AWARD NUMBER: W81XWH-14-2-0130

TITLE: Permethrin Exposure Dosimetry: Biomarkers and Modifiable Factors

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REPORT DATE: August 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Aug 2018	Annual	15 Jul 2017 – 14 Jul 2018

4. TITLE AND SUBTITLE

Permethrin Exposure Dosimetry: Biomarkers and Modifiable Factors

5a. CONTRACT NUMBER
W81XWH-14-2-0130

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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the Advancement of Mil Med, Inc.
6720-A Rockledge Drive
Bethesda, MD 20817

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel 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

The primary aim of this project is to investigate the relationship between various modifiable factors and the absorption of permethrin as a result of wearing permethrin-treated Army Combat Uniforms (ACU-Permethrin). The research objective is to examine the effect of body weight/BMI and total energy expenditure on permethrin absorption and dose, as determined by measurement of urinary biomarkers (3PBA and cis- and trans-DCCA) levels. There are two studies involved in our project – the first is a study among Army recruits during Basic Training (Study 1) and the second involves Army National Guard Soldiers during Annual Training (Study 2). Data collection for Study 1 and for Study 2 was completed in 2015 and 2017 respectively. Data analyses are complete. Manuscript preparations are in progress.

15. SUBJECT TERMS
Permethrin, biomarkers, military, dose, exposure dosimetry, military, energy expenditure

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

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Section 1: Introduction

The primary aim of this project is to investigate the relationship between various modifiable factors and the absorption of permethrin as a result of wearing permethrin-treated Army Combat Uniforms (ACU-Permethrin). The research objective is to examine the effect of body weight/BMI and total energy expenditure on permethrin absorption and dose, as determined by measurement of urinary biomarkers (3PBA and cis- and trans-DCCA) levels. There are two studies involved in our project – the first is a study among Army recruits during Basic Training (Study 1) and the second involves Army National Guard Soldiers during Annual Training (Study 2).

Section 2: Keywords

Permethrin, biomarkers, military, dose, exposure dosimetry, military, energy expenditure

Section 3: Accomplishments

3:1 - What were the major goals of the project?

As described in the approved Statement of Work (see Table of Tasks below), the major goals during the Year 4 of this project are outlined.

Year 1	***Task 1***	*Months 1-4*	-Project set up and approvals
	Task 2	*Months 4-8*	-Plan logistics for Study 1
	Task 3	*Months 4-8*	-Study 1 protocol approval
	Task 4	*Months 8-12*	-Initiate Study 1 data collection
	Task 5	*Months 10-12*	-Initiate laboratory analyses of Study 1 samples
	Task 6	*Months 10-12*	-Initiate Study 1 data management steps; integrate with USARIEM research database system
Year 2	***Task 7***	*Months 13-15*	-Prepare analytic dataset for data analyses
	Task 8	*Months 15-17*	-Initiate Study 1 data analyses to address hypotheses
	Task 9	*Months 14-20*	-Plan logistics for Study 2
	Task 10	*Months 14-20*	-Study 2 protocol approval
	Task 11	*Months 16-24*	-Report/summarize Study 1 results
	Task 12	*Months 20-24*	-Initiate Study 2 data collection
	Task 13	*Months 25-26*	-Initiate laboratory analyses of Study 2 samples

Year 3	***Task 14***	*Months 25-30*	-Initiate Study 2 data management steps
	Task 15	*Months 30-36*	-Complete Study 1 and Study 2 laboratory sample analyses
Year 4	***Task 16***	*Months 37-39*	-Complete integration of analytic dataset for project data analyses
	Task 17	*Months 37-39*	-Complete Study 2 data analyses to address hypotheses
	Task 18	*Months 39-48*	-Report/summarize Study 2 results -Prepare Project technical reports/manuscripts for publication
	Task 19	*Months 39-48*	-Provide/disseminate evidence-based guidance

TASKS 1-3 were completed in Year 1 (See Year 1 Annual Report.)
TASKS 4-10, 12, and 14 were completed in Year 2 (See Year 2 Annual Report.)
TASK 13 was completed in Year 3 (See Year 3 Annual Report.)

3:2 - What was accomplished under these goals?

In Year 4, Tasks 11, 15-17 were completed.
Below is a bulleted list of the projected goals and accomplishments over this Year 4 study period:

Task 11 *Report/summarize Study 1 results* [**COMPLETE**]

- Data analyses to address the Study 1 hypotheses are complete.

  Summary of Study 1 conducted among Active Duty Army recruits**.** The age range of the 60 participants was between 18-29.5 years and the average age was 20.8 years old. Thirty percent of the group was female. Among the 44 persons who completed all aspects of the longitudinal study, baseline percent body fat (% BF) was 14.72%, and the average % BF decreased 2.83 % (males decreased 3.17%; females decreased 2.14%) by the end of the study (10 week period of Basic Combat Training (BCT)). The highest level of urinary permethrin metabolites were observed during week 1 of BCT, which makes intuitive sense as the uniforms worn were new with limited washing. Mixed models were run to examine the hypothesis of whether permethrin exposure/absorption was associated with % BF and total daily energy expenditure (TEE). Analyses show that 10% higher % BF is statistically associated with a 4.42% higher concentration of 3-phenoxybenzoinc acid, a permethrin metabolite; ($F = 5.93$, $p = 0.0223$) a 10% higher TEE is statistically associated with a 10.57% increase in 3-phenoxybenzoinc acid concentration ($F = 4.93$, $p = 0.0357$). This association holds after controlling for known confounders including sex, age, time that uniform was worn, times uniform was washed, and the duration of time in BCT.

- Results were presented via a poster at the American Public Health Association Annual Meeting in Atlanta in Nov 2017. (Abstract is provided in the Appendix.)

- Oral presentations were delivered on study results at:

- ✓ 21st Triennial DoD Pest Management Workshop (coordinated by the Armed Forces Pest Management Board (AFPMB)), held at Lackland AFB, on 20 March 2018
- ✓ US Army Research Institute of Environmental Medicine Seminar Series, Natick, MA, on 24 May 2018
- ✓ US Army Public Health Center, Aberdeen, MD, 6 June 2018

Task 15 -*Complete Study 1 and Study 2 laboratory sample analyses* [**COMPLETE**]

- CDC provided the remaining urine sample analyses results for permethrin metabolite concentrations from Study 2 to USARIEM.

Task 16 *Complete integration of analytic dataset for project data analyses* [**COMPLETE**]

- Data were integrated into the USARIEM study database in Q2 of the past year. All data management tasks have been completed and the entire dataset has been optimized for analyses.

Task 17 *Complete Study 2 data analyses to address hypotheses* [**COMPLETE**]

- Data analyses to address the Study 2 hypotheses are complete.

  Summary of Study 2 conducted among Army National Guard Soldiers. Participants (n = 49) ranged in age between 20-53 years old, with an average age of 28.6 years. Over the 9 day study period, average %BF was 16.88% and did not vary significantly over time. Mixed models run to examine the hypothesis that permethrin exposure was associated with %BF and TEE found no significant association. However, a significant interaction effect was observed between %BF and the number of times a uniform was washed on permethrin biomarker concentration ($F = 6.90$, $p = 0.0099$). Among participants wearing uniforms washed <50 times, a 10% increase in %BF was associated with a 15.5% increase in 3-phenoxybenzoic acid concentration, while there was no association between %BF and 3-phenoxybenzoic acid concentration among participants wearing uniforms washed >50 times. The association held after controlling for known confounders including sex, age, and the time duration that uniform was worn.

Task 18 *Report/summarize Study 2 results; Prepare project technical reports/manuscripts for publication* [**~75% COMPLETE]**

- Two manuscripts describing Study 1 results have been drafted: both were recently submitted to journals for publication consideration

- A manuscript describing Study 2 results is being drafted.

Task 19 *Provide/disseminate evidence-based guidance* [**IN PROGRESS**]

- As described above, manuscripts describing Study 1 and Study 2 results are in the process of being submitted to peer-reviewed journals.

- In the Fall 2017, an executed Transition Agreement with the Army Public Health Center was established, for the dissemination of USARIEM research pertaining to permethrin exposure from wearing permethrin-treated uniforms. The PI and team have reported study findings in several different settings over the past year (see Task 11 above).

3:3 - What opportunities for training or professional development has the project provided?

As reported in earlier Annual Reports for this award:

- A Boston University School of Public Health graduate student (MPH candidate) worked on this project from July 2016-June 2017; her primary role on the project was performing data management and analytic tasks for the project. She graduated with her MPH in Dec 2016.

- A Boston University School of Public Health graduate student (PhD candidate) has worked on this project for Oct 2015- June 2018; her primary role on the project has been to help direct field data collection activities and in preparation of reports, abstract, manuscripts, and presentations. She graduated with her PhD in May 2017.

3:4 - How were [are] the results [being] disseminated to communities of interest?

- Presentation of study results have been made at national conferences, as well as direct briefings to DoD stakeholders (i.e., USA Training and Doctrine Command, Federal and State National Guard stakeholders, Armed Forces Pest Management Board, and US Army Public Health Center). See Task 11 above.

3:5 - What do you plan to do during the next reporting period to accomplish the goals?

- During the next reporting period, we will
  - Finalize manuscripts and submit them for publication in peer-reviewed journals
  - As noted below, the PI (Dr. Proctor) has been invited as a subject matter expert to attend the upcoming World Health Organization meeting in Helsinki Finland, 29-31 Aug that will examine the human health risk assessment models for repellants and treated clothing.

Section 4: Impact

4:1 - What was the impact on the development of the principle discipline of the project?

This project permethrin research, in addition to others previously conducted by the PI (Dr. Proctor), are contributing to the knowledge base pertaining to human exposure assessment to permethrin through treated clothing. As such, Dr. Proctor has been invited by the Armed Forces Pest Management Board to attend the upcoming World Health Organization meeting in Helsinki Finland, 29-31 Aug; the meeting is being

convened to examine the human health risk assessment models for repellants and treated clothing.

4:2 - What was the impact on other disciplines?

- Nothing to report at this point.

4:3 - What was the impact on technology transfer?

- Nothing to report at this point.

4:4 - What was the impact on society beyond science and technology?

- Permethrin treated clothing are worn by general population groups (through commercially available outdoor treated clothing), as well as the military. As permethrin is a pesticide regulated by US Environmental Protection Agency (EPA), findings from this study will contribute to the knowledge base for human exposure and human health policies for the US and the international community.

Section 5: Changes/Problems

5:1 - Changes in approach and reasons for change

- Nothing to report.

5:2 - Actual or anticipated problems or delays and actions or plans to resolve them

- In Q3, we anticipated that we would have all of our study results summarized in manuscripts, with the manuscripts submitted and accepted in peer-review literature by July 2018. However, this timeline has not been met, so we requested and been granted a no-cost extension for 12 months to July 2019 to complete the remaining project Tasks (Tasks 18 and 19). We anticipate completing these plans on schedule.

5:3 - Changes that had a significant impact on expenditures

- Nothing to report.

5:4 - Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

- Nothing to report

Section 6: Products

6:1 - Publications, conference papers, and presentations

- Poster presentation delivered at the APHA Annual Meeting in Nov 2017: Submitted Abstract (See Appendix A): Effect of body composition on permethrin dose when wearing treated military uniforms during initial military training

6:2 - Websites and/or Internet Sites

- Nothing to report

6:3 - Technologies or techniques

- Nothing to report

6:4 - Inventions, patent applications, and/or licenses

- Nothing to report

6:5 - Other products

- Nothing to report

Section 7: Participants and other Collaborating Organizations

7:1 - What individuals have worked on the project?

Over the following Year 4 period:

✓ *Name:* Susan P. Proctor, DSc
Project Role: Principal Investigator
Nearest person-month worked: 15% of 12 person-months (1.8 person-months)
Contribution to Project: Handling all PI responsibilities for the project, including interactions with the IRB, the grantee (HJF), CIMT, Army recruit training POCs, Fort Sill, NGB/ARNG, and CDC and PBRC staff.
Funding Support: Army Civilian employee

✓ *Name:* Matthew M. Scarpaci, MPH
Project Role: Project Coordinator
Nearest person-month worked: 100% of 12 person-months (12 person-months)
Contribution to Project: Mr. Scarpaci has assumed the role of project coordination, assisting the PI in the day-to-day planning of the project, IRB tracking, HJF administrative tasks, data collection preparations, and data management etc.

✓ *Name:* Alexis Maule, PhD
Project Role: Research Associate
Nearest person-month worked: 100% of 11 person months (11 person months)
Contribution to Project: Ms. Maule has continued to assist the PI and project coordinator on IRB-related tasks and training additional study staff on data collection processes.

Prior years of the Award:

Name: Caitlin Dillon, MPH
Project Role: Data Analyst
Nearest person-month worked: 50% of 12 months (6 person-months)- started June 2015 (ending late July 2016) 5-10% of 12 months (.5-1 person-months)- started July 2016-June 2017
Contribution to Project: Ms. Dillon has worked on the set-up of data management tasks and work on database and analyses.

✓ *Name:* Nicole Murphy, BS
Project Role: Research Associate
Nearest person-month worked: 50% of 5 months (2.5 person-month)- May –Oct 2016
Contribution to Project: Ms. Murphy has assisted in data collection and data entry processes.

7:2 - Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- Nothing to report

7:3 - What other organizations were involved as partners?

- Nothing additional to report

Section 8: Special Reporting Requirements

8:1 – See Quad Report

Section 9: Appendices

Abstract: Presented at the Annual American Public Health Association meeting in Atlanta in Nov 2017

Title: Effect of body composition on permethrin dose when wearing treated military uniforms during initial military training

Authors: Scarpaci MM, Dillon CC, Maule AL, Taylor KM, Ospina M, Calafat AM, Heaton KJ, Proctor SP

Background: As of 2013, US Army policy requires all new uniforms issued be permethrin-treated for protection against insect bites and vector-borne illnesses. Permethrin is a low-toxicity insecticide with neurotoxicant properties and research demonstrates that body fat may influence dermal absorption. We investigated the effect of percent body fat (%BF) on permethrin absorption among Soldiers wearing permethrin-treated uniforms in a 10-week training period.

Methods: This prospective study involved US Army recruits and three data collection visits occurring during the first, middle, and last week of Basic Combat Training (BCT) period. Individual measurements of %BF (skinfold method) were obtained a total of six times (start and end of each visit). Spot urine samples were collected daily during each visit and analyzed for permethrin metabolites (3-phenoxybenzoic acid, *cis*- and trans-2,2-(dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid) and creatinine. The relationships between %BF and metabolite concentrations were examined via linear mixed modeling. All models were adjusted for creatinine, age, sex, days in BCT, number of times worn uniform was washed, and daily number of hours uniform was worn.

Results: Sixty participants started the study (average age=20.77 years; 30% female); 44 recruits completed the study. For a 10% higher %BF level permethrin metabolites concentrations were 3.43-3.58% higher ($p<0.0001$).

Conclusion: Higher %BF was a significant factor in the absorption of permethrin. The independent effect of body composition will be discussed in relationship to other risk factors including uniform wash history and wear time duration.

Disclaimer: The views expressed are those of the authors and do not reflect the official policy of the US Department of the Army, the US Department of Defense or the Centers for Disease Control and Prevention.

Permethrin Exposure Dosimetry: Biomarkers and modifiable factors

Log Number: 13063057 Task Area: Biomarkers to monitor for injury and disease processes

Contract #: W81XWH-14-2-0130

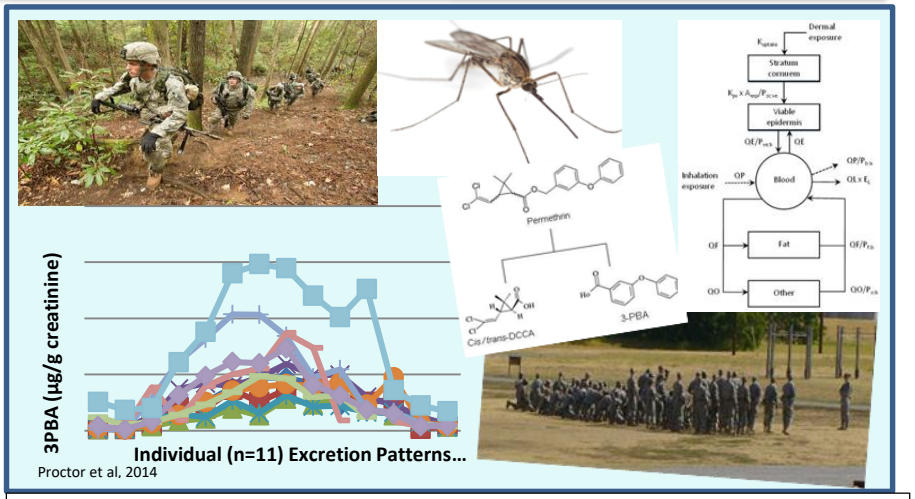
PI: Susan P. Proctor, DSc **Org:** Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF) **Award Amount:** $1,861,959

Study/Product Aim(s)

- Address the influence of permethrin exposure from wearing treated uniforms (ACU-Permethrin) on human dose and monitor the potential role of exposure on health and performance for accurate policy guidance regarding potential health risk.
- The study aims to determine the modifiable factors that significantly influence human permethrin dosimetry as a result of wearing the ACU-Permethrin. Specifically, determine whether body weight/body mass index and physical activity patterns influence the absorbed permethrin dose.

Approach

The project will define relationships between ACU-Permethrin wear-time scenarios among Army recruits (at Basic Training, Study 1) and Army National Guard Soldiers (during Annual Training, Study 2), urinary biomarkers of dose (3PBA, cis- and trans-DCCA), and modifiable factors (body mass index and physical activity levels) to provide valid predictive models.



Accomplishments [Yr4]: Analyses for all studies are completed. One manuscript has been submitted to a peer-reviewed journal for publication, two additional manuscripts under preparation.

Timeline and Cost

Activities:	**Yr 1** 7/14-6/15	**Yr 2** 7/15-6/16	**Yr 3** 7/16-6/17	**Yr 4** 7/17-6/18
Project Start-Up/Approvals				
Study 1 and Study 2 Data Collection and Sample Analysis				
Data analyses & Preparation of Manuscript & Reports				
Estimated Budget ($K)	**$718**	**$521**	**$307**	**$316**

Updated: 15 July 2018

Goals/Milestones

Yr1 Goals – Study approvals and Initiation of Study 1

☑ USARIEM IRB approval; HRPO approval granted Oct 2014

☑ Complete Study 1 site planning steps and initiate data collection

Yr2 Goals– Initiate Study 1 data analyses and Study 2 data collection

☑ Initiate Study 1 data analyses

☑ Complete Study 2 site planning steps and initiate data collection

Yr3 Goals– Complete Study 2 data collection and sample analyses

☑ Initiate Study 2 data analyses

☑ Complete Study 1 and 2 laboratory sample analyses (90% complete)

Yr4 Goals–Complete data analyses and manuscript(s) preparation

☑ Finalize data analyses and modeling

☑ Prepare technical reports/manuscript(s)

Comments/Challenges/Issues/Concerns

- We have been granted a no-cost extension to July 2019 to complete the three manuscript submissions

Budget Expenditure to Date:

Projected Expenditure: ~$1.8K

Actual Expenditure (as of July 2018): ~$1.35K