AWARD NUMBER: W81XWH-21-2-0048

TITLE: The Impact of Gulf War Inhalant Pollutant and Chemical Exposures on the Upper Sinonasal Airway

PRINCIPAL INVESTIGATOR: Jivianne T. Lee, MD

CONTRACTING ORGANIZATION: VA Greater Los Angeles Healthcare System

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Fort Detrick, Maryland 21702-5012

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OMB No. 0704-0188

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OCTOBER 2022	ANNUAL	15SEPT2021 - 14SEPT2022

4. TITLE AND SUBTITLE

The Impact of Gulf War Inhalant Pollutant and Chemical Exposures on the Upper Sinonasal Airway

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5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jivianne T. Lee, MD

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

VA Greater Los Angeles Healthcare System

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:

Despite upper respiratory disease (i.e. chronic rhinosinusitis) debilitating a significant proportion of Gulf War (GW) Veterans, data regarding the impact of GW exposures on the sinonasal airway remain limited. The proposed research aims to investigate the effects of GW chemical and pollutant exposure on tissues of the sinonasal tract, which to date has not been fully characterized. We hypothesize that GW toxicant and particulate matter exposures from pesticides and oil well fire effluents contribute to the underlying pathophysiology of Gulf War Illness associated sinonasal disease. This translational study utilizes 3 parallel but complementary systems to elucidate the molecular, cellular, and microbiome changes in sinonasal epithelia that arise from GW airborne chemical and pollutant exposures.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	24	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

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

Despite upper respiratory disease (i.e. chronic rhinosinusitis) debilitating a significant proportion of Gulf War (GW) Veterans, data regarding the impact of GW exposures on the sinonasal airway remain limited. The proposed research aims to investigate the effects of GW chemical and pollutant exposure on tissues of the sinonasal tract, which to date has not been fully characterized. We hypothesize that GW toxicant and particulate matter exposures from pesticides and oil well fire effluents contribute to the underlying pathophysiology of Gulf War Illness associated sinonasal disease. This translational study utilizes 3 parallel but complementary systems to elucidate the molecular, cellular, and microbiome changes in sinonasal epithelia that arise from GW airborne chemical and pollutant exposures.

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

Gulf War Illness, toxicant, pollutant, particulate matter, pesticide, chronic rhinosinusitis

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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.

Aim 1- To determine *in vitro* effects of Gulf War toxicant and particulate matter exposure on human sinonasal epithelial cells (SOW target date completion: 24 months; percentage of completion to date: 40%)

1.) **SOW Milestone(s) Achieved: SOW target date 8-12 months**
   a. Local IRB Approval: Completed
   b. HRPO approval: Completed
   c. Optimal dose for Permethrin/ DEET for combined exposures: Completed
   d. Cytotoxicity/oxidative stress: 70% complete

2.) **Major Task 1:** To determine *in vitro* effects of Permethrin and DEET on cultured cells (SOW target completion date: 8-12 months; percentage of completion to date: 70%)
   a. **Subtask 1**- Grow primary cell cultures from human sinonasal tissue specimens (SOW target completion date: 2-3 months; percentage of completion to date: 100%)
   b. **Subtask 2**- Determine the *in vitro* effects of Permethrin on cultured cells (SOW target completion date: 2-3 months; percentage of completion to date: 70%)
   c. **Subtask 3**- Determine the *in vitro* effects of DEET on cultured cells (SOW target completion date: 2-3 months; percentage of completion to date: 70%)
   d. **Subtask 4:** Determine the *in vitro* effects of Permethrin/DEET in cultured cells (SOW target completion date: 2-3 months; percentage of completion to date: 70%)

3) **Major Task 2:** To determine *in vitro* effects of PM on primary human sinonasal epithelial cells (target completion date per SOW: 8-12 months/Year 2). Will commence work on this major task next month.

B. Aim 2- To assess the impact of GW toxicant and ultrafine nanoparticulate matter (nPM) exposure on the upper sinonasal airway *in vivo* (SOW target date completion: 30 months; percentage of completion to date: 30%)

1. **Milestones achieved SOW target date: 30 months**
    a. Local IACUC Approval: Completed
    b. ACURO Approval: Completed
    c. Determine optimal dosage of Permethrin/DEET for combined exposures: 70% completed
    d. Differential effects of ultrafine nPM with and/without Permethrin/DEET versus controls on sinonasal tissue/nasal fluid: 30% completed

2. **Major Task 1**: To determine toxicity thresholds/effect levels for Permethrin/DEET (SOW target date of completion 4 months: 70% completed)
    a. **Subtask 1:** Dose ranging study for Permethrin/DEET (n = 5-10 mice): completed one time for each agent but plan to repeat
    b. **Subtask 2:** To harvest sinonasal tissue and nasal lavage fluid for cytokine analysis: completed one time for each agent but plan to repeat

3. **Major Task 2**: To determine the effects of ultrafine nPM/Permethrin/DEET exposure (SOW target date of completion 8 months; 30% completed)
    a. **Subtask 1:** To randomize mice into control/exposed groups and perform exposure experiments: completed for ultrafine nPM, Permethrin, and DEET individually one time but not yet in combination. Plan to repeat.
    b. **Subtask 2:** To obtain nasal lavage fluid and sinonasal tissue following exposures for cytokine, histopathologic, and microbiome analysis: completed for ultrafine nPM, Permethrin, and DEET individually one time but not yet in combination with Permethrin/DEET. Plan to repeat.
    c. **Subtask 3:** To obtain serum and brain tissue following exposures for histopathology and biomarker analysis: completed for ultrafine nPM and controls one time but not yet in combination with Permethrin/DEET. Plan to repeat.

4. **Major Task 3 and 4:** Repeat the exposure experiments in Major Task 2 (SOW target date 14-18 months; will commence in Year 2)

C. Aim 3- To compare sinonasal microbiome composition, cytokine profiles, and serum biomarkers of Veterans with GWI associated chronic rhinosinusitis (CRS) to healthy controls. (SOW target date of completion 36 months)

1. **Milestones achieved: SOW target date 30 months**
    a. Local IRB approval: complete
    b. HRPO approval: complete
    c. Fort Benning IRB approval: pending
    d. SOW Target enrollment during 3-year study period: 30 CRS without GWI, 30 CRS with GWI patients
    e. SOW Target enrollment for Year 1: 20 patients; percentage completion 25%

2. **Major Task 1**: Procure sinus tissue and blood samples from study participants
    a. **Subtask 1**: Enroll Veterans with CRS with and without GWI (SOW target enrollment for Year 1 was 20 patients; percentage complete: 25% as 5 patients have been enrolled with specimens procured)
    b. **Subtask 2:** Disseminate SNOT-22, MDFI, MPQ-SF and obtain specimens from study participants. (SOW target enrollment for Year 1 was 20 patients; percentage complete: 25% as 5 patients have been enrolled with specimens procured and questionnaires completed)

3. **Major Task 2:** Compare microbiome composition and cytokine profiles of study participants (will commence in Year 2 once more samples are procured)

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.

A. Aim 1- To determine *in vitro* effects of Gulf War (GW) toxicant and particulate matter exposure on primary human sinonasal epithelial cells

1.) **Major activities:** Sinonasal tissue was collected from 5 patients undergoing endoscopic transsphenoidal surgery without a history of sinonasal disease or GW exposures. A portion of the tissue was transported to the laboratory in serum free RPMI media and processed for *in vitro* tissue culture as previously described. 96 well tissue culture plates were exposed to different concentrations of DEET and Permethrin both individually and in combination. The IncuCyte system was set to scan the wells every 2h from 0h to 9.5 days (228 h) after exposure. The data are presented as fold changes in cell density from initiation (0h) to specific time points during the assay. Cell density was calculated with IncuCyte software and phase contrast images. Each experiment was repeated 5 times per exposure combination during the study period.

2.) **Specific objectives:** To determine *in vitro* effects of Permethrin and DEET on cultured human sinonasal epithelial cells

a. Grow primary cell cultures from human sinonasal tissue specimens
b. Determine the *in vitro* effects of Permethrin on cultured cells with respect to cytotoxicity, cell viability, and proliferation
c. Determine the *in vitro* effects of DEET on cultured cells with respect to cytotoxicity, cell viability, and proliferation
d. Determine the *in vitro* effects of Permethrin/DEET in combination on cultured cells in terms of cytotoxicity, cell viability, and proliferation

3.) Significant results: Similar to our preliminary data (**Figure 1A**), when cells were treated with DEET (4000 cells/well, in quadruplicate) (**Figure 2A**), the IncuCyte results showed that cell proliferation is inhibited in a concentration dependent manner (0.625 mM-5mM). Treatment with 2.5mM of DEET was most effective for inhibition of primary cell growth. Likewise, when cultured cells (1500 cells/well, in quadruplicate) (**Figure 2B**), were treated with Permethrin (0.625mM-5mM), cell viability was reduced in a dose dependent manner. Our results indicate that at lower concentrations the toxicants are not killing the cells but may slow the cellular proliferation rate. When cultured cells were exposed to Permethrin and DEET in combination, a synergistic inhibition in cell proliferation was observed at certain concentrations; such that reduction in cell density was greater than the additive effects of the agents when used separately (**Figure 2C & 2D**). Similar results were obtained when the experiments were repeated (**Figure 3**).

Figure 1

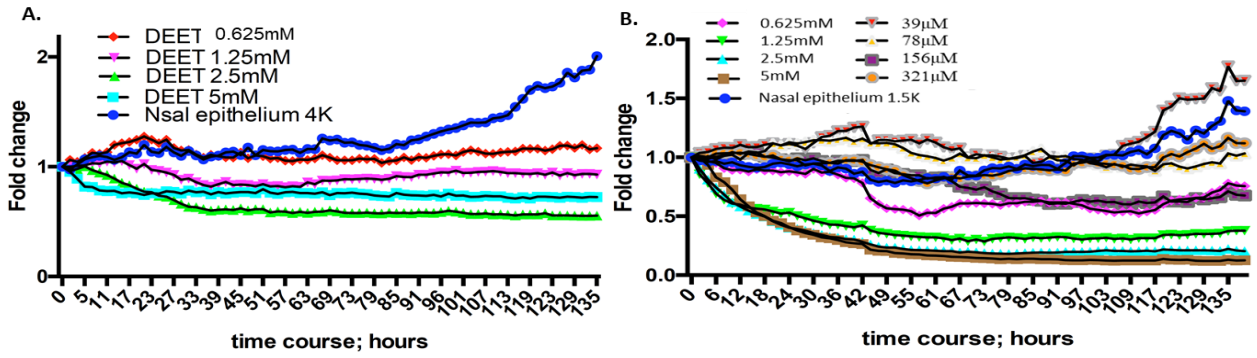

Figure 1: Time courses (x-axis, hours) for real time cell confluency (y-axis, fold changes in cell density) of primary sinus epithelial cells treated with (A) 0.625mM to 5mM DEET and (B) 39µM to 5mM Permethrin.

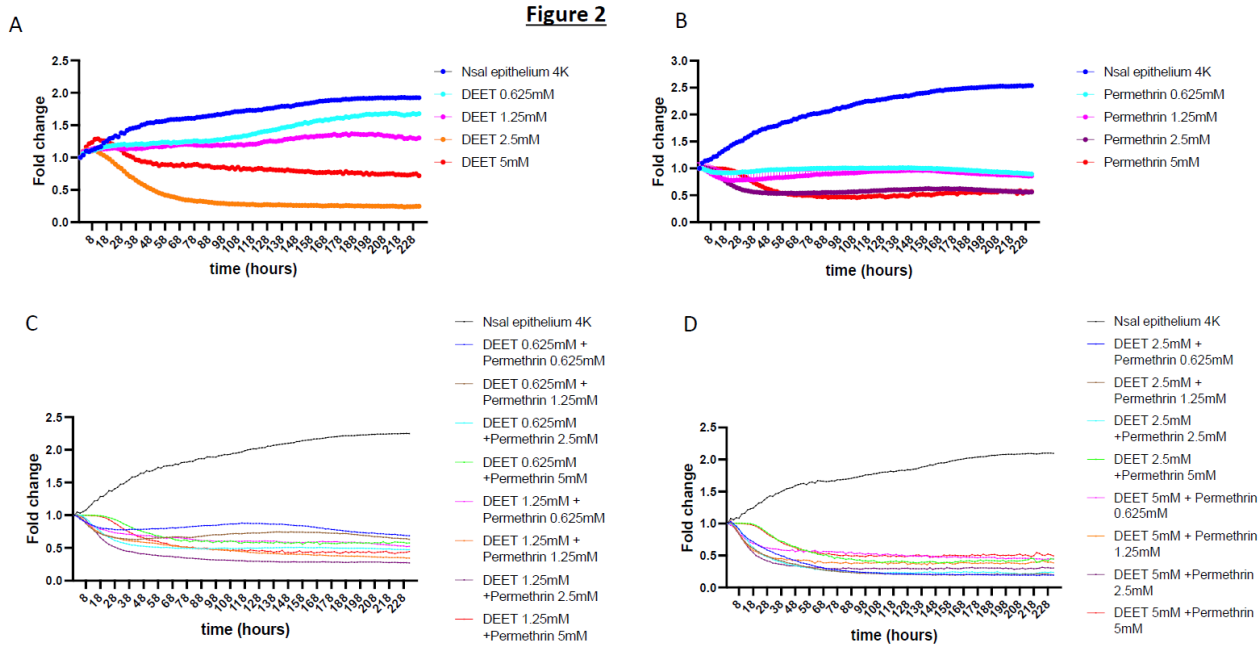

Figure 2: Time courses (x-axis, hours) for real time cell confluency (y-axis, fold changes in cell density) of primary sinus epithelial cells treated with (A) 0.625mM to 5mM DEET, (B) 0.625mM to 5mM Permethrin, (C) 0.625mM to 1.25mM DEET and 0.625mM to 5mM Permethrin, and (D) 2.5mM to 5mM DEET and 0.625mM to 5mM Permethrin.

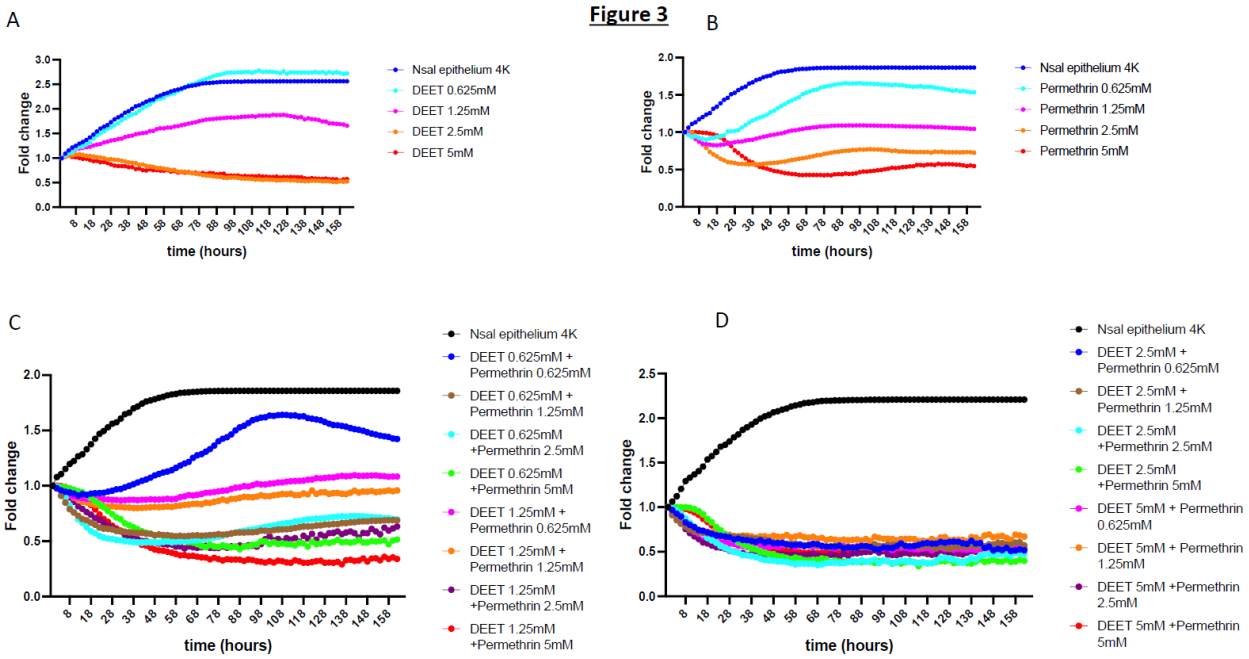

Figure 3: Time courses (x-axis, hours) for real time cell confluency (y-axis, fold changes in cell density) of primary sinus epithelial cells treated with (A) 0.625mM to 5mM DEET, (B) 0.625mM to 5mM Permethrin, (C) 0.625mM to 1.25mM DEET and 0.625mM to 5mM Permethrin, and (D) 2.5mM to 5mM DEET to 0.625mM to 5mM Permethrin.

4.) Stated goals not yet met: While the results to date have illustrated the cytotoxic effects of Permethrin and DEET on cultured sinonasal epithelial cells, exposure experiments will have to be repeated to verify reproducibility and accuracy. Specific concentrations of Permethrin/DEET eliciting a synergistic reaction need to be confirmed. Oxidative stress studies and analysis of inflammatory biomarkers need to be conducted to investigate potential underlying pathomechanisms causing reduction in cell viability and proliferation from pesticide exposures. Particulate matter exposure experiments also need to be performed to examine the impact of pollutants on cultured sinonasal epithelial cells in isolation and when administered in conjunction with pesticides.

B. Aim 2: To assess the impact of GW toxicant and ultrafine nanoparticulate matter (nPM) exposure on the upper sinonasal airway *in vivo*

1.) **Major activities:** Following IACUC and ACURO approval, 3 murine experiments were conducted during the study period to investigate the effects of Permethrin, DEET, and ultrafine nPM on sinonasal epithelia *in vivo*
   a. **Permethrin:** Sixteen mice were randomized to receive either saline (controls n=4), Permethrin (4mg/day; n=6), or Permethrin (8mg/day; n=6), for 7 days via transnasal instillation (volume 10 µL). Following the exposure period, sinonasal and brain tissue were harvested and collected in Z-fix and RNA later for subsequent histopathologic and microbiome analysis.
   b. DEET: Sixteen mice were randomized to receive either saline (controls n=4), DEET (0.06mg/day), or DEET (1mg/day), for 7 days via transnasal instillation (volume 10µL). Following the exposure period, sinonasal and brain tissue were harvested and collected in Z-fix and RNA later for subsequent histopathologic and microbiome analysis.
   c. Ultrafine nPM: Twenty-two mice were randomized to receive either filtered air (controls n=11) or ultrafine nPM (n=11) for 14 weeks. Following the exposure period, sinonasal and brain tissue were harvested and collected in Z-fix and RNA later for subsequent histopathologic and microbiome analysis. Sinonasal lavage fluid was also collected for analysis of inflammatory cells.

2.) **Specific objectives:**
   a. To determine individual toxicity threshold/effect levels for Permethrin and DEET on sinonasal epithelia *in vivo*
   b. To harvest sinonasal tissue and nasal lavage fluid following exposure to Permethrin, DEET, or nPM and analyze for inflammatory changes

3.) **Significant results:** Exposure to DEET at 1mg/day for 7 days demonstrated neutrophilic infiltration in murine sinonasal epithelia **(Figure 4**). Increased mucinous cells were also present in the underlying stroma (**Figure 5**).

Figure 4:

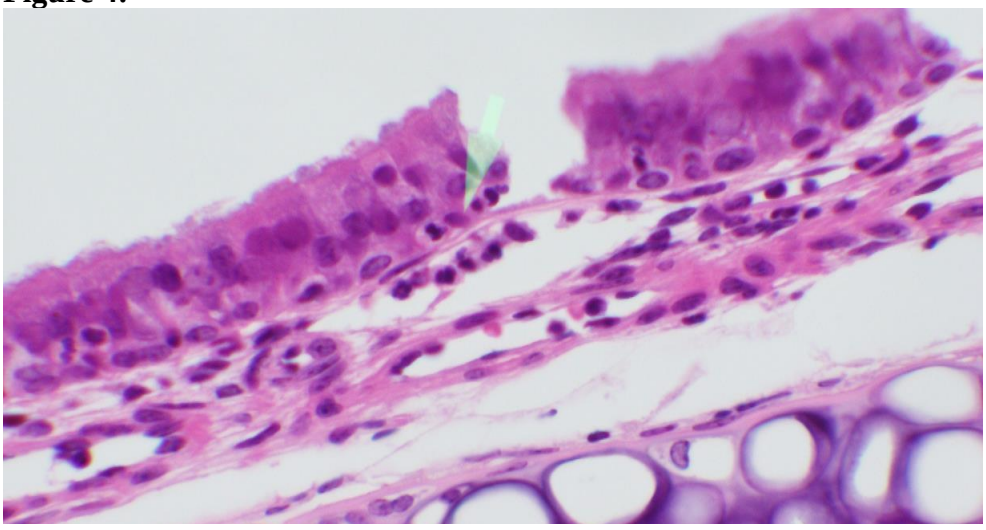

Figure 4: H&E stained cross section of nasal cavity of DEET treated mouse at 40X demonstrating neutrophilic (arrow) infiltrate within the underlying stroma and ciliated pseuodostratified columnar epithelia

Figure 5:

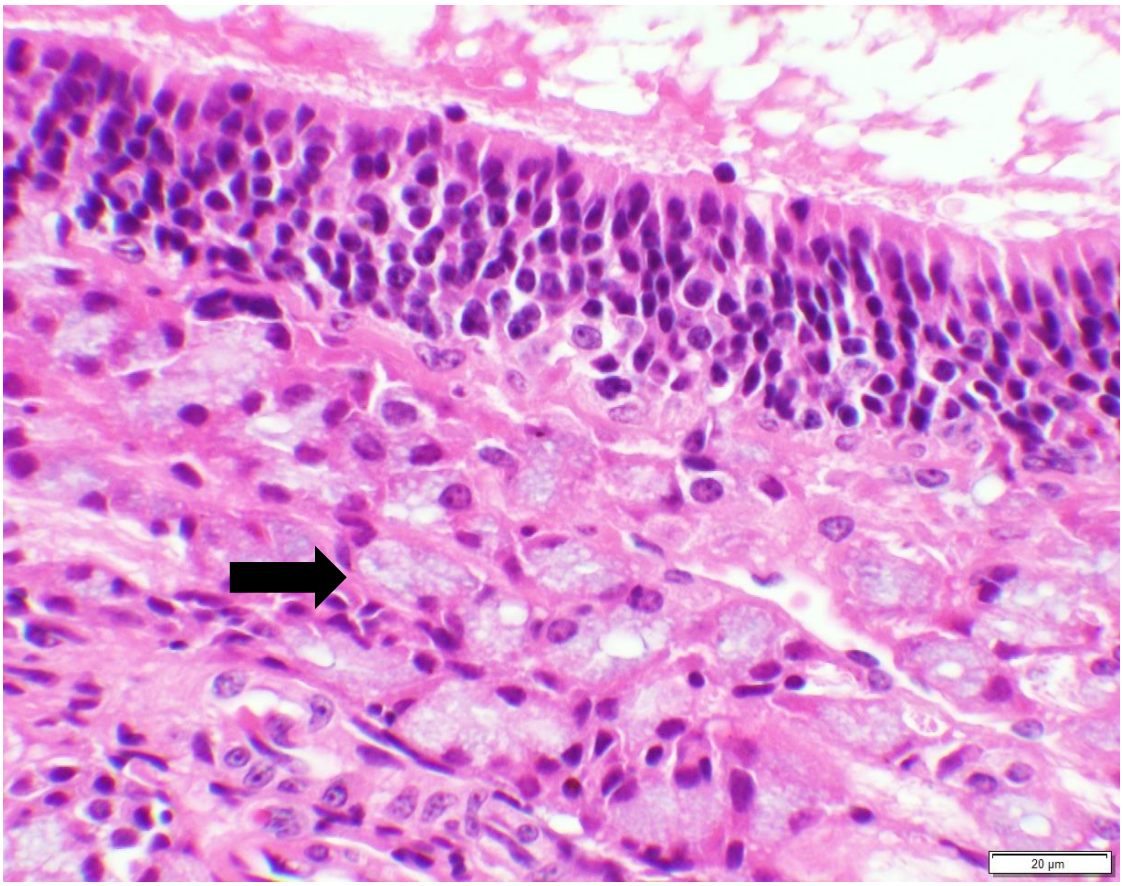

Figure 5: H&E stained cross section of nasal cavity of DEET treated mouse at 40X showed increased mucinous cells (arrow) and submucosal edema.

5.) Stated goals not yet met: Analysis of murine specimens from Permethrin and ultrafine nPM exposures must still be conducted to assess the impact of those exposures, respectively. Combined Permethrin, DEET, and ultrafine NPM exposure experiments need to be completed to determine potential additive/synergistic deleterious effects on the sinonasal airway.

C. Aim 3- To compare sinonasal microbiome composition, cytokine profiles, and serum biomarkers of Veterans with GWI associated chronic rhinosinusitis (CRS) to healthy controls.

1.) **Major activities:** Following local IRB and HRPO approval, sinus tissue and blood were collected from 4 control patients and 1 GWI patient with CRS during the study period. Sinus tissue was stored in RNA later and flash frozen, respectively, for subsequent microbiome and cytokine analysis. Blood was centrifuged and serum collected. Fort Benning IRB process has been initiated and is pending. During the study period, the PI (Jivianne T. Lee, MD) visited the DOD BBRAIN in person and met with the lab director. Supply kits were created for blood sample shipping to the DOD that would be used by both the Greater Los Angeles VA and Fort Benning.

2.) **Specific objectives:**

a. To procure sinonasal tissue and blood from 20 CRS patients with and without GWI. To date, biospecimens have been obtained from 4 CRS patients without GWI specimens, and 1 CRS patient with GWI.
b. To compare sinonasal epithelial cytokine profiles, microbiome composition, and serum biomarkers of GWI associated CRS to healthy controls.

3.) **Significant results**: Pending

4.) **Stated goals not yet met:** Increased enrollment is necessary to reach yearly target of 20 CRS patients with and without GWI. Once sufficient specimens are obtained, cytokine and microbiome analysis will commence.

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.

Training activities: Dr. Lee (PI) engaged in additional animal training during the study period and trained Dr. Basak in murine sinonasal tissue harvest and nasal fluid collection. Dr. Lee had further training in growing cell cultures and *in vitro* work from Dr. Basak as well. In addition, Dr. Lee and Dr. Basak were trained by Dr. Araujo's (Co-Investigator) lab personnel on exposure protocols, methods of administration, and murine specimen processing techniques. Dr. Lee also visited the DOD BBRAIN during the study period, directed by Dr. Sullivan and Dr. Klimas, Mentors and Co-Investigators on the project. During the visit, Dr. Lee met with lab personnel, who instructed her on creation of proper supply kits for biospecimen collection and shipping to the BBRAIN that would be used by the Greater Los Angeles VA, Fort Benning, and other potential enrollment sites.

Professional Development activities: Dr. Lee engaged in one-on-one discussions with Dr. Araujo and met with his lab personnel on a regular basis to expand her knowledge of animal exposure experiments and gain experience in running a toxicant/pollutant exposure laboratory. During these sessions, preliminary results, adjustments for future experiments, and grant writing were discussed. Due to COVID, the toxicology conference Dr. Lee had planned to attend was cancelled but plans have been made to participate in the upcoming year.

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.

For AIM 1, we plan to repeat the Permethrin/DEET exposures individually and in combination on cultured human sinonasal epithelial cells to verify our results to date. We will commence the *in vitro* particular matter exposure experiments both alone and in combination with Permethrin/DEET. Cytotoxicity, cell viability, and proliferation will be measured using the IncuCyte Live Cell System. Oxidative stress will be determined by assessing superoxide dismutase activity. Fluid from the cultured cells will be collected and inflammatory cytokine analysis conducted using the Luminex assay.

For AIM 2, we plan to repeat the individual Permethrin, DEET, and particulate matter murine exposure experiments to verify our results to date. We will commence the combined Permethrin/DEET/ particulate matter versus filtered air exposure protocol. Sinonasal tissue will be harvested for histopathologic and microbiome analysis. Nasal lavage fluid will be collected and analyzed for inflammatory cell accumulation and cytokine expression.

For AIM 3, we plan to continue enrollment of Veterans with Gulf War Illness associated chronic rhinosinusitis and healthy controls. Fort Benning should have IRB approval within the next study period. With COVID restrictions lifting and contribution of patients from Long Beach VA and Loma Linda VA, we anticipate more robust recruitment and meeting the Year 2 enrollment target of 40 patients. Sinonasal tissue and blood samples will be procured and microbiome composition, cytokine profiles, and serum biomarkers studies conducted. Biospecimens will also be sent to the DOD BBRAIN.

4. IMPACT: Describe 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 Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

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.

Due to COVID, there were significant setbacks in sinus tissue and blood sample procurement in controls and CRS with and without GWI patients (AIM 3). Elective sinus/skull base surgeries were suspended multiple times during the study period due to COVID surges which hampered recruitment and specimen collection. Supply chain issues, shutdowns, and labor shortages associated with the pandemic also impaired surgery scheduling and execution of research activities. As such only 5 patients were enrolled for AIM 3 instead of the anticipated 20 patients.

Action plan: With the COVID restrictions lifting, enrollment is anticipated to improve in the next study period as surgery scheduling is ramping up. Recruitment of subjects has expanded to include the Long Beach VA and Loma Linda VA in the coming year. Fort Benning is anticipated to have IRB approval as well in the upcoming study period.

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

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

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).*

Nothing 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).*

Nothing 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.*

Nothing 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.

Not applicable

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Not applicable

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. 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.

Not applicable

- **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;*
  - *biospecimen 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."

Name:	Jivianne T. Lee, MD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-0711-855X
Nearest person month worked:	6
Contribution to Project:	Dr. Lee has been involved in overall execution and supervision of all experiments to date. This includes but is not limited to experimental design, *in vitro* and *in vivo* animal exposure studies, murine tissue/fluid collection, human subject enrollment, biospecimen procurement, and analysis of study findings.

Name:	Saroj Basak, PhD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	None
Nearest person month worked:	3.6
Contribution to Project:	Dr. Basak has been involved in conducting the *in vitro* exposure experiments as well as the animal exposure studies.

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

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:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (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.*

Organization Name: University of California Los Angeles David Geffen School of Medicine
Location of Organization: Los Angeles, California
Partner's Contribution to the Project: Dr. Araujo, a Co-Investigator on the project, directs two Inhalational Exposure Facilities in the UCLA Department of Laboratory and Animal Medicine where the *in vivo* exposure experiments to ultrafine nPM took place during the study period. .

Organization Name: GWI Boston Biorepository, Recruitment, and Integrated Network (BBRAIN)
Location of Organization: Miami, Florida
Partner's Contribution to the Project: Dr. Lee visited the DOD BBRAIN site at Nova Southeastern University/Miami Veterans Affairs Medical Center directed by Dr. Klimas and Dr. Sullivan, Mentors and Co-Investigators on the project. Supply kits were created with lab personnel for biospecimen collection and shipping to the BBRAIN.

8. **SPECIAL REPORTING REQUIREMENTS**
   COLLABORATIVE AWARDS: N/A
   QUAD CHARTS: N/A

9. **APPENDICES:** N/A