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TITLE:	An Investigation of Serum Levels of Per- and Polyfluoroalkyl Substances and Testicular Cancer Risk Within the Department of Defense Serum Repository
PRINCIPAL INVESTIGATOR:	Dr. Mark Purdue
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Tacoma, WA 98402

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are substances used until recently in the manufacture of aqueous film forming foams (AFFF) for firefighting, non-stick cookware, and other products. Military sites with a history of using AFFFs have been identified as a major source of PFAS water contamination for several communities. However, the level of exposure to PFAS experienced by military personnel is unclear. Limited evidence suggests that perfluorooctanoic acid (PFOA), one of the most produced and studied PFAS, is associated with increased risk of testicular cancer. To clarify this research question, we plan to conduct a case-control study of serum PFAS levels and testicular cancer risk nested within the Department of Defense Serum Repository (DoDSR). We will measure PFAS levels in the earliest and latest available banked serum samples stored in DoDSR from 500 Air Force servicemen who later developed testicular cancer, identified through the DoD Automated Central Tumor Registry, and 500 Air Force cancer-free controls. Serum levels of PFOA and nine other PFAS analytes will be measured using automated solid-phase extraction coupled to reversed-phase high-performance liquid chromatography-tandem mass spectrometry at the Centers for Disease Control and Prevention.

15. SUBJECT TERMS

Air Force, PFAS, PFOA, PFOS, testicular cancer, serum, Department of Defense Serum Repository, epidemiology, nested case-control study, etiology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

Page

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

PFAS are man-made chemicals that for several decades had been used to make firefighting foams, non-stick cookware coating and other products. PFAS are now widespread contaminants in the blood of Americans due to exposures through contaminated food and water. Military sites with a history of using PFAS-containing firefighting foams have been identified as a major source of PFAS water contamination. The Department of Defense recently identified 401 bases (including 203 Air Force installations) with a known or suspected release of PFAS, including 126 bases (50 Air Force) with tested drinking water or groundwater PFAS concentrations exceeding current safety guidelines. It is not known to what extent military personnel have been exposed to PFAS. The PFAS chemical perfluorooctanoic acid (PFOA) has been classified as a possible cancer-causing agent by the International Agency for Research on Cancer, with suggestive evidence from epidemiologic studies of an increased risk of testicular cancer, the most commonly diagnosed cancer among US males aged 15-39 and among male active-duty servicemen. The cancer-causing potential of other PFAS has not yet been evaluated. To clarify whether exposure to PFOA and other PFAS is associated with testicular cancer risk, we are conducting a nested case-control study within the Department of Defense Serum Repository. We will compare measurements of PFOA and 11 other PFAS chemicals in stored serum samples from 500 Air Force servicemen who later developed testicular cancer and 500 controls (male cancer-free Air Force personnel matched to cases on several factors). We will also investigate whether US Air Force servicemen stationed at installations that used PFAS-containing firefighting foams have higher serum levels of PFAS than servicemen stationed at locations that did not use these foams.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Air Force, PFAS, PFOA, PFOS, testicular cancer, serum, Department of Defense Serum Repository, epidemiology, nested case-control study, etiology

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: IRB Approvals and Study Design

1) Subtask 1: Obtain IRB approval from USUHS and NCI
   Original target dates for completion: months 1 to 6 (Sept 2019 – Feb 2020)
   Percentage of completion: 100%

2) Subtask 2: Select 500 eligible cases and 500 matched controls with serum samples available in DoDSR; aliquot and send serum samples to CDC laboratory
   Original target dates for completion: months 6 to 11 (Feb 2020 – July 2020)
   Percentage of completion: 100%

Major Task 2: Measure 12 serum PFAS using Automated Solid-Phase Extraction Coupled to Reversed-Phase High-Performance Liquid Chromatography-Tandem Mass Spectrometry

Original target dates for completion: months 11 to 15 (Aug 2020 to Dec 2020)
Percentage of completion: 50% *(This task is expected to be completed by January 2022)*

Major Task 3: Analyze PFAS Dataset and Prepare Results of Project for Publication in Peer-Reviewed Journals

Original target dates for completion: Dec 2020 to Sep 2021
Percentage of completion: 0% *(This task is expected to be completed by September 2022)*

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Please note that due to project delays in Study Year 1 related to the COVID-19 pandemic, we requested and were approved for a one-year no-cost extension to the grant period.

In Study Year 2 we completed the selection of study cases and controls, requisition and aliquotting of their banked serum specimens, and shipment of the samples to the laboratory at the Centers for Disease Control (i.e., Subtask 2 of Major Task 1) for PFAS measurements.

In Study Year 2 we also began working on Major Task 2 (PFAS measurements in banked serum samples). At the time of the preparation of this report, the laboratory had measured PFAS concentrations in nearly half of the study samples. It is expected that Major Task 2 will be completed by January 2022.

As the project is still ongoing, we do not yet have results / key outcomes to report.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period, we plan to:

1) Complete the measurement of PFAS concentrations in serum samples (Major Task 2); and
2) Complete the data analysis and preparation of manuscripts (Major Task 3).

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

In Study Year 2 we encountered some delays. The completion of the case & control selection by the Armed Forces Health Surveillance Branch and the requisition and aliquotting of serum specimens by the Department of Defense Serum Repository took longer than expected, due to limited manpower at the time. Both tasks however were eventually completed. The CDC laboratory has also experienced some staffing turnover and a COVD-related reduction in the number of employees who can work in the laboratory at a time which has affected the speed with which they can analyze our samples. However, they have recently made good progress and expect to be completed in January of 2022.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

There were no significant changes in the approved protocol to report. (Note: this question is not applicable to our project, which involves the use of banked serum specimens and data from the Defense Medical Surveillance System and Automated Central Tumor Registry.)

Significant changes in use or care of vertebrate animals

Not applicable to this project.

Significant changes in use of biohazards and/or select agents

Not applicable to this project.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  As the project is still in progress, there are no journal publications as yet to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

As the project is still in progress, there are no publications as yet to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

As the project is still in progress, there have been no presentations or publications as yet to report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Dr. Mark Purdue, National Cancer Institute
Project Role:	Principal Investigator
ORCID ID:	https://orcid.org/0000-0003-1177-3108
Nearest person month worked:	3
Contribution to Project:	Dr. Purdue drafted the IRB protocol, Defense Health Agency DSA application and the request for testicular cancer case records from the DoD Cancer Registry. He also led discussions with AFHSB regarding case and control selection methods and serum selection and organized the serum sample shipment to the CDC laboratory. Dr. Purdue has had regular teleconferences with Dr. Rusiecki to advance the study.
Funding Support:	Not applicable. Dr. Purdue is a federal government employee.
Name:	Dr. Jennifer Rusiecki, Uniformed Services University
Project Role:	Co-Investigator
ORCID ID:	Dr. Rusiecki does not have an ORCID ID
Nearest person month worked:	2
Contribution to Project:	Dr. Rusiecki edited and submitted the IRB protocol at USU and contributed to the drafting of the DHA DSA application. She has contributed to the discussions and (and, as a DoD-affiliated researcher, is the PI of record on these documents) and contributed to the discussions with AFHSB regarding case and control selection. Dr. Rusiecki has had regular teleconferences with Dr. Purdue to advance the study.
Funding Support:	Not applicable. Dr. Rusiecki is a federal government employee.
Name:	Ivy Hung, Uniformed Services University
Project Role:	Research Assistant
ORCID ID:	Ms. Hung does not have an ORCID ID
Nearest person month worked:	0.25
Contribution to Project:	Ms. Hung was responsible for coordinating the submission of necessary applications and took minutes on teleconferences.
Funding Support:	Ms. Hung's salary is supported through several grants of Dr. Rusiecki.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

National Cancer Institute, Division of Cancer Epidemiology and Genetics
Rockville, MD
Contribution to the project:

- Financial support (NCI is providing additional funding to include additional samples beyond those covered by this grant);
- Facilities

Uniformed Services University of the Health Sciences, Preventive Medicine and Biostatistics
Bethesda, MD
Contribution to the project:

- Facilities

Armed Forces Health Surveillance Branch
Silver Spring, MD
Contribution to the project:

- Collaboration

Centers for Disease Control & Prevention
Atlanta, GA
Contribution to the project:

- Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.