UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

September 30, 2015

MEMORANDUM

SUBJECT: Ethics Review of Completed Study entitled, "Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military"

FROM: Maureen Lydon, Human Research Ethics Review Officer
Office of the Director *(on Detail)*
Office of Pesticide Programs

TO: Marietta Echeverria, Chief, Invertebrate-Vertebrate Branch 1
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REF: Bernier, U.; Staeben, J.; Hummel, R. (2015) Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military. Unpublished document prepared by the United States Department of Agriculture – Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology. July 30, 2015. 285 p. (MRID 49684002) (D429130)

I have reviewed available information concerning the ethical conduct of the research study entitled, "Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military." If the research is determined to be scientifically acceptable, I find no barrier in regulation to the U.S. Environmental Protection Agency's (EPA's) reliance on this study in actions under the Federal Insecticide, Fungicide, or Rodenticide Act (FIFRA) or the Federal Food, Drug, and Cosmetic Act (FFDCA). The Human Studies Review Board (HSRB) will be asked to comment on this study.

Completeness of Submission

All requirements of §26.1303 were satisfactorily addressed in the completion of this study as noted in the checklist in Attachment 1.

Background and Ethics-related Chronology

The purpose of this study was to determine the bite protection level of the etofenprox-treated U.S. military Fire Resistant Army Combat Uniforms (FRACUs) treated initially at an application rate of approximately 0.9% (wt/wt), and to assess bite protection performance prior to washing (0x) and after washing 20 times (20x), 50 times (50x), and 75 times (75x). Among the insecticide-treated uniform types that the U.S. military personnel use, the more open weave construction of the FRACU uniform makes this uniform the most difficult to prevent bite-through. It has relatively low efficacy compared to other military uniform types; as a result, it was selected as the worse-case scenario for treatment of military uniforms.

The protocol for this study was approved by the overseeing institutional review board, the Western Institutional Review Board (WIRB), and submitted to EPA for review. The protocol and EPA's review, dated March 20, 2014, were discussed in a public meeting by the Human Studies Review Board (HSRB) on April 9, 2014. As documented in the final HSRB meeting report, dated June 25, 2014, the HSRB concluded, with respect to ethics, that "the protocol submitted for review, if modified in accordance with EPA…and HSRB recommendations, is likely to meet the applicable requirements of 40 CFR 26, subparts K and L."

In follow-up to the April 9, 2014 HSRB meeting, the protocol, self-certification questionnaire, telephone screening script, consent form and advertisement were revised to address comments, including EPA and HSRB comments described in Attachment 2, and submitted to the Western Institutional Review Board (WIRB) for review and approval prior to initiating the study. The last WIRB approval for revisions was issued on July 20, 2015.

The WIRB approvals which occurred from March 2015 through July 2015 and impacted the ethical conduct of the study are listed below, along with pertinent discussions with the U.S. Environmental Protection Agency (EPA):

<u>Chronology of WIRB Approvals</u>

a) 3/19/15 – The study director submitted revisions to the protocol, subject self-certification questionnaire, telephone screening script and consent form to the WIRB for review and approval. These revisions addressed EPA and HSRB comments.

b) 4/7/15 – WIRB approved the revised protocol dated 3/19/15, subject self-certification questionnaire, telephone screening script, and consent form.

c) 5/14/15 – The U.S. Department of Agriculture's Agricultural Research Service (USDA/ARS) discussed with EPA's Office of Pesticide Programs (OPP) their proposal to include 75 x wash cycle specimens in the study consistent with the rest of the protocol.

<u>Inclusion of 75x wash cycle</u>:

The inclusion of the 75x wash cycle specimens in this study, consistent with the protocol, resulted in not having to test 8 new subjects with controls in the future. The additional test time for each subject was 15 minutes for mosquito species Aedes aegypti (1 hour and 15 minute total test time) and 15 minutes for Anopheles Albimanus (1 hour and 15 minute total test time). As proposed to EPA, the extra two tests would increase compensation from $200 to $250 and would be reflected in the revised consent form and advertisement.

d) 5/21/15 – EPA approved the changes described above. The revised protocol, including the revised consent form and advertisement, with changes incorporated as noted above, were submitted to the WIRB for review and approval. Revisions submitted to the WIRB also addressed recommendations from the Quality Assurance (QA) advisor for Good Laboratory Practices (GLPs).

e) 5/28/15 – Using an expedited review process, the WIRB approved changes submitted on 5/21/15.

The May 21st protocol, which includes the WIRB-approved changes, is in Appendix I to the completed study submitted to the HSRB for review.

f) 7/13/15 – The study director submitted two protocol amendments, described on pages 5-6 of this memo, to the WIRB, along with corresponding language revisions for the protocol and consent form.

g) 7/20/15 – Using an expedited review process, the WIRB approved the revised protocol dated 7/13/15 and consent form reflecting the amendments.

The required forms requesting the aforementioned changes and the WIRB approval certificates are provided in separate files for the HSRB entitled “WIRB correspondence.”

Subject Recruitment

A printed advertisement in the Gainesville Sun (local newspaper) and on bulletin boards in the University of Florida buildings recruited subjects from the general population in Gainesville, Florida. The advertisement briefly described the testing and financial compensation for participation. Subjects were compensated for participating in the initial consent meeting and for each set of sleeves worn in the testing paradigm. Subjects received $250 for completion of testing with the full set of 10 pairs of sleeves. The advertisement provided a phone number where interested respondents were instructed to leave a message. The study director reviewed the messages and called respondents, who underwent preliminary screening via telephone to determine if they met the inclusion/ exclusion criteria. (The study discusses preliminary screening on page 19 of the study.) None of the test subjects had a relationship with the study director or any test personnel.

Subject Inclusion/Exclusion Criteria

The following inclusion/exclusion criteria were used for subject selection:

1. Must be between 18 – 62 years old.
2. Must speak and read English.
3. Children (under the age of 18) and pregnant or lactating women were excluded.
4. People in poor health or physical condition were excluded.
5. People hypersensitive to or phobic of mosquito bites were excluded.
6. People known to be sensitive to the test material, pesticides or other chemical products were excluded.
7. People with open cuts, scrapes or skin conditions (e.g. psoriasis or eczema) on their hands or forearms were excluded.
8. People with latex sensitivity or allergy were to be offered nitrile gloves.
9. People with a relationship to the study director or sponsor (students or employees of the study director or sponsor) were excluded.

Exclusion of Pregnant Women

Potential female subjects completed the home pregnancy test (HPT) in a private setting at the mosquito testing lab at the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE). After self-reading the results, these participants were asked if they would like to continue as a participant (irrespective of results). Those requesting to continue were asked to show the test results to a female staff member for verification of negative results. The results were recorded in the raw data, but will be kept confidential.

Random Selection of Subjects

A list of at least 20 qualified potential subjects was created after the telephone screening process. Eight test subjects were chosen at random from the list and enrolled for testing. The remaining qualified subjects were considered to be alternates. During the course of the study, a ninth subject was enrolled to supplant one of the original eight subjects. After completion of testing with the initial four female subjects, inspection of the data revealed that the non-treated control mosquito rate for both mosquito species was extremely low for one female subject. Rather than retest this subject against both species, data were acquired from a fifth female subject, selected from the list of alternates, to satisfy minimum testing requirements. Due to one alternate being enrolled, there were nine subjects, including four male and five females. None of the subjects withdrew from the study.

Informed Consent

The study director and each subject held consent meetings; a representative of the study sponsor attended in order to pay subjects for participating in the consent meeting. Respondents were asked to complete self-certification forms attesting that their responses given during the telephone pre-screening were true and accurate. The study director provided a detailed explanation of study procedures and the consent form, outlining risks of participating in the study. The participants were informed of how many bites they were likely to obtain and what symptoms of arthropod-borne reactions they should be alert for

after participating in the study. (The representative of the study sponsor only commented during the study director's explanation if she believed a particular point needed to be added.) There was consistency in information delivered in each consent meeting. After the detailed explanation of the procedures, the study director escorted the subject to the conference room where the video of the testing process was set up; a member of the study director's trained staff played the video of the testing process. (The study staff members were the same trained individuals who helped to implement the study.) The candidate was allowed to ask questions at any time, including afterwards. After the video was viewed and questions were answered, the study director recalled the sponsor's representative to provide payment for participating in the consent meeting. The subject then left with their compensation for attending the consent meeting and their unsigned consent form.

The process allowed sufficient opportunity for subjects to consider whether or not to participate before signing the consent form. On the day of testing, subjects entered the laboratory with their signed consent form and verbally confirmed their intent to engage in the study. The signed consent form was then accepted by the study director. Two copies were made of the consent form and one copy was provided to the subject. (The second copy, with the subject's name and signature blacked out, was inserted into the study notebook for documentation that the subject provided consent.)

Protocol Amendments

The study protocol, dated May 21, 2015, was amended twice after it was approved by the Western Institutional Review Board (WIRB) on May 28, 2015. The modifications did not negatively affect participants' rights, health or safety. As reflected in the "WIRB Correspondence" files provided to the HSRB, the protocol amendments were approved by the Western Institutional Review Board (WIRB) on July 20, 2015, using an expedited review process, and are summarized as follows:

Amendment 1:

- Approve additional subject to be tested to replace Subject 3 for a total of 9 subjects.

- Reason: Additional testing of subject was to ensure that data was collected with mosquitoes behaving avidly for each subject. After completion of testing for Subject 3, inspection of the data set revealed that the non-treated control mosquito bite rate was extremely low for this subject. Data from a 9^{th} subject was to be used to satisfy testing requirements.

- Additional Ethics-related Information: Consistent with the compensation section of the protocol, Subject 3 was compensated the full amount of $250.

Amendment 2:

- Allow Subject 4 to be retested against a mosquito species because it was known that a set of data was collected with mosquitoes that had been incorrectly maintained.

- Reason: Additional testing of subject was to ensure that data were collected with mosquitoes behaving avidly for each subject. Subject 4 had one set of sleeves with very low bite through amounts for controls. It was determined that this was the result of mosquitoes that had been incorrectly maintained and therefore did not respond as avidly. The subject was willing to be retested, and the results from the retest were used in place of the original results, thereby allowing analysis of data based on mosquitoes that were not compromised.

- Additional Ethics-related Information: Subject 4 was compensated at the rate of $125 for retesting 5 pairs of sleeves and therefore received an additional $125 above the $250 for the original tests. The same precautions were observed for the retest as for the original test. There was a break of about three weeks between the original test and the retest.

  The amendments did not negatively affect participants' rights, health or safety.

WIRB Approvals in Response to Amendments

Additional Language Approved for Protocol

On July 20, 2015, the WIRB approved additional language for the protocol in response to the amendments. The latest WIRB-approved clean version of the protocol is provided in a separate electronic file for the HSRB; the electronic file is dated July 20, 2015. (For the HSRB's convenience, the same protocol, in track changes format, with the WIRB-approved changes highlighted, is also provided and dated July 13, 2015.)

The additional language approved by the WIRB for the revised protocol includes the following:

1) In section 3.6, under "Summary of Experimental Design" the following statement was added: "Should it be determined that tests have been conducted with mosquitoes that are not behaving properly (e.g. very low control biting rates), then a subject may be asked to repeat the test set."

2) In section 8.1.1, under "Recruitment," the following statement was added: "In the event that a subject repeats a set of sleeves, then they will be compensated at the rate of $25 per pair."

3) In section 8.4, under "Summary of Human Subject Test Procedure," the following statement was added: "Should a situation arise where mosquito bite through rates are very low for a test set, and the cause of this [is] even suspected to be due to unhealthy mosquitoes, then a subject may be asked to repeat tests with an entire set of sleeves, against the mosquito species in question."

(On page 24 in the red-line/strike-out version of the revised protocol, a fourth sentence is highlighted. This statement is not new to the protocol; instead, it was simply moved from a different location.)

Additional Language Approved for Consent Form

Related to the protocol amendments, on July 20, 2015, the WIRB approved similar revisions to the consent form. The most recent version, with the July 20th WIRB approval stamp, is provided in a separate file for the HSRB. The additional language approved by the WIRB for the revised consent form includes the following:

1) At the end of the study procedures section, language was added which reads: "In the event that we discover that the mosquito bite rates for a set of tests is too low, we may ask you to repeat the tests on another day. If you agree, we will ask you to test the control sleeves and 4 sets of treated sleeves. This additional testing will take about 2 hours."

2) Under costs and payment, the following clarification was added: "If you are repeating a set of tests, you will be paid at the same rate of $25 per pair."

Application Rate Amendment

There was another change in implementing the protocol that EPA recommends the study director report to the WIRB. The original protocol stated that etofenprox-treated U.S. military Fire Resistant Army Combat Uniforms (FRACUs) would be treated initially at an application rate of 1% wt/wt, and not at a rate of approximately 0.9% (wt/wt), as indicated in the final study. This change did not negatively impact participants' rights, health or safety, but should be reported to the WIRB.

Follow-up action by EPA:

EPA will ask the study director and sponsor to ensure that all amendments are reported to and approved by the WIRB in future studies.

Protocol Deviations

The study documents eight protocol deviations, on pages 228-230, which occurred during 2015. The study director consulted with the WIRB with regard to their guidance on reporting deviations. The WIRB confirmed for the study director that the nature of these deviations did not require prompt reporting to the WIRB within five days; protocol deviations which require prompt reporting include those that harmed a subject or placed a subject at risk of harm and protocol deviations made without prior IRB approval to eliminate an immediate hazard to a subject. (If you look at the second page of the WIRB Certificates of Approval provided to the HSRB in the separate files entitled "WIRB Correspondence," item # 7 outlines the information that must be reported to the WIRB within five days.)

The eight protocol deviations which occurred during 2015 are outlined below:

Deviation 1

- A sponsor representative attended consent interviews to pay subject candidates in order to streamline the interview process.

  Additional Ethics-related Information for HSRB:
  The language of the protocol stipulated that the study director would perform the consent meeting and did not mention that a sponsor's representative might sit in in order to pay subjects for participating in the consent meeting.

  The sponsor representative honored the confidentiality of the consent process. No information about the subject's identity or participation in the study was disclosed by the sponsor representative as a result of her attending the consent interview. Also, the consent form indicated that information from the study would be given to the sponsor.

  Related to the consent meeting, the protocol did not explicitly state that the study director's trained staff would play the video of the testing process or respond, along with the study director, to subjects' questions after they watched the video; this occurred during the study. Section 8.1.6 of the protocol stated, "During the consent meeting, respondents will be given a detailed explanation of the procedures of the study and be asked to watch a movie of the testing process."

  Follow-up action by EPA:

  Deviation 1 did not negatively impact the rights of the subjects or the informed consent process. However, EPA will request that the study director and sponsor adhere closely to the specifics of the consent meeting and consent process, as explicitly outlined in approved protocols, in future studies.

Deviation 2

- Chain of custody letters were not shipped with fabric samples from Warmkraft, Inc. and Natick Soldier Research, Development and Engineering Center (NSRDEC).

Deviation 3

- NSRDEC provided chemical analysis information to CMAVE prior to the bite-protection assay. The reason was to determine whether fabric swatches were treated in accordance with the label rate.

Deviation 4

- Some mosquito test populations were outside of the range required by the protocol due to inaccurate estimation of the number of females collected for testing.

Deviation 5

- Sleeves from the coat fabric were placed on the left arm and sleeves from the trouser fabric were placed on the right arm. This was an oversight; previous protocols consistently had coat fabric placed on the right arm and trouser fabric places on the left arm.

Deviation 6

- The number of blood-fed mosquitoes per each set of tested mosquitoes was determined by counting the number of blood-fed mosquitoes twice instead of in triplicate. This was an oversight. As a result, counting ceased once the same number of blood-fed mosquitoes was determined twice.

Deviation 7

- Human subject number codes were not preceded by a gender code (M or F) as stated on page 27 of the protocol. However, the gender of the participants was still noted using the gender code (M or F) in a separate column on the data sheet.

Deviation 8

- Bite protection data were not analyzed using a generalized linear model (GLiM) with a log link. GLiM-based confidence intervals are inappropriate when there is subject-to-subject variation. A t-distribution confidence interval was used instead because it provided more accurate confidence intervals.

The deviations, listed above, did not negatively affect participants' rights or their health or safety, and did not adversely impact the scientific integrity of the study.

Regulatory and Statutory Standards

The following provisions of 40 CFR 26 Subpart Q, as amended, define the applicable ethical standards, which read in pertinent part:

> §26.1703: Except as provided in §26.1706, EPA must not rely on data from any research subject to this subpart involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.
>
> §26.1705: Except as provided in §26.1706, EPA must not rely on data from any research subject to this section unless EPA determines that the research was conducted in substantial compliance with all applicable provisions of subparts A through L of this part. . . .

In addition, §12(a)(2)(P) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) applies. This passage reads:

> In general, [i]t shall be unlawful for any person . . . to use any pesticide in tests on human beings unless such human beings (i) are fully informed of the nature and purposes of the test and of any physical and mental health

consequences which are reasonably foreseeable therefrom, and (ii) freely volunteer to participate in the test.

Findings

Prior HSRB and EPA Review

Because this study was initiated after April 7, 2006, prior submission of the protocol and supporting materials to EPA was required by 40 CFR §26.1125. The requirements of 40 CFR §26.1125 for prior submission of the protocol to EPA and of §26.1606 for HSRB review of the protocol were satisfied. The study protocol was approved by the Western Institutional Review Board (WIRB) prior to submittal to EPA. The HSRB discussed the protocol at its April 9, 2014 meeting, and concurred with EPA's assessment that the protocol, if revised as suggested by the Agency and the HSRB, would meet the applicable requirements of 40 CFR part 26, subparts K and L.

Responsiveness to HSRB and EPA reviews

EPA's and the HSRB's ethics comments on the protocol were addressed before the research was conducted. Please see Attachment 2 for details.

Prohibition of research involving intentional exposure of pregnant or nursing women or of children

All enrolled subjects were at least 18 years old and there were no pregnant or nursing female subjects. The prohibition in 40 CFR §26.1703 of research involving intentional exposure of pregnant or nursing women or of children under age 18 was satisfied.

Substantial compliance with 40 CFR 26 subparts A through L

40 CFR §26.1705 requires that EPA not rely on data from any research subject to this section unless EPA determines that the research was conducted in substantial compliance with all applicable provisions of subparts A through L of this part. Within this range, only subparts K and L are directly applicable to the conduct of third-party research such as this. The study documents substantial compliance with subparts K and L.

Compliance with 40 CFR 26 subpart M

As documented in Attachment 1 to this review, the central requirements of 40 CFR 26 subpart M, §26.1303 to document the ethical conduct of the research were satisfactorily addressed.

Compliance with FIFRA §12(a)(2)(P)

The requirement of FIFRA §12(a)(2)(P) that human subjects of research be "fully informed of the nature and purposes of the test and of any physical and mental health consequences reasonably foreseeable therefrom," and "freely volunteer to participate in the test," was met for this study.

EPA Conclusion

The study entitled, "Laboratory Evaluation of Bite Protection from Repellent-Impregnated Clothing for the United States Military," reports research conducted in substantial compliance with the applicable requirements of 40 CFR 26, subparts K and L. The conduct of the study met all applicable ethical standards for the protection of human subjects of research, and requirements for documentation of ethical conduct of the research were satisfied.

For future studies, EPA will recommend to the study director and sponsor that they closely adhere to the details of the consent process as described in the approved protocol and ensure that all amendments are reported to the WIRB.

If this study is determined to be scientifically valid and relevant, there is no regulatory barrier to EPA reliance on it in actions under FIFRA or §408 of FFDCA.

Attachment 1: §26.1303 completeness check
Attachment 2: Responsiveness to EPA and HSRB Comments on Protocol

Attachment 1
§ 26.1303 Checklist for Completeness of Reports of Human Research Submitted for EPA Review

Any person who submits to EPA data derived from human research covered by this subpart shall provide at the time of submission information concerning the ethical conduct of such research. To the extent available to the submitter and not previously provided to EPA, such information should include:

	Requirement		Y/N	Comments/Page References
(a) Copies of all of the records relevant to the research specified by §26.1115(a) to be prepared and maintained by an IRB	§1115(a)(1): Copies of • all research proposals reviewed, • scientific evaluations, if any, that accompany the proposals, • approved sample consent documents, • progress reports submitted by investigators, and reports of injuries to subjects.		Y n/a Y n/a	
	§1115(a)(2): Minutes of IRB meetings which shall be in sufficient detail to show • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; • a written summary of the discussion of controverted issues and their resolution.		Y	Separately provided to HSRB members.
	§1115(a)(3): Records of continuing review activities.		Y	
	§1115(a)(4): Copies of all correspondence between the IRB and the investigators.		Y	WIRB correspondence which occurred in follow-up to the April 9, 2014 HSRB meeting was provided in a separate file to the HSRB.
	§1115(a)(5): • A list of IRB members identified by name; earned degrees; representative capacity; indications of experience such as board certifications, licenses, etc., sufficient to describe each member's chief anticipated contributions to IRB deliberations; • any employment or other relationship between each member and the institution		Y Y	Previously provided to HSRB members with the protocol. Updated information was included in a separate file provided to HSRB members with the completed study.
	§1115(a)(6): Written procedures for the IRB in the same detail as described in § 26.1108(a) and § 26.1108(b).		Y	Previously provided to EPA with the protocol.
	§1115(a)(7): Statements of significant new findings provided to subjects, as required by § 26.1116(b)(5).		n/a Pls. see note	Note: As discussed in the study and EPA review, one subject had to be retested. This does not constitute a significant new finding from the entire study for all subjects; it does constitute a finding which needed to be provided to <u>one</u> subject.
(b) Copies of all of the records relevant to the information identified in §26.1125(a) (§1125(a) A discussion of:		(1) The potential risks to human subjects;	Y	Discussed in consent form and p. 39-40 of revised protocol.
		(2) The measures proposed to minimize risks to the human subjects;	Y	Discussed in consent form and p. 39-40 of revised protocol.
		(3): The nature and magnitude of all expected benefits of such research, and to whom they would accrue;	Y	Discussed in consent form and p. 41 of revised protocol.
		(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	Discussed in consent form and p. 42 of revised protocol.
		(5) The balance of risks and benefits of the proposed research.	Y	Discussed on p. 41 of revised protocol.
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.		Y	Provided in separate file to EPA and HSRB
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.		Y	Pages 18-19 of study, Appendix 1 and pages 19-20 of revised protocol.

Requirement		Y/N	Comments/Page References
	§1125(d): A description of the circumstances and methods proposed for presenting information to potential human subjects for the purpose of obtaining their informed consent.	Y	Page 20 of study, revised consent form, and p. 21 of revised protocol.
	§1125(e): All correspondence between the IRB and the investigators or sponsors.	Y	Separate file provided to HSRB with requests sent to WIRB and certificates of approval from WIRB.
	§1125(f): Official notification to the sponsor or investigator, in accordance with the requirements of this subpart, that research involving human subjects has been reviewed and approved by an IRB.	Y	
(c) Copies of sample records used to document informed consent as specified by §26.1117, but not identifying any subjects of the research		Y	Revised consent form approved by WIRB was provided to HSRB.
(d) If any of the information listed in paragraphs (a) through (c) of this section is not provided, the person shall describe the efforts made to obtain the information.		n/a	

Attachment 2 - Responsiveness to EPA's and HSRB's Ethics Comments

Comments from EPA and HSRB	Has comment been addressed?
1. In section 8.6 of the protocol, entitled "Detailed Stepwise Test Procedure," insert an explanation of how, when, and by whom subjects' hands and arms will be inspected for cuts or other skin conditions.	Yes. The following statement was added to step 3 of section 8.6 in the protocol: "The laboratory technician or study PI will request the subject wash their hands with soap and water and then inspect the arms of the subject for cuts and scrapes."
2. In section 8.6 of the protocol, entitled "Detailed Stepwise Test Procedure," insert an explanation of how the pregnancy testing for female subjects will be handled, clarifying where in the sequence of stepwise procedures the pregnancy testing will occur.	Yes, in section 8.6, the following statement was added to step 3: "If the participant is female, they will be required to take a pregnancy test as following the procedures of section 8.1.4."
3. Please clarify which member of the research team will verify pregnancy test results of female subjects who desire to remain in the study after taking the pregnancy test.	Yes, section 8.1.4 of the protocol was revised to read in part, "Those requesting to continue will be asked to show the test results to a female staff member for verification of negative results."
4. Please revise the first sentence in section 11.0 of the protocol, titled "Benefits and to Whom Benefits Accrue," as follows: "While there are no direct benefits to the subjects participating in this research study beyond a small compensation for their time, there are…" The proposed payment to subjects is considered compensation for lost time and inconvenience, not a benefit of participating in the research. This study provides no direct benefits to subjects.	Yes, the reference to compensation in this sentence was deleted from the protocol as requested. The revised sentence reads, "While there are no direct benefits to the subjects participating in this research study, there are indirect benefits to both the subjects and society."
5. In section 8.1.2 of the protocol, titled "Inclusion/Exclusion Criteria," consider expanding exclusion #6 as follows: "Exclusion of people known to be sensitive to the test material, pesticides, or other chemical products."	Yes, exclusion criteria #6 was expanded as requested.
6. In section 8.1.2 of the protocol, titled "Inclusion/Exclusion Criteria", add an exclusion for people with open cuts or scrapes or skin conditions such as psoriasis or eczema on their hands or forearms. Cut, scrapes or other skin conditions might increase the risk of skin reactions or sensitivity during the testing. Also, add this exclusion to the "Restrictions" section of the consent form.	Yes, the requested exclusion was added as exclusion #7. The exclusion for people with cuts, scrapes or skin conditions was also added to the restrictions section of the consent form.
7. In the "Study Procedures" section of the consent form, insert text similar to what appears below as #2, and adjust the numbering of the subsequent procedures accordingly: "2.	Yes, the requested language was added to the consent form and the steps which followed in the study procedures section were renumbered accordingly.

It is important that you NOT be in this study if you are pregnant. So, before the testing begins, each female volunteer will be asked to go to a private area and will be given a home pregnancy test kit. A female researcher will be able to explain how to use it and answer questions. If you are a female, you will be asked to take the test in a private restroom. If, after taking the test, you still wish to participate in the study, you will be asked to show the result of the test to a female laboratory technician so that she can verify that you are not pregnant. If you withdraw from the study after taking the pregnancy test, you will not be asked to share the result of the test with anyone."	
8. Although a question about allergy/sensitivity to latex is listed on the enrollment questionnaire, latex allergy/sensitivity does not appear as an exclusion criterion in the recruitment interview script or consent document. Latex allergy/sensitivity should be included in all statements of exclusion criteria. Alternatively, the protocol could be modified to use nitrile gloves instead of latex.	Yes, the language, "People with latex sensitivity or allergy will be offered nitrile gloves" was added to inclusion/exclusion criteria in the protocol and the restrictions list in the consent form.
9. The protocol currently calls for test sleeves to be made in a single size, despite the likely variation in arm size among participants and 34 different uniform sizes available to military personnel. The single sleeve size may both skew the results of the testing across participants of various arm sizes and it may also cause embarrassment to participants who attempt to put on sleeves that do not fit easily. The Board recommended that researchers include a question about shirt sleeve size in the recruitment interview and have test sleeves available in different sizes.	Yes, as discussed on p. 20 of the study, a range of sleeve sizes, designated small, medium and large, were constructed prior to study initiation. The fabric dimensions were altered by one-half inch increments, as needed, to yield sewn sleeves that were snug on the forearms of subjects. Due to the ability to alter the sleeves as noted, it was unnecessary to include a question about shirt sleeve size in the recruitment interview.
10. Currently, stopping rules depend on researchers' judgment on key criteria of subjects' attractiveness and sensitivity, and mosquitoes' biting pressure. The Board recommended that the investigators articulate objective measures for stopping criteria.	Yes, stopping rules in the protocol were expanded in response to this comment. The revised stopping rules in section 14 of the protocol read as follows: "The study will be stopped if the test site becomes unsafe for any reason, biting pressure falls below threshold needed (<50% of the mosquitoes in a cage contact the fabric worn by a subject), biting pressure rises too high for subject comfort (expressed verbally by subject) or safety, subject asks to withdraw irrespective of the point they are in the study, subject is unattractive to target species (<50% of mosquitoes land on fabric surface during test

	interval), subject exhibits hypersensitivity to insect bites during test (large areas of swelling generally over 0.5 cm per bite), subject exhibits sensitivity to the test materials during the test (redness, swelling or other skin reaction), study is terminated or discontinued."
11. The Board noted that clarification is needed in the consent form about a nurse being on call but not present at the study site.	The study director's interpretation of the comment was that it wasn't clear on the consent form that the nurse was on call; therefore, language was added to indicate this. Page 39 of the original and revised protocol included language indicating that the nurse would be on call. The original consent form submitted to the HSRB with the protocol did not include a reference on the first page that a nurse would be on call. This language was added to the consent form, along with the name and telephone number of the on-call nurse.
12. Currently, partial (per sleeve) compensation is provided to participants who leave the study early for any reason. The HSRB recommended that, to prevent coercion or to compensate for dropping out or being excluded by the Principal Investigator (PI), full compensation be provided to all participants.	With respect to payment of subjects and prevention of coercion, the study director stated that he tried to ensure that the study did not include any factors that could lead to coercion. The payment system was established for fair compensation of participants for the time spent on the study and to pay subjects for each set of sleeves on which they initiated testing; the approved consent form states, "If you begin the study but do not complete it, you will be paid for each pair of sleeves at a rate of $25 per pair". If a subject was to start a study and withdraw for any reason, they would still be compensated for any sleeves tested, regardless of whether or not they finished the 15 minute test period. This represented a compromise to compensate participants, regardless of their ability to complete a full set of sleeves. In fairness to other participants who completed testing on all sets of sleeves, a participant who did not complete a full set of sleeves had less time invested, and therefore did not receive the same level of compensation as a participant who completed all sets.
13. Language in the consent form indicating that laboratory mosquitoes pose no risk of transmitting disease needs to be modified. To address the unlikely possibility that any of the test mosquitoes is found to carry a disease, investigators should articulate a standard	Yes, the language in the protocol under "risk of exposure to disease" was revised in response to this comment. This section currently reads, "There are risks of getting sick when you are bitten by mosquitos in the outdoors. The test mosquitos for this study

method and message for notifying participants of exposure.	have been grown in the laboratory and should not have had a chance to carry disease. In the unlikely event that they are found to carry disease, you will be notified immediately and will receive appropriate treatment at the hospital."
14. It is recommended that researchers complete a course in human subject protections within three years of study initiation and completion. Depending on when the study occurs, some investigators may exceed this recommended time limit.	Yes, the study director and his research staff completed a number of courses, including two University of Florida courses (HIPAA for Researchers) and an IRB training course. The latter course is every 3 years. They also completed NIH training. They updated their training earlier this year per the recommendation of the HSRB.