the current study data. Upon receipt of the signed consent form, each enrollee will be randomly assigned a new participant ID. This unique identifier will be used throughout the remainder of the study to ensure an additional level of confidentiality. Only one member of the study staff will have access to the personal identifying information.

Subsequent surveys (both paper- and web-based) will include the pre-printed participant IDs. These IDs will then be used to link follow-up surveys to the participant's initial survey and serum-PFAS levels from the previous studies. Only study personnel will have access to these randomly assigned participant IDs

and the link to the participant's identity. This will be done in accordance with acceptable practices ensuring the protection, confidentiality, and integrity of the data contents.

Outcomes

The outcomes of interest include occurrence and frequency of common respiratory infections (e.g., flu, COVID-19) and common gastrointestinal infections (e.g., stomach flu). This will include self-reported laboratory-confirmed outcomes as well as self-reported number of episodes of selected combinations of symptoms that might be associated with infections such as influenza, the common cold, pneumonia, gastrointestinal illness, and COVID-19. Symptoms will include fever, chills or repeated shaking with chills, cough, shortness of breath or difficulty breathing, nasal congestion, runny nose, sore throat, new loss of taste or smell, headache, fatigue, muscle pain or body aches, nausea or upset stomach, abdominal pain, vomiting, diarrhea, and unexplained rash. Symptoms will be analyzed individually (Dalsager et al., 2016); additionally, these symptoms will be analyzed using syndrome definitions. These outcomes will be considered separately in the analysis:

- Selected individual symptoms collected on the follow-up survey, considered individually, including
    - Fever (either subjective or temperature >100.5° F)
    - Cough
- Predefined symptom syndromes, including
    - Influenza-like illness, defined as either: (1) an acute respiratory illness with a measured temperature of ≥100° F and cough (Fitzner et al., 2018); or (2) Cough and at least 1 or more of fever/feverishness, chills, or body aches (Aiello et al., 2012)
    - Severe acute respiratory infection, defined as an acute respiratory illness with a history of fever or measured fever of ≥100° F and cough, with onset within the past 10 days, requiring hospitalization (Fitzner et al., 2018)
    - COVID-like illness, defined as fever and one of the following symptoms: (cough) OR (shortness of breath) OR (difficulty breathing); OR positive COVID-19 test result (CDC, 2021)
    - Upper respiratory illness ("common cold"), defined as 2 of the following symptoms for 1 day or 1 of the following symptoms for 2 days: runny nose, cough, sneezing, stuffy or blocked nose, fever, sore throat (Sandora et al., 2005)
    - Gastrointestinal illness ("stomach flu"), defined as any 2 of the following symptoms: diarrhea, abdominal pain, vomiting, headache, fever, and chills (Eckardt & Baumgart, 2011; Hall et al., 2011)
    - Other combinations of symptoms could be considered in sensitivity analyses.
- Diagnoses
    - Positive test for COVID-19
    - Positive test for influenza
    - Physician diagnosis of pneumonia.
    - Self-reported, self-diagnosed cold (upper respiratory infection)

The first survey will include questions on COVID-19 dating back to January 2020. Because of the nature of the pandemic and the global attention paid to this, we feel that questions about COVID-19 will not be

subject to recall bias as much as questions about the common cold. Each of the follow-up surveys will ask about the three-month period since the last survey.

Methods of Data Analysis

The primary exposures of interest will be previously measured serum-PFAS concentrations. The PFAS that were measured in the EAs, PEATT assessments, and Pease study include PFOA, PFOS, PFNA, PFHxS, PFDA, PFUnA, and MeFOSAA. The analysis will consider each of these PFAS separately as well as a sum of the PFAS measurements. Further analyses examining the effects of mixtures may be considered. Serum concentrations that are below the limit of detection will be replaced by the limit of detection divided by the square root of 2.

Serum-PFAS will be analyzed in quartiles based on the distribution of PFAS levels among the study population, whenever possible. A test for trend for each analysis will be done using the natural log of the median values from each quartile as a continuous measure. In addition, for PFAS that have a high detection frequency (>90%), analyses using a continuous log-transformed variable for the serum-PFAS level will also be considered. In these analyses, values below the limit of detection will be assigned a value of the limit of detection divided by the square root of 2. Analyses will not be lagged because of the long half-lives of these chemicals.

Primary outcomes of interest include those listed above. The primary exposure of interest is serum-PFAS levels collected through the previous cohorts as described above. Covariates of interest are discussed below under the Confounders section.

Confounders

All models will control for age at serum sample collection, vaccination status (both COVID-19 and influenza vaccinations), race/ethnicity (categorized as non-Hispanic White, non-Hispanic Black, and Other), smoking status (in categories of current smoker, former smoker, and never smoked), and body mass index. Other potential confounders (e.g., combinations of underlying illnesses) will be considered in sensitivity analyses. Additionally, we will consider including a variable for how participants chose to respond to surveys (e.g., via REDCap or via paper-based) in order to account for mode effects.

All data analysis will be done using SAS statistical software (SAS Institute, Cary, North Carolina).

Descriptive Analyses

Descriptive statistics will be used to describe the baseline cohorts (both separately and together) in terms of number of participants, demographics (age, sex, race/ethnicity), categories of potential confounders, other baseline characteristics that might be relevant to exposure or susceptibility to COVID-19 or other viral infections (receipt of influenza and COVID-19 vaccines; underlying health conditions; activities that could put them at risk for exposure to viruses; and history of known COVID-19 exposure, testing, symptoms, diagnosis and treatment), frequency of each symptom reported on the follow-up surveys, frequency of the pre-defined outcomes of interest (as described above), and measured serum-PFAS levels. Serum-PFAS concentrations will be compared with serum-PFAS concentrations measured in NHANES among people of similar ages.

Analysis of association between serum-PFAS concentrations and symptoms of viral infection

Analyses to examine the association between serum-PFAS and incidence of symptoms of viral infection will differ for outcomes (such as a diagnosis of COVID-19) that would typically occur only once for a given person during the study period (referred to as single-event outcomes), and outcomes (such as upper respiratory illnesses) that could typically occur several times for a given person during the study period (referred to as multiple-event outcomes). If recurrent COVID-19 infections appear to be common, we will consider it as an outcome expected to occur more than once.

Single-event outcomes: For single-event outcomes, the analysis will use survival analysis. Each person's time under observation will start on the date of completion of the initial survey and end at the time of their first break in response coverage due to lack of continued survey responses. Survival analysis will use Cox proportional hazards models with calendar time as the time scale, to account for expected seasonal variations in incidence. Participants will remain in the analysis until the date of onset for their first occurrence of the outcome of interest, or until they are censored (at the time when they are lost to follow up or the end of the study period, whichever occurs first). Models will control for the potential confounders listed above. The baseline hazard will be stratified by cohort to account for potentially different patterns of viral transmission in different geographic areas. The proportional hazards assumption will be checked for each variable in the model, and appropriate measures will be used to address any violations of that assumption (e.g., inclusion of interactions with time or further stratification of the baseline hazard). Multivariate frailty (random effects) models will be explored to account for clustering effects within households; this should account for household clustering since households were randomly selected in the initial cohorts. Methods to account for infectious disease transmission will be explored during data analysis

Multiple-event outcomes: For multiple-event outcomes, the analysis will use recurrent event survival analysis, using the counting process approach. For this analysis, each person will be included in the analysis for all periods for which they complete a survey. A data set will be created that allows for multiple lines per participant, for different time periods. Time periods will end either when the person experiences an event or when they are lost to follow up. Subsequent periods will start either right after the event (if still under observation) or when the person is observed again. Each line will have a start date and a stop date. Time periods that end with an event will have an event status of 1 and periods that end without an event (censoring) will have an event status of 0. Cox proportional hazards models, with calendar time as the time scale, will be run in SAS statistical software (SAS Institute, Cary, North Carolina). As for single-event outcomes, models will control for the potential confounders listed above. Again, as for single-event outcomes, the baseline hazard will be stratified by cohort to account for potentially different patterns of viral transmission in different geographic areas. The proportional hazards assumption will be checked for each variable in the model, and appropriate measures will be used to address any violations of that assumption (e.g., inclusion of interactions with time or further stratification of the baseline hazard). Because the data in these analyses will have two levels of clustering (within households and within individuals), these analyses will be done using the SURVIVAL procedure in SAS-callable SUDAAN (which fits proportional hazards models, allows for stratification of baseline hazards, and can account for the two levels of clustering). Multivariate Bayesian frailty models that include random effects for both household and individual will also be considered, using SAS proc

MCMC. Methods to account for infectious disease transmission will also be explored during data analysis.

For missing data, if appropriate (e.g., data appear missing at random and the percentage missing is not high [less than 40% missing (Jakobsen, Gluud, Wetterslev, & Winkel, 2017)]), multiple imputation will be considered, in which missing values will be replaced by five simulated complete data sets. In this method, the missing values are imputed based on predictions from regression models with relevant covariates. Each data set is analyzed with standard methods, and the results are combined to produce estimates and confidence intervals, which incorporate missing-data uncertainty.

All models will be run for children (younger than 18 years) and adults (ages 18 years and older) separately, if there is sufficient power. Models will yield hazard ratios and confidence intervals.

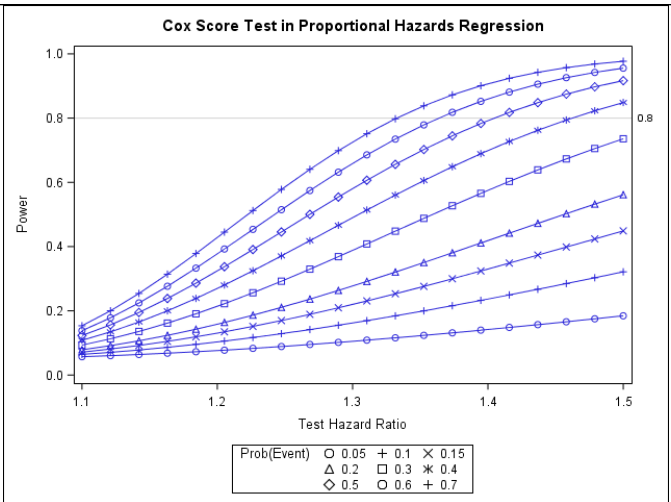
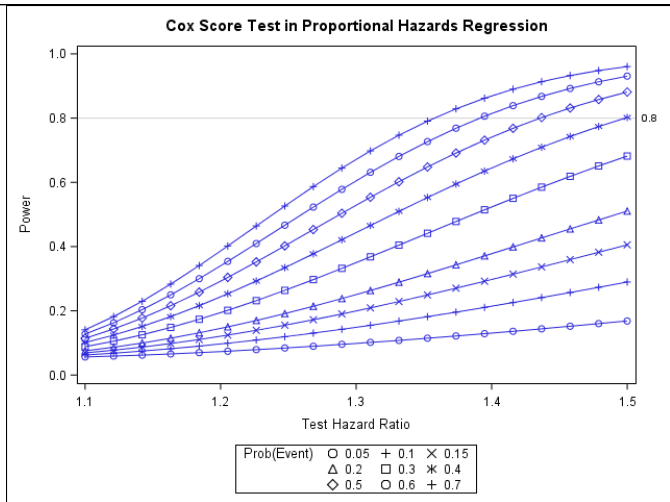
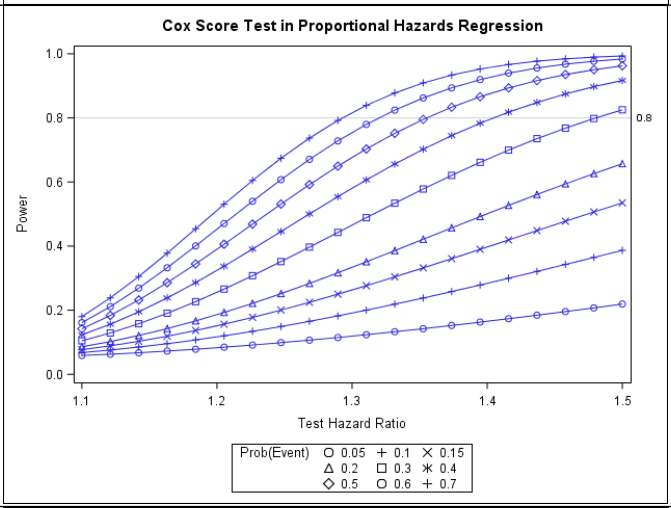
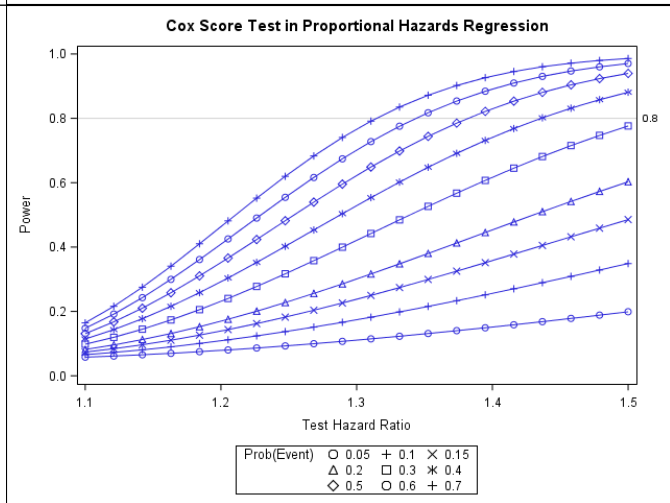
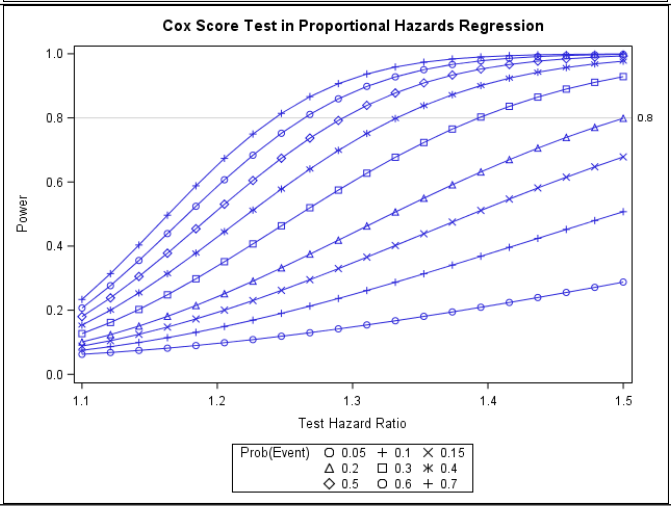
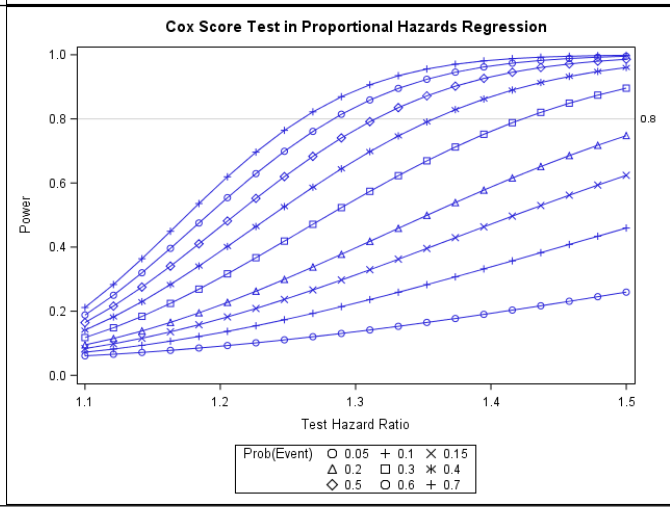
Quality control for data analysis

All SAS code for data preparation and analysis will be reviewed by a second CDC/ATSDR statistician; all analytic results will be reviewed by epidemiologists at CDC/ATSDR.

Statistical Power Calculation

Power calculations were done using the proc power procedure in SAS version 9.4 with the coxreg option, which computes power for Cox proportional hazards models. The calculations were done for hazard ratios comparing the 4th and 1st quartiles of the distribution of PFAS measurements varying from 1.1 to 1.5 (represented on the x-axis of the graphs below), using a two-sided score test with a type I error (α) of 0.05, and assuming event probabilities (Prob(event) represented by the various lines in the graphs below) ranging from 0.05 to 0.7, response rates of 40%, 50% and 70%, and various degrees of correlation between the predictor variables in the model (r^2 values of 0.15 and 0.25). A low event probability (likely ~0.05) would be expected for COVID-19, based on current (as of April 2021) seroprevalence estimates (https://covid.cdc.gov/covid-data-tracker/#national-lab). Higher event probabilities would be expected for more common viral syndromes, such as “flu” (estimated event probability ~0.18, (Vugia, et al. 2004)) and upper respiratory infections (“cold”, estimated event probability ~0.7 (Looker, et al., 2014)). The anticipated power under the various scenarios are shown in the graphs below. For example, with a response rate of 50% among a population of 3200 and correlation between predictors of r^2=0.15, we expect to have 80% power to detect a risk ratio slightly larger than 1.48 for an outcome with an event probability of 0.3.

	r^2 for correlation between predictors=0.15	r^2 for correlation between predictors=0.25

40% response rate		
50% response rate		
70% response rate		

Timeline

The table below presents the anticipated implementation timeline for this study:

ACTIVITY	*Months 1-3*	*Months 4-6*	*Months 7-9*	*Months 10-12*	*Months 13-15*	*Months 16-18*	*Months 19-21*

Recruitment							
Initial Survey							
Follow-up Survey 1							
Follow-up Survey 2							
Follow-up Survey 3							
Follow-up Survey 4							
Data Analysis							
Manuscript Development							
Publication Submission							

Anticipated Risks and Benefits

The anticipated risks of this study are low. There is the potential for the loss of privacy and confidentiality if a data breach were to occur. Participants' identities will be linked to serum samples using a randomly assigned participant ID, which only one member of the study staff will have access to. Therefore, no identifying information will be attached to the data during the data analysis phase. Moreover, any final reports will contain aggregated data that will not be identifiable at the individual level.

Participants will not directly benefit by taking part in this study; however, their participation will help scientists understand if there may be an association between the amount of PFAS in a person's body and susceptibility to viral infections. These risks and benefits are described for participants in their informed consent forms (Appendix H, I, J).

Limitations of the study

There are several limitations of this study. The expected number of confirmed COVID-19 cases in the available ATSDR-supported cohorts could be very small. Therefore, we might not have high enough statistical power to draw strong conclusions specifically for COVID-19. However, the power is expected to be better for other viral syndromes. If PFAS exposure impacts susceptibility to respiratory viral infection, there is no *a priori* reason to think that it would affect susceptibility to one respiratory viral infection over another.

Power to detect associations between serum-PFAS concentrations and our outcomes could also be reduced by non-participation and by loss to follow-up. We have accounted for potential non-participation in our power calculations, but levels of participation could be lower than we expect. In addition, as in any longitudinal study, loss to follow-up could lead to bias if loss to follow-up is not independent (i.e., if it results in the remaining study population having a different average risk for the outcomes than would have been observed in the absence of loss to follow-up, within any relevant strata).

There is the potential for recall bias when participants retrospectively answer questions on self-reported COVID-19 symptoms dating from the beginning of the pandemic in January 2020 to the time of

enrollment. However, previous studies have investigated viral infections in similar ways (Ait Bamai et al., 2020; Dalsager et al., 2016; Goudarzi et al., 2017; Huang et al., 2020; Kvalem et al., 2020). Efforts will be made to minimize the potential effect of recall bias by collecting information at baseline as well as at varying follow-up periods. We are providing the participants with a symptom diary to encourage them to prospectively keep track of any symptoms they may experience between follow-up surveys. We are offering participants different formats for the symptom diary, as well as having the diary available in paper and electronic versions. Additionally, there are reminders at the end of each survey to encourage participants to use their symptom diaries in between each survey. By spacing the surveys only three months apart, we hope to overcome some of the potential recall issues.

There can also be difficulty with categorizing the outcomes of interest. Due to the nature of these infections, there is often not a confirmed diagnosis (e.g., typically testing is not done to identify the viruses causing the common cold). For this reason, we will examine viral syndromes defined through combinations of symptoms. However, people might define the relevant symptoms differently. Previous studies have used similar syndromic analyses, which lends credence to the approach in the present study (please see discussion of outcomes below for more details and references on this). Moreover, although individual symptoms may be non-specific, examination of combinations of symptoms may help to improve specificity for various groupings of respiratory viral infections (e.g., requiring the presence of fever, cough, and myalgias together). In addition, we note that there is the potential for measurement error due to inaccuracies in self-reporting (e.g., over or under-reporting symptoms).

Participants' serum-PFAS levels may have changed in the time between these surveys and the initial cohort blood draw, and they may have changed at different rates. People with the highest levels of PFAS at the time of the blood draw may not all have the highest levels during this study; this is uncertain as this study will not seek to capture updated PFAS blood measures. However, there is a question on drinking water source on the initial surveys. Since we are not collecting new blood samples for this study, the inclusion of these questions will allow us to potentially understand if people could have had additional exposure to PFAS in drinking water after PFAS levels in their blood were measured. For individuals on municipal water, the timings of the municipal water supply changes are known to the investigators and took place prior to PFAS blood level sampling.

Finally, participants know their serum-PFAS concentrations and they are aware of how their PFAS concentrations compare to others. People with higher levels in their cohort might be more likely to participate in this follow-up study, report more symptoms, or seek testing for viral infections (i.e., flu or COVID-19) because they perceive they are at increased risk or are more sensitive to symptoms. This might lead to bias in study findings.

Anticipated Products

The results of the study will be disseminated through abstracts, professional meeting presentations and manuscripts for publication in peer reviewed journals.

We will share the results from this study through community-facing mechanisms. As the study findings are being finalized, the study team will work with ATSDR communications to develop a roll-out plan on how to best share these results with community members. This could potentially include a webinar for community members, distribution of fact sheets, etc.

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Peer Review

This Protocol was reviewed through ATSDR's external peer review process by 3 leading experts in the field:

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- Jamie DeWitt, PhD, DABT, Associate Professor, Brody School of Medicine, East Carolina University
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