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

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

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MEMORANDUM

SUBJECT: Science and Ethics Review of Protocol for Laboratory Evaluation of Bite Protection from Insecticide-Impregnated Fabrics

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REF: Bernier, Ulrich, (2017) Laboratory Evaluation of Bite Protection for Repellent-Impregnated Fabrics. Unpublished document prepared by Uli Bernier and sponsored by Pinebelt Processing, Inc., August 09, 2017. 211 p. (MRID 50360801)

Bernier, Ulrich, (2017) Supplemental Materials to Address Ethical Requirements for the Protocol Entitled: Laboratory Evaluation of Bite Protection for Repellent-Impregnated Fabrics. IRB Correspondence Report. Unpublished document prepared by Uli Bernier and sponsored by Pinebelt Processing, Inc., November 7, 2017. 95 p.

We have reviewed the referenced protocol for a laboratory test of insecticide-treated fabrics from both scientific and ethics perspectives. This EPA review evaluates the scientific aspects of the proposed research for an efficacy study to assess fabrics impregnated with either 0.52% permethrin or 0.9% etofenprox after 0, 25, 50, and 75 washings. Ethical aspects of the proposed research are assessed in terms of the standards defined by 40 CFR 26 subparts K and L.

A. Completeness of Protocol Submission

The submitted protocol was reviewed for completeness against the required elements listed in 40 CFR §26.1125. EPA's checklist is appended to this review as Attachment 2. All elements of required documentation are provided in the submitted protocol package and supplementary documentation of review by Western Institutional Review Board (WIRB).

B. Summary Assessment of Ethical Aspects of the Proposed Research

Here is a summary of observations about the ethical aspects of the proposed protocol. Attachment 1 provides supporting details and a point-by-point evaluation of this protocol.

1. **Societal Value of Proposed Research:** This study is designed to determine the bite protection level of fabrics treated or impregnated at the factory level with either etofenprox (0.9%) or permethrin (0.52%). The research will be conducted in a laboratory setting. Fabrics may include those representative of consumer clothing and military physical training gear. The fabrics may be woven and/or knits. Each type of treated fabric will be tested unwashed, 20 times washed, 50 times washed, and 75 times washed for protection against bites by mosquitoes. The research has societal value because U.S. military personnel and civilians, both domestically and abroad, are at risk of contracting mosquito-borne diseases. The rationale for this testing is to collect data to show that fabrics impregnated or treated with etofenprox or permethrin will provide protection against mosquitoes for up to 75 washings. As intended, the data resulting from this proposed study will be used to support registration of specific treated fabrics.

2. **Subject Selection:** Subjects will be recruited from the Gainesville, Florida area. Advertisements will be placed in a local newspaper (Gainesville Sun) and posted on bulletin boards around the University of Florida. The advertisement, provided in Appendix F to the protocol, will note that the researchers are seeking volunteers to test treated fabric against mosquitoes, provide an estimate of the time required and the potential compensation, and include some of the eligibility requirements for subjects. It also includes contact information for Dr. Bernier, the study director, for those who wish to learn more about the study or to volunteer to participate.

   Candidates who contact Dr. Bernier for more information about the study or to volunteer will be contacted by Dr. Bernier or a member of the study team to complete a preliminary screening over the phone. The screening will cover the eligibility criteria and provide more information about the study. According to p. 18 of the protocol, the eligibility criteria are as follows:

   "*1. Must be between the ages of 18-62 years old.*
   2. Must be able to speak and read English.
   3. Children (under the age of 18), and pregnant or lactating women will be excluded.
   4. Exclusion of people in poor health or physical condition.
   5. People who are hypersensitive to or phobic of mosquito bites will be excluded.
   6. Exclusion of people known to be sensitive to the test material, pesticides or other chemical products.

7. *People with open cuts, scrapes, or skin conditions (e.g. psoriasis or eczema) on their hands or forearms.*
8. *People with latex sensitivity or allergy will be offered nitrile gloves.*
9. *Exclusion of people with a relationship to the study director or sponsor (students or employees of the study director or sponsor).*"

If the candidate meets the criteria during the phone screening, they will be invited to meet with Dr. Bernier and/or a designated member of the study team. At the start of the meeting, they will complete a "Self-Certification Form", as provided in Appendix G to the protocol. The study team member will provide a detailed description of the research. In addition, candidates will watch a movie of the testing process, which has been reviewed and approved by the IRB. Those who are qualified and interested in continuing will be invited to complete the consent form and enroll in the study.

The recruitment materials and consent form will only be available in English. One of the screening criterion is that candidates must be able to speak and read English. Current repellent product labels are in English and the language that someone speaks does not directly affect attractiveness to mosquitoes. This research does not offer benefits to the subjects, so limiting recruitment to English speakers will not result in equity-of-access issues.

For each type of treated fabric (e.g., etofenprox-treated woven fabric), a list of 20 qualified potential subjects will be created. The study team will strive to ensure that this list has an equal distribution of males and females. From this group, a set of 10 test subjects will be randomly selected, ensuring that 5 males and 5 females are enrolled. The remaining candidates on the qualified potential subjects list will serve as alternates. If necessary, an alternate will be selected randomly to replace a test subject who withdraws. Alternates will be the same gender as the subject withdrawing. The sample size of 10 subjects is supported by an EPA analysis conducted as part of EPA's "Science and Ethics Review of Protocol for Laboratory Evaluation of Mosquito Bite Protection for Permethrin-treated Clothing for the United States Army after 0, 20, and/or 50 Washings."[1]

To determine the bite protection against each mosquito species (*Aedes aegypti* and *Anopheles albimanus*) individually, and to limit the discomfort associated with mosquito bites, only one mosquito species will be tested on a single test day against all washing levels of a single type of treated fabric (e.g., permethrin-treated knit fabric washed 0, 20, 50, and 75 times), as well as against the untreated control. As a result, a subject completing testing of a treated fabric against both mosquito species will participate in two test days for a single type of treated fabric.

Testing for control attractiveness will be conducted on each test day.

3. **Risks to Subjects:** The protocol discusses four potential hazards associated with these tests including risks of exposure to arthropods, risk from exposure to disease vectors, risks from exposure to the test material, psychological risks related to

[1] Ciarlo, T., E. W. Bohnenblust and M. Lydon. Science and Ethics Review of Protocol for Laboratory Evaluation of Mosquito Bite Protection from Permethrin-treated Clothing for the United States Army after 0, 20 and/or 50 Washings. US EPA. September 29, 2016.

pregnancy testing, and adverse reaction to the test substances. Risks are minimized in the protocol by excluding candidates known to be hypersensitive to or phobic of mosquito bites; using disease-free colony-raised mosquitoes; excluding candidates known to be sensitive to insect repellents or insecticide-treated fabrics and subjects with open cuts, scrapes, skin disease and skin problems; and incorporating procedures to keep the results of pregnancy testing private. Practical steps to minimize risks to subjects have been described in the protocol, and the remaining risks have a low probability of occurrence.

To eliminate the risk of contracting any mosquito-borne diseases, the study will be conducted only with laboratory-reared mosquitoes, which are not known to harbor any pathogens. The mosquitoes have been reared in colony at the Center for Medical, Agricultural, and Veterinary Entomology (USDA facility) in Gainesville Florida, and have not had a human blood meal prior to use in the study. The colonies are maintained using bovine blood that has been certified as sterile (pathogen-free) by the supplier. In addition, mosquitoes will only be used in one test period with a single subject; after the test period, the mosquitoes are removed from test cages, frozen, and killed to determine the total number of mosquitoes used in the test cage and the number of blood-fed mosquitoes.

4. **Benefits:** This research offers no benefits to subjects. The target levels of mean bite protection are ≥90% for the unwashed, 20 times washed, 50 times washed, and 75 times washed treated fabrics. Depending on the results of the research, it may provide benefits to society by potentially leading to data that could be used by EPA to register fabrics treated with either etofenprox or permethrin that provide mosquito bite protection equal to or greater than the target levels of mean bite protection; this would facilitate protection of military service members and civilians from nuisance bites and bites that lead to mosquito-borne diseases.

5. **Risk/Benefit Balance:** The protocol describes measures to further reduce risk to subjects while maintaining the robustness of the scientific design. Due to the risk mitigation measures put in place, the residual risk to subjects is low and reasonable in light of the potential benefits of the data to society.

6. **Independent Ethics Review:** The Western Institutional Review Board (WIRB) has reviewed and approved the protocol, informed consent form, and recruitment materials. Western IRB is independent of the investigators and sponsors. Satisfactory documentation of the IRB procedures and membership is on file with the Agency. Documentation regarding IRB approval of the protocol has been provided to the HSRB members with the background materials for this protocol.

7. **Informed Consent:** The protocol contains a complete and satisfactory description of the process by which potential subjects will be recruited, informed and trained in preparation for the test day, and the process for seeking subjects' consent to participate. A copy of the IRB-approved consent document meeting requirements of 40 CFR §§26.1116 and 26.1117 is included in the background materials.

8. **Respect for Subjects**: The subjects' identities will be protected as follows: each subject will be assigned a code number, and only subjects' code numbers will appear

on data sheets. The subjects' names will not appear anywhere on the data sheet, or in the reports. The study records will be maintained at the testing facility in locked cabinets and electronic files kept on a password-protected computer server. Provision is made for discrete handling of the pregnancy testing that is required of female subjects on each day of testing. Candidates and subjects will be informed that they are free to decline to participate or to withdraw at any time for any reason. Subjects will be compensated as described in the protocol - $20 for attending a consent meeting and $20 per sleeve tested ($200 if the subject completes all testing for a treated fabric sample against both species of mosquitoes). Breaks for subjects between exposures have been incorporated into the study design.

C. Compliance with Applicable Ethical Standards

This is a protocol for third-party research involving intentional exposure of human subjects to a pesticide, with the intention of submitting the resulting data to EPA under the pesticide laws. Thus the primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply. A point-by-point evaluation of how this protocol addresses the requirements of 40 CFR 26 Subparts K and L and the criteria recommended by the HSRB is appended as Attachment 1.

EPA's Ethics Comments

Dr. Bernier and the study sponsor were notified that before the research is conducted, the protocol and supporting documents should be revised to address EPA's comments and recommendations resulting from the review by the HSRB. Dr. Bernier and the study sponsor have already agreed to address EPA's comments. After the HSRB completes its review of the protocol and relays its recommendations to the EPA, the EPA, Dr. Bernier, and the study sponsor will reach agreement on implementation of the HSRB's recommendations; the revised protocol and supporting documents will be resubmitted for review and approval to the overseeing IRB prior to initiating the research.

The EPA's ethics comments are provided below and organized by section headings used in the protocol.

Contact Information

1. Include information about the institutional review board in this section.

Identification of the Test System – Section 3

2. Revise the protocol to include the representative type of knit fabric to be tested. If you plan to test other woven or knit fabrics, revise the protocol as follows: "In addition to the representative types of fabrics included here, additional fabrics may be tested. Before testing any additional fabrics, the protocol will be amended to include the specific type of fabric being tested."

GLP Compliance – Section 4

3. Either as a subsection or as a new section following this section, include language about compliance with the EPA's regulation on the protection of human subjects in research (40 CFR 26). For example:
"This study will be conducted in accordance with EPA's final regulation published in 40 CFR Part 26 that establishes requirements for the protection of subjects in human research. The protocol, informed consent form, and other required documentation for this study will be approved by an independent institutional review board (Western Institutional Review Board, WIRB) and submitted to the EPA as required by 40 CFR 26.1125. The report of the completed research is subject to 40 CFR 26.1303 requirements to document its ethical conduct.

"Written approval from WIRB will be obtained prior to study initiation. Following approval by WIRB, the study protocol, approved informed consent form (ICF) and supporting information will be submitted to the EPA and Human Studies Review Board (HSRB) for review. Recruitment of subjects into the study will not be initiated until the EPA and HSRB reviews have been completed, recommendations have been addressed, and WIRB approval of the revised final protocol has been granted.

"All protocol changes (amendments and deviations) must be reported to the WIRB as specified by WIRB's written policies and procedures. All amendments must be reviewed and approved by WIRB prior to implementation in the study, except for amendments deemed necessary to eliminate apparent immediate hazards to human subjects. Proposed changes (amendments) deemed necessary to eliminate apparent immediate hazards to the human subjects may be implemented without prior IRB approval; these must be reported to WIRB as outlined in WIRB's policies and procedures. All other amendments must be reviewed and approved by WIRB prior to implementation in the study. The protocol amendment procedure detailed in AEATF SOP 2C.3 will be followed. The final study report will contain a summary of all protocol changes and the associated documentation as specified in 40 CFR 26.1303. The IRB may provide expedited review of minor changes as defined by 40 CFR Part 26.1110 at its discretion.

"Unplanned protocol changes (deviations) which occur during conduct of the study cannot, by definition, be reviewed by the IRB prior to implementation. All deviations must be reported to WIRB as soon as possible following the change. A dated acknowledgment from WIRB of receipt of the deviations must be received and included in the final study report as specified in 40 CFR 26.1303."

Methods and Materials - Section 8

4. Clarify whether pre-screening telephone calls will be conducted by Dr. Bernier or a member of the study team. In some areas, the protocol notes that the Study Director will conduct the screening (8.a.*i.* – Recruitment), and in other areas the protocol notes that the screening and consent will be done by a study team member in consultation with the Study Director.

5. Revise the protocol (and consent materials) to state that pregnancy testing for female subjects will be conducted on each day of testing for the female subject.
6. Specify that the consent meeting will cover the detailed stepwise test procedure as described in 8.f.
7. Rather than recording the results of pregnancy tests, EPA recommends recording that a pregnancy test was conducted and that the subject was qualified to enroll or that the subject withdrew, instead of recording the results of negative pregnancy tests.
8. Ensure that a subject who withdraws is replaced by an alternate of the same gender, if possible.
9. Clarify the protocol and consent materials to clearly state that a subject testing a single treated fabric against both species of mosquitoes will participate in two days of testing.
10. Provide information about when and how payment will be provided to subjects.
11. Include the minimum amount of time that will elapse between a subject testing the treated fabric against different species of mosquitoes. EPA suggests allowing 48-72 hours to elapse between test days involving the same subject. For example, "When a test subject participates in more than one test day, their two test days will be spaced apart a minimum of [48/72] hours in order to minimize any possible discomfort or complications such as an allergic response." Please reflect this change in all areas of the protocol where this timeframe is mentioned.
12. Revise the protocol to indicate whether subjects will be permitted to test more than one type of treated fabric, and if so, the minimum waiting period between testing one set of fabric sleeves and the second set of treated fabric sleeves. If subjects are permitted to participate in more than one day, consider including this language: "Subjects may choose to test more than one fabric sample, if they desire and are eligible." Include the amount of time that must elapse between participating in testing involving different types of fabrics or treatments.
13. Revise the protocol to indicate that data from subjects who withdraw for any reason or who are removed by the study director will not be used. Ensure that this language is consistent throughout the protocol.
14. In "d. Summary of Human Subject Test Procedure", ensure that subjects' arms are washed with soap and water before the untreated control fabric sleeves are donned for the first test period.

Risks to the Subjects – Section 10

15. Add risk of exposure to latex to 10.c., "Risks from Exposure to the Test Material."
16. Add the names of the test substances to 10.c. – etofenprox and permethrin.
17. Add a section on "Risks from Unanticipated Loss of Confidential Information." Sample language for this section includes: "The information obtained from subjects taking part in these studies will be used by the researchers, funders, and the sponsor, and will become part of one or more reports on the study. All reports (as well as all study-related records) and will be kept as confidential as possible under local, state, and federal laws. The results of this study are not intended for publication; however, if any of the study-related data are published, subjects' identities will remain confidential. All efforts will be taken to maintain the confidentiality of the pregnancy test results. The test results

will not be recorded, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director."

Benefits and to Whom Benefits Accrue – Section 11

18. The research offers no benefits to subjects. Revise the first sentence as follows: "~~While t~~There are no direct benefits to the subjects participating in this research study.~~, t~~There are indirect benefits to ~~both the subjects and~~ society."

Respect for Subjects – Section 13

19. Include a statement that subjects are free to withdraw at any time, *without forfeiting any benefits to which they are entitled*.

Informed Consent Document (IRB-approved version dated 9/25/17)

20. Under "Study Enrollment" include a statement that women will be required to take a pregnancy test on each day of testing in which they participate.
21. Under "Study Procedures":
    a. Revise #5 to note that a sleeve will be placed on one or both forearms.
    b. Clarify that a testing day will only include one type of treated fabric and one species of mosquitoes. Move #13 to the end of the numbered list and revise as follows:

"~~13.~~ 15. On a separate day, at least [48 or 72] hours after your first test day, we will ask you to return to complete the same set of steps to test fabric sleeves treated with the same substance against a different species of mosquitoes. ~~These steps will be repeated until all the sleeves have been tested with both mosquito species. In the event that we discover that the mosquito bite rates for a set of tests are too low, we will as you to test the control sleeves and 4 sets of treated sleeves.~~ This additional testing will take about two hours."

22. Under "Risks and Discomforts":
    a. Include permethrin in "Risk of allergic reaction to test chemicals (etofenprox and permethrin)".
    b. EPA's understanding is that there is almost no risk of the mosquitoes raised in colony and used in this study to contract disease or to transmit any disease to a subject. Based on this, revise "Risk of exposure to disease" to delete the last sentence: "In the unlikely event that they are found to carry disease, you will be notified immediately and will receive appropriate treatment at the hospital."
23. Under "Benefits", delete the last sentence: "This may indirectly benefit you."

Self-Certification Form (Appendix G)

24. Revise question #4 to note that females will be required to take a home pregnancy test on each day of testing.
25. Revise the numbering for the follow up questions:
    a. "If you answered yes to question ~~#6~~#8 please describe the type of reaction you have had to mosquito bites."
    b. "If you answered yes to question ~~#15~~#18 please list the other studies you are enrolled in."

D. Summary Assessment of Scientific Aspects of the Proposed Research

The objective of this protocol is to determine the bite protection provided by fabric treated with 0.9% w/w etofenprox or 0.52% w/w permethrin after 0x, 20x, 50x, and 75x washes.

This protocol is for a non-guideline study; therefore, it is not designed to fulfill the requirements of a specific OCSPP (formerly OPPTS) Guideline. Studies under this protocol will be conducted in accordance with EPA, FIFRA (Federal Insecticide, Fungicide and Rodenticide Act), and Good Laboratory Practice Standards (GLP); 40 CFR, Part 160 (October 1989). (pg. 16 of 211).

This protocol is a modified version of a protocol testing efficacy of fabrics against mosquitoes for the U.S. military that was reviewed and accepted by the EPA and Human Studies Review Board (HS RB) in April 2014. EPA and HSRB reviewed and accepted the subsequent study in October 2015. In this version, the number of subjects is increased from 8 to 10; testing is not restricted to military fabrics; 0.52% permethrin treated fabrics are added; and treated knit fabrics will be included to test fabrics representative of consumer clothing and military physical training gear.

The tests to be conducted under the following protocol focus on factory-level produced insecticide/repellent treated fabrics. The basic experimental unit in this study is a sleeve test. Each test exposure involves a subject exposing for 15 minutes a fabric-sleeved arm into a cage (59,000 cm^2) containing 200 ± 25 individual female mosquitoes of one species. Each subject will expose one arm to mosquitoes 3 times and the other arm 2 times for a total of 2.5 hours of exposure split between two days (pp. 15-16 of 211). Two mosquito species will be tested, with each species tested on different days. The data obtained from each 15-minute exposure with each experimental subject will be counts of the number of blood-fed female mosquitoes and the total number of female mosquitoes in each test cage. The observed bite-through proportion (or 'rate') for the control treatment (untreated fabric sleeve) is the proportion of blood-fed female mosquitoes to the total number of mosquitoes in each test cage. Rates of bite-through for the insecticide-treated fabrics will be corrected using Abbott's formula for 'background' bite-through rates in the control treatment. To increase testing precision, each subject will serve as their own control subject for each fabric type and mosquito species. Therefore, the experiment consists of 5 exposures per fabric type (e.g., Fire Resistant Army Combat Uniform (FRACU); see example using FRACU below) for each mosquito species in the following order:

- 1 test with an untreated FRACU fabric-sleeve, which serves as the control.
- 1 test with treated washed (75x) FRACU fabric.
- 1 test with treated washed (50x) FRACU fabric.
- 1 test with treated washed (20x) FRACU fabric.
- 1 test with treated unwashed (0x) FRACU fabric.

Fabric types will be tested as described in Table 1 below. Subjects will test each fabric type per number of launderings once per mosquito species for a total of 5 treatments per species per subject using 10 subjects per fabric washing level, resulting in 10 total replicates per fabric treatment level per mosquito species for this experiment. The rationale for the number of human test subjects is provided in the statistical design section (section 2) of this review.

Table 1*: Experimental Design

Fabric and Treatment Condition[1]	Number of Fabric	Number of	Number of	Total Replicates

	Specimens	Subjects	Species[2]	per Fabric Type
Untreated Unwashed Cotton Control[3]	1	10	2	20
Treated Washed 75x	1	10	2	20
Treated Washed 50x	1	10	2	20
Treated Washed 20x	1	10	2	20
Treated Washed 0x	1	10	2	20

[*] Table 1 in this report denotes Table 7 in the submitted protocol (p. 15 of 211, §3.e).
[1] Fabric treatment conditions are either untreated and unwashed (Control) or treated and unwashed (0x), treated and washed 20 times (20x), treated and washed 50 times (50x), or treated and washed 75 times (75x).
[2] The test species are *Aedes aegypti* or *Anopheles albimanus.*
[3] Each subject serves as their own control for the bite protection calculation.

Because repeated wash cycles will progressively remove some of the impregnated permethrin/etofenprox, the 75x washed fabric samples will be tested first followed by the 50x washed samples, the 20x washed samples, and finally the 0x unwashed samples. This order will reduce the possibility of any "carryover" contamination effects. Subjects will wash their forearms with soap between each exposure period to further reduce the potential for carryover of permethrin/etofenprox residues on skin from one exposure period to the next.

The widely accepted method of evaluating efficacy of insecticide treated clothing includes laboratory aging of treated clothing by laundering through standardized wash cycles per the American Association of Textile Chemists and Colorists (AATC) laundering protocol (Appendix E). Testing will be conducted with treated and untreated clothing prior to laundering (0x wash cycle) and at the 20x, 50x, and 75x wash cycles for the treated clothing.

The endpoint for determining efficacy in this proposed experiment (percent bite protection based on the proportion of blood-fed to total mosquitoes in a cage) differs from skin-applied repellent evaluations where the "Landing with Intent to Bite" measure is used and efficacy is measured as Complete Protection Time. In brief, the repellent effect created by skin-applied repellents is instantaneous and non-toxic, whereas mosquitoes exposed to treated clothing must remain in contact with the treated cloth for a longer time period to elicit an effect. The resulting repellent effect is a toxic effect that results in 'excito-repellency' or incapacitation due to exposure to the fast-acting insecticide. The target level of bite protection across fabric types and number of washes is ≥ 90% § 3.e. (p.13 of 211).

As in the previous version, this protocol also proposes to evaluate the repellent effect (percent bite protection) of treated clothing using only two mosquito species, unlike skin-applied repellent studies conducted under field conditions where three species are evaluated. The proposed study will assess % bite protection of treated fabrics from two mosquito species, *Anopheles albimanus* (malaria vector) and *Aedes aegypti* (vector of dengue, yellow fever, chikungunya, and Zika). A mosquito species from the genus *Culex* (vector of West Nile virus or St. Louis encephalitis) will not be tested. Considering the anthropophilic nature of the proposed *Anopheles* and *Aedes* mosquito species and their proper response in laboratory assays, prior studies of this nature have shown little difference between these species in their bite protection results. Therefore, the addition of *Culex* spp. would not contribute sufficiently distinct data to offset the burden to subjects from participation in this kind of study. *Culex* mosquitoes were also not proposed for testing in either of the two previous protocols reviewed

and accepted by EPA and the HSRB in April 2014, and October 2016 (p. 6 of 211).

The objective of the data analysis is to estimate the mean level of bite protection and associated 95% confidence intervals for different 'treatments' [i.e. different combinations of fabric types (FRACU and knits), number of washes, and mosquito species].

1. **Study design:**

   Replicate subjects will be used in this study to evaluate bite protection for FRACU and knit clothing fabrics treated with either permethrin or etofenprox. A fabric's "bite protection" is a measure of the relative level to which a treated fabric prevents bites compared to the untreated control fabric. As described in § 3.e. (p. 13 of 211) of the protocol, the observed bite protection for a subject is calculated using the bite-through rates for the treated fabric and a corresponding untreated/unwashed control fabric. Each subject serves as their own control. The purpose of the control is to compensate for the subject's individual attraction level, the general host-seeking response of the test mosquito population, and to correct for bite-through rate of the untreated fabric. The treatment and control values for a subject are then used in Abbott's formula to calculate the observed bite protection level of the fabric for that subject.

   Treated fabric will be evaluated at the following wash intervals: unwashed (0x), 20x, 50x, and 75x washes. Separate fabric specimens for each wash interval are tested, similar to that described in U.S. military GL/PD specifications. Two species of mosquitoes, *Aedes aegypti* and *Anopheles albimanus*, will be tested separately. Ten subjects will be used to test each fabric and mosquito species combination. Ten alternate subjects will also be recruited for testing of each fabric type. Each subject will test only one species on a given day; therefore, it will take commitments on two different days to complete all tests. Should a subject withdraw from the study for any reason or be removed from participation by the Study Director, his or her data will not be used in the study, and an alternate will be selected to replace the withdrawn subject.

   For each fabric treatment (untreated and treated), the subjects' will expose their right and left arms to mosquitoes for 15 minutes per arm in accordance with the testing paradigm denoted in Tables 2 and 3. Table 2 outlines the exposure to *Aedes aegypti*; Table 3 outlines the exposure to *Anopheles albimanus*. A detailed summary of the experimental design is described in § 3.e. (pp. 13-16 of 211). The fabric type used on each arm (right vs. left) will be determined *a priori* as denoted in Tables 2 and 3.

   For quality control, the study will be stopped if the test site becomes unsafe for any reason. In addition, subjects will not continue in the study if biting pressure falls below 50% or a subject is unattractive to mosquitoes (i.e., less than 50% of mosquitoes in a cage contact the fabric worn by the subject during a test interval), biting pressure rises too high for subject comfort or safety, a subject asks to withdraw (pp. 39 of 211).

Table 2: Testing Paradigm using *Aedes aegypti***

Test Set[1]	Subject Right Arm		Subject Left Arm	
	Treatment Condition[3]	Specimen Designation	Treatment Condition[3]	Specimen Designation
1	Untreated Unwashed	Sleeve 1	No Sleeve	---

	Control[2]			
2	Treated Washed 75x	Sleeve 2	Treated Washed 50x	Sleeve 3
3	Treated Washed 20x	Sleeve 4	Treated Washed 0x	Sleeve 5

[**]Table 2 in this report denotes Table 8 in the submitted protocol (p. 15 of 211, §3.e).
[1]Each test set runs for 15 minutes.
[2]Each subject serves as their own control for the bite protection calculation. Each subject will test the control on a randomly selected arm as Test 1 (in the sample above it is listed as Right Arm).
[3]Each subject will have both their right arm and left arm tested simultaneously for all treated specimens and complete Test Sets 2-3 for *Aedes aegypti*. Each subject will have a break between test sets when new cages are being filled with mosquitoes. All cages will be washed after all test sets for each participant are complete.

Table 3*: Testing Paradigm using *Anopheles albimanus***

Test Set[1]	Subject Right Arm		Subject Left Arm	
	Treatment Condition[3]	**Specimen Designation**	**Treatment Condition[3]**	**Specimen Designation**
4	Untreated Unwashed Control[2]	Sleeve 6	No Sleeve	---
5	Treated Washed 75x	Sleeve 7	Treated Washed 50x	Sleeve 8
6	Treated Washed 20x	Sleeve 9	Treated Washed 0x	Sleeve 10

[***]Table 3 in this report denotes Table 9 in the submitted protocol (p. 16 of 211, §3.e).
[1]Each test set runs for 15 minutes.
[2]Each subject serves as their own control for the bite protection calculation. Each subject will test the control on a randomly selected arm as Test 1 (in the sample above it is listed as Right Arm).
[3]Each subject will have both their right arm and left arm tested simultaneously for all treated specimens and complete Test Set 5-6 for *Anopheles albimanus*. Each subject will have a break between test sets when new cages are being filled with mosquitoes. All cages will be washed after all test sets for each participant are complete.

Laboratory-reared 6-11 day old adult female mosquitoes from colonies maintained at USDA-ARS, CMAVE, (Gainesville, FL) will be used for the bite protection assay (pp. 20 - 21 of 211, § 8.c.). Adult female mosquitoes of two aggressive and anthropophilic species will be tested. One species will be *Aedes aegypti*, a species that vectors the agents that cause yellow fever, dengue fever, Zika, and chikungunya and is found in tropical and subtropical regions of the world, including the most of the Southern US. The second species will be *Anopheles albimanus*, an aggressive biter native to the eastern US and a competent vector for malaria transmission. Mosquitoes from a colony typically respond more aggressively to attractant stimuli than strains reared from freshly collected wild-types.

Female mosquitoes will be preselected from stock cages by using a specially designed draw box that uses odors from the hand of a laboratory staff person to attract mosquitoes upwind in to a trap. The trap containing the mosquitoes will then be transferred to the test cage for subsequent testing by subjects (p. 21 of 211, § 8.c.). After the test period, the mosquitoes will be removed from the test cage by a laboratory staff person, frozen, and counted to determine the total number mosquitoes present in the test cage and the number of blood-fed mosquitoes.

2. Statistical design:

The primary objective of the data analysis is to estimate the overall (or 'mean') level of bite protection and associated 95% confidence interval for different 'treatments' (i.e., different combinations of fabric type, number of washes, and mosquito species). Subject-specific bite protection values will be calculated for each treatment using Abbott's formula as described in Section 8(e) (p. 23 of 211). These values will be averaged across all subjects to obtain mean observed bite protection values to confirm any model-based bite protection estimates.

The numbers of blood-fed and total female mosquitoes recorded following exposure to treated and control fabric for each subject will be analyzed as binomially distributed data in a generalized linear mixed model (GLIMMIX) using a log link function. Subjects will be treated as a random effect and the within-subject correlation accommodated using a mixed effect GLIMMIX (Appendix K). Use of the log link makes it possible to obtain an estimate and confidence interval for the ratio of the treatment and control bite-through rates. The estimates and confidence intervals for percent bite protection are obtained from the relationship:

Percent Bite Protection = [1 – (treatment rate) / (control rate)] × 100%

The GLIMMIX model-based bite protection estimates could be obtained by analyzing multiple models each with just a single treatment group and the matched control group. However, it may also be of interest to compare the bite protections of different types of treated fabric, number of washes, or mosquito species. In this case, all treatments (and species) of interest will be included in the same model. Because the GLIMMIX model uses a log link, hypothesis tests concerning ratios of bite protection can be formulated as linear contrasts in the GLIMMIX.

For a previous protocol reviewed by EPA and HSRB (October 2016), EPA conducted a power analysis for FRACU fabric treated with permethrin based on a similar study assessing bite protection of etofenprox treated uniforms previously reviewed by HSRB in October 2016. In the original protocol, the bite-through rate of the control group (non-treated FRACU fabric) was assumed to be set as 20% and 50%. In the EPA's power analysis, bite-through rate in the control treatment was assumed to be 75% based on results of the study reviewed in October 2016. Similar to the FRACU, many knit fabrics have an open construction with interstitial spaces in the fabric that are easily penetrated by the mouthparts of a biting mosquito; therefore, bite-through rates in the control group for knit fabrics are expected to be similar to bite-through rates in the control group for the FRACU. Thus, bite-through rate in the control group is not expected to change the results of the Agency's previous power analysis for the fabric types proposed to be tested in this protocol.

In accordance with findings from the previous protocol, the current protocol indicates 10 individuals will serve as test subjects for each type of treated fabric. EPA expects the study design with 10 subjects to have sufficient power to achieve the half width of the 95% confidence interval of the estimated percent bite protection of less than 6% if the bite-through rate for the control fabric is 75% and the true percent bite protection of the fabric is at least 80% (Table 4). The Agency's previous simulations indicate that to

reach 80% power of achieving the half width of the 95% confidence interval of the estimated percent bite protection of less than 3%, the study requires a sample size of 10 subjects, given that a true bite-through rate in the control is 75% and the true percent bite protection is 80% (Table 4). Similarly, the Agency's simulations demonstrated that to reach 95% true bite protection with 80% power, the half width of the 95% confidence interval of the estimated percent bite protection is less than 2% when using a sample size of 10 (Table 4). Because the Agency requires a mean bite protection to be 90% which falls between 80 and 95% true bite protection, the Agency is confident the statistical analysis will provide adequate power provided that the assumptions are correct.

Table 4[*]: Impact of the Number of Replications on the Number of Subjects when Control Bite-Through is 75%.

True bite-through Rate in control	True Percent Protection	Nr Subs	subject as fixed effect					GLIMMIX: subject as random effect				
			N[**]	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile	N[**]	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile
75	80	5	998	3.6	3.9	4.1	4.3	955	5.0	5.3	5.5	5.7
		6	995	3.3	3.5	3.7	3.9	945	4.2	4.5	4.6	4.8
		7	990	3.1	3.3	3.4	3.6	912	3.7	3.9	4.1	4.2
		8	985	2.8	3.0	3.1	3.3	898	3.3	3.5	3.6	3.7
		9	982	2.7	2.9	3.0	3.1	894	3.1	3.3	3.4	3.4
		10	**967**	**2.6**	**2.7**	**2.8**	**2.9**	**885**	**2.9**	**3.0**	**3.1**	**3.2**
		15	915	2.1	2.2	2.3	2.3	817	2.2	2.3	2.4	2.4
		20	831	1.8	1.9	1.9	2.0	717	1.9	2.0	2.0	2.0
	95	5	1000	2.0	2.3	2.4	2.6	982	2.8	3.2	3.4	3.7
		6	1000	1.8	2.0	2.2	2.3	984	2.4	2.7	2.9	3.0
		7	1000	1.7	1.9	2.1	2.2	980	2.1	2.4	2.5	2.7
		8	1000	1.6	1.8	1.9	2.0	965	1.9	2.1	2.2	2.4
		9	1000	1.5	1.7	1.8	1.9	978	1.8	1.9	2.1	2.2
		10	1000	1.4	1.6	1.7	1.7	982	1.6	1.8	1.9	2.0
		15	999	1.2	1.3	1.3	1.4	951	1.3	1.4	1.4	1.5
		20	987	1.0	1.1	1.1	1.2	946	1.1	1.1	1.2	1.2

*Table 4 in this report denotes Table 6 in the submitted protocol (p. 14 of 211, §3.e).
**Number of datasets analyzed by the model. Model used log link function. Variation between logit values between subjects SD = 1

The primary objective of the data analysis is to estimate the overall (or 'mean') level of bite protection and associated 95% confidence interval for different 'treatments' (i.e., different combinations of fabric type, number of washes, and mosquito species). Subject-specific bite protection values will be calculated for each treatment using Abbott's formula as described in §8.3.2. These values will be averaged over all subjects to obtain mean observed bite protection values that can be used to confirm any model-based bite protection estimates.

$$\% \text{ Bite Protection} = \frac{(B_{NC}/F_{C}) - (B_T/F_C)}{(B_{NC}/F_C)}$$

Where:

B_{NC} = bites recorded on the arm covered by the negative control fabric

F_C = female insects in the cage that are capable of biting at the start of the 15-minute exposure period

B_T = bites recorded on the arm that was covered by the treated fabric

3. How and to what will human subjects be exposed?

Subjects will be exposed to test material (permethrin- or etofenprox-impregnated fabric) and two species of caged mosquitoes in the laboratory. Each subject will have either permethrin-treated or etofenprox-treated sleeves placed on one or both forearms. A subject will only test a single fabric/test material combination against a single mosquito species per test day. Subjects will expose sleeved arms to caged mosquitoes for 15 minutes [The step-wise procedure is described in detail in §8.f, pp. 24-36 of 211]. This exposure period allows mosquitoes to land, probe, and blood-feed. Test subjects are expected to receive the greatest number of bites during the first set of tests with the untreated, unwashed control sleeves. Subsequent tests will involve treated sleeves and test subjects are expected to receive far fewer bites on arms covered with treated fabric.

4. Endpoints and Measures:

Efficacy will be measured as percent bite protection. The proposed study will estimate the mean level of bite protection and associated 95% confidence interval for different 'treatments' (i.e., different combinations of fabric type, number of washes, and mosquito species). Subject-specific bite protection values will be calculated for each treatment using Abbott's formula as described in §8.e based on exposure to mosquitoes during a 15-minute bioassay every hour for a total of 2.5 hrs. These values will be averaged over all subjects to obtain mean observed bite protection values that can be used to confirm any model-based bite protection estimates.

B. Compliance with Applicable Scientific Standards

This protocol adequately addresses the following elements according to applicable scientific standards:

- Acute toxicity research to characterize toxicological profile of the formulation and calculate margin of exposure (MOE)
- Experimental design and statistical power
- Pre-training of subjects

<u>EPA Science Comments</u>

The following elements in the protocol require revision before the research goes forward:

- Add clarification on page 15 to indicate "… it will take commitments on two different days to complete a test for a single fabric type with one active ingredient."
- Subjects should refrain from using fragrant items that such as deodorant, perfumes, skin lotions, or drinking alcohol for 24 hours prior to the test day.
- To ensure consistency and reduce potential for carryover, subject's arms should be washed with unscented soap prior to testing control sleeves and between each exposure period.

- Describe the method (e.g., random number generator) for randomly selecting which arm will receive the control and subsequent treatments.
- Describe the fabrics a bit more, e.g., composition of fabric types and openness vs. tightness of the weave, etc. (p. 21 of 211, §3.a)
- Specify length of the drying cycle. (p. 135 of 211, Appendix E)
- Storage of fabric samples prior to cage testing and retention analysis should be described in more detail than what was provided. (p. 17 of 211, §7)
- Please include a description of the retention analysis. If methods are similar to chemical analysis detailed in Appendix B, then please indicate in the protocol.
- Both Tables 2 and 3 denote "No Sleeve" in a section describing the test paradigm. Please clarify that this does / does not mean a subject will insert their bare arm into a cage of mosquitoes. If an arm will not be inserted in a cage, then consider something like "---" to replace the text "No Sleeve."
- In Table 3 the third footnote mentions test set "5-6." Reviewers think the author intends test set "7-8" given the context.

cc: Rick Keigwin

Attachment 1: EPA Protocol Review
Attachment 2: EPA Completeness Checklists

Attachment 1 - EPA Protocol Review

Title: Laboratory evaluation of bite protection for repellent-impregnated fabrics

Date: August 09, 2017

Principal Investigator and any sub-investigators: Dr. Uli Bernier

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1. Societal Value of Proposed Research

(a) What is the stated purpose of the proposed research?

In this protocol, military uniform and/or consumer knit fabric will be treated with permethrin or etofenprox. Treated fabric will be compared to untreated fabric to determine to determine the bite protection provided by treated fabric. Specifically, this protocol will determine against two species of mosquitoes (*Aedes aegypti* and *Anopheles albimanus*) the bite protection provided by U.S. Military Fire Resistant Army Combat Uniforms (FRACUs) and knit fabrics impregnated with 0.52% permethrin or 0.9% etofenprox after 0x, 20x, 50x, and 75x washes (p. 9 of 211, § 1).

(b) What research question does it address? Why is this question important? Would the research fill an important gap in understanding?

The purpose of this protocol is to develop a study to be used to evaluate the bite protection provided by fabrics that are treated or impregnated with substances that repel or prevent mosquito bites.

The rationale for testing is to collect data to show that military uniforms or knit fabrics (consumer products) impregnated with 0.52% permethrin or 0.9% etofenprox will provide ≥90% mean bite protection against mosquitoes for up to 75 washings. Although there are efficacy data for etofenprox-impregnated FRACU, there are currently no data demonstrating efficacy of etofenprox-impregnated knit fabrics with an open construction, and no adequate data demonstrating efficacy of permethrin-impregnated woven fabrics.

A standardized protocol will enable the EPA to receive consistent and scientifically reliable data for new clothing treatments. The bite protection data will provide information about: 1) the relative level to which bites are received through the fabric with insecticide treatment; 2) the bite protection efficacy of permethrin and etofenprox on knit fabric(s) with an open construction to support EPA registration.

(c) How would the study be used by EPA?

EPA will review the study to satisfy efficacy data requirements for registration and acceptable label claims for efficacy for the test material.

(d) Could the research question be answered with existing data? If so, how? If not, why not?

EPA requires product-specific efficacy data to support product registration. Data for permethrin-impregnated fabrics are not adequate or are not available. Efficacy data are not available for etofenprox-impregnated knit fabrics.

(e) Could the question be answered without newly exposing human subjects? If so, how? If not, why not?

Human subjects are required because they represent the target system for the test material, and sufficiently reliable non-human models for repellency testing have not been developed. Because the nature of these repellents is not fully understood, use of a non-human model is unlikely to deliver representative data.

2. Study Design

(a) What is the scientific objective of the study? If there is an explicit hypothesis, what is it?

The objective of this proposed study is *"To determine the bite protection level of 0.9% w/w etofenprox or 0.52% w/w permethrin treated fabric products and to assess their bite protection performance after 0x, 20x, 50x, and 75x washes."*

(b) Can the study as proposed achieve that objective or test this hypothesis?

The objective cited may be achieved by the study as proposed if the protocol is revised to address the recommendations in EPA's review.

2.1 Statistical Design

(a) What is the rationale for the choice of sample size?

The protocol indicates 10 individuals will serve as subjects for each fabric/treatment combination tested.

EPA has done a power analysis for a similar study previously reviewed by HSRB.[2]

[2] Ciarlo, T, Bohnenblust, E, Lydon, M. Science and Ethics Review of Protocol for Laboratory Evaluation of Mosquito Bite Protection from Permethrin-treated Clothing for the United States Army after 0, 20, and/or 50

In a previous HSRB study submission, the bite-through rate of the control group (non-treated FRACU fabric) was assumed to be set as 75%. The bite-through rate for knit fabrics is expected to be similar to the bite-through rate for FRACU.

EPA requires the study design to have sufficient power to achieve the half width of the 95% confidence interval of the estimated percent bite protection of less than 6% if the bite-through rate of the control fabric is 80%. To reach 80% power of achieving the width of the 95% confidence interval of the estimated percent bite protection of less than 3%, our simulations indicate that the study requires a sample size of 10 subjects, given that a true bite-through rate in the control is 75% and the true percent bite protection is 80% (Table 5 and Table 6 of protocol). Similarly, the Agency's simulations demonstrated that to reach 95% true bite protection with 80% power, the half width of the 95% confidence interval of the estimated percent bite protection is less than 2% when using a sample size of 10 (Table 5 and Table 6 of protocol). Because the Agency requires a mean bite protection to be 90% which falls between 80 and 95% true bite protection, the Agency is confident the statistical analysis will provide adequate power provided that the assumptions are correct.

(b) What negative and positive controls are proposed? Are proposed controls appropriate for the study design and statistical analysis plan?

Each subject will serve as their own treatment and negative control for each test fabric as described on p. 13 of 211 in §3.e. The controls are appropriate to calculate the overall bite protection because percent bite protection will be calculated by counting blood-fed female mosquitoes in the treatments and comparing them to the untreated control. One arm will serve as a control treatment replicate for each combination of fabric, treatment, and mosquito. Positive controls were not proposed.

(c) How is the study blinded?

The study is not blinded. Untreated fabric sleeves will be tested first, followed by treated fabric sleeves washed 75x, 50x, 20x and 0x.

(d) What is the plan for allocating individuals to treatment or control groups?

To obtain a statistically robust data set, this study requires a minimum of 10 subjects for each fabric/treatment combination tested. A total of 20 subjects will be enrolled to have 10 participants (5 female and 5 male) and 10 alternates for each fabric. Alternates will be randomly selected, though efforts will be made to replace withdrawn subjects with an alternate subject of the same gender.

(e) Can the data be statistically analyzed?

Washings. September 29, 2016. https://www.epa.gov/sites/production/files/2016-11/documents/epa_science_and_ethics_review_of_i2lresearch_launchbay_protocol_for_permethrin_treated_fabric_sept_29_2016.pdf

Yes, the data are appropriate for statistical analysis. See (f) below.

(f) What is the plan for statistical analysis of the data?

Based on past recommendations from the HSRB, a generalized linear mixed model (GLiM) procedure with the subject level treated as a random effect will be used for data analysis. There are several industry-standard statistical software packages than can be used to perform the analyses. These include SAS, JMP, or EXCEL (p. 37 of 211, §9).

(g) Are proposed statistical methods appropriate to answer the research question?

The analysis will provide the overall bite protection values and 95% confidence intervals for each treatment group and the controls. As proposed, the analysis addresses mean bite protection values and associated uncertainties. The statistical analysis is appropriate to determine bite protection provided by the different fabric treatments.

(h) Does the proposed design have adequate statistical power to definitively answer the research question?

The current protocol submitted by Pine Belt Processing, Inc. indicates that 10 individuals will serve as test subjects for each type of treated fabric tested. EPA's power analysis demonstrated that a sample size of 10 provides enough power to the statistical analysis. See section (a) above for detailed rational.

2.2 How and to what will human subjects be exposed?

Subjects will be exposed to test material and mosquitoes in the laboratory. The trapezoidal test material will be cut out of treated fabric and formed into "sleeves" by using clips to secure the two leading edges (connecting the parallel edges) (p. 20 of 211, §8.b). The active ingredient in the test material, permethrin or etofenprox, has a low acute and chronic risk profile (see section 4 below). Subjects with known allergic reactions (§8.1.2) are excluded from participation in the test.

Subjects will be exposed to laboratory reared populations of mosquitoes free of mosquito-borne pathogens (p. 20 of 211, §8.c). Subjects with known allergic reactions to mosquito bites will be excluded from research participation (p. 22 of 211, §8.d).

(a) What is the rationale for the choice of test material and formulation?

Efficacy data to satisfy product performance requirements and to support label claims for this product are required by EPA for registration. EPA requires submission of product performance data for all products claiming efficacy against public health pests.

(b) What is the rationale for the choice of dose/exposure levels and the staging of dose administration?

The rationale for testing is to collect data to show that different fabrics impregnated with 0.52% permethrin or 0.9% etofenprox will provide ≥ 90% mean bite protection against mosquitoes for up to 75 washings. There are no adequate data supporting currently registered fabric impregnated with 0.52% permethrin showing ≥ 90% efficacy through 50 washes using human subjects.

(c) What duration of exposure is proposed?

The exposure period is five 15-minute periods (2.5 hours total); three periods for one arm and two periods for the other arm of each subject (Tables 2 & 3).

2.3 Endpoints and Measures

(a) What endpoints will be measured? Are they appropriate to the question(s) being asked?

Endpoints/Measures for efficacy evaluation:

- Number of blood-fed and total number of female mosquitoes in each test. Tests will be replicated across two species of mosquitoes. The proportion of blood-fed mosquitoes/total mosquitoes will be calculated and expressed as a percentage value. This calculation will be performed for untreated control sleeves and treated sleeves (0x, 20x, 50x, and 75x washes).
- For each test subject, the treatment % bite values will be corrected to account for the bite-through values in the untreated control using Abbott's Formula.
- The overall % bite protection will be calculated and expressed as a mean value for each treatment: 0x, 20x, 50x, and 75x washes for each fabric.

The endpoints are appropriate to the questions being asked and address uncertainty associated with the samples size, between subject variation, treatment % bite values and the overall bite protection value.

The data form for each 15-minute sleeve exposure is presented in Appendix I on page 183 of 211.

(b) What steps are proposed to ensure measurements are accurate and reliable?

- Standard Operating Procedures (SOPs) will be in place that must meet Good Laboratory Practices requirements.
- Laboratory technicians will prepare cages of mosquitoes.
- Laboratory technicians will assist subjects with double-glove procedure to ensure gloves are worn correctly.
- Laboratory technicians or the Primary Investigator (PI) will assist subjects with placing the test sleeves on their arms and excluding all exposed skin from mosquito exposure.

- Laboratory technicians or the PI will assist subjects with insertion and removal of their arms in/from the cages.
- Laboratory technicians and the study director will track test sleeve samples, closely monitor the testing, and record all the data for the definitive study.
- Alternate subjects will be enrolled to ensure adequate sample size.
- Counts of blood-fed mosquitoes and the total number of mosquitoes in the cage will be determined by a research technician.
- The test sleeve samples will be assayed by the Analytical Unit (p. 24 of 211, §8.f) and the amount of permethrin and or etofenprox reported as a surface concentration of permethrin or etofenprox in units of mg/cm^2, which is commonly done for treated fabrics.

(c) What QA methods are proposed?

As explained in §7 on p.17 of 211, a separate, professional Quality Assurance Unit (QAU) will inspect the study: "Quality assurance of this study will be carried out in accordance with Good Laboratory Practice (GLP) Standards 40 CFR 160. Written reports of all findings from the Quality Assurance Officer will be provided to the study director and management. Any part of the study found by the Quality Assurance Officer to be likely to affect the integrity of the study will be brought the attention of the study director. A statement signed by the Quality Assurance Officer listing the phases inspected, inspection dates, and dates reported to the study director and management will be included in the final report. All deviations and amendments will be recorded and reported as per GLP guidelines. Additionally, fabric samples will be retained indefinitely for further analysis and verification as requested by analytical facility for quality control. The quality assurance unit of the analytical laboratory will provide the study director and the study director's management with relevant data, process, and report audits to meet Environmental Protection Agency GLP requirements."

(d) How will uncertainty be addressed? Will point estimates be accompanied by measures of uncertainty?

Uncertainty is addressed in the experimental design and selection of the number of subjects as described in §6. The objective of the data analysis is to estimate the mean level of bite protection and associated 95% confidence intervals for different 'treatments' [i.e. different combinations of fabric types (e.g., FRACU), number of washes, and mosquito species]. The numbers of blood-fed and total female mosquitoes in cages with treated and control fabric for each subject will be analyzed as binomial distributed data in a generalized linear model (GLiM) using a log link, generalized estimating equations or a mixed effect GLiM. This is largely dependent on the 'subject term', which may be treated as a fixed or random effect to adjust for within-subject differences (p. 17 of 211, § 6).

3. Subject Selection

3.1 Representativeness of Sample

(a) What is the population of concern?

The populations of concern are U.S. military personnel and civilians who would wear woven or knit fabrics treated with permethrin or etofenprox.

(b) From what populations will subjects be recruited?

Subjects will be recruited from the Gainesville area through advertising in the local newspaper and through postings on bulletin boards at the local university. Enrollment will be open to all interested candidates who meet the eligibility criteria.

(c) Are expected participants representative of the population of concern? If not, why not?

The expected participants will be recruited from the general population, which is one of the target populations of concern.

(d) Can the findings from the proposed study be generalized beyond the study sample?

Yes.

3.2 Equitable Selection of Subjects

(a) What are the inclusion/exclusion criteria? Are they complete and appropriate?

The inclusion/exclusion criteria are complete and appropriate.

(b) What, if any, is the relationship between the investigator and the subjects?

None. People with a relationship to the study director or sponsor (including employees and students) are excluded from becoming subjects.

(c) Are any potential subjects from a vulnerable population?

No.

(d) What process is proposed for recruiting and informing potential subjects?

Recruiting Subjects:

Subjects will be recruited from the Gainesville area through advertising in the local newspaper and through postings on bulletin boards at the local university. The advertisement will be approved by WIRB. It will include general information about the study, and information about how to contact the study director if interested in participating.

Informing Subjects:

Individuals from the pool will be contacted by telephone to determine whether they meet the basic inclusion criteria. They will be given a brief outline of the study and undergo a pre-screening to see if they meet basic eligibility criteria. If they are interested in enrolling in the study and qualified, they will be given a time, date and location to meet with study staff for an in-person meeting to learn more about the study and their potential role in it, go over the inclusion/exclusion criteria, review the consent form, watch a video about the study, listen to the other information to be provided by researchers during training as described the protocol, and receive answers to any questions the subjects may have. Contact information is included on the consent form for any individual who has additional questions or if further clarification is desired, after they have attended the training session. If an individual still wishes to enroll in the study, he or she will be asked to sign the consent form, which will be witnessed by the staff member who led the consent discussion. The subject will then be given a copy of the signed ICD. Enrolled subjects will be assigned randomly to serve as test subjects or alternates.

(e) If any subjects are potentially subject to coercion or undue influence, what specific safeguards are proposed to protect their rights and welfare?

Subjects will be recruited from the Gainesville, Florida area, via advertising through newspaper ads and ads posted on bulletin boards at the local university. Employees and students of the study director or sponsor are excluded from participation. Subjects should not be subject to coercion or undue influence.

3.3 Remuneration of Subjects

(a) What remuneration, if any, is proposed for the subjects?

Each subject will be paid $20 for attending the informational meeting, whether or not they enroll in the study. Subjects who enroll and show up on the test day but do not participate in the testing will be paid $20. Subjects will be compensated for participation at the rate of $20 per sleeve tested. For a subject who tests all washes of a treated fabric and the untreated control against both species of mosquitoes, this would mean being compensated $200. Subjects who have to repeat testing due to low biting pressure or other will be paid for the repeat tests at the rate of $20 for each sleeve re-tested.

If the Study Director or staff ask a subject to withdraw from the test or if a test subject needs to withdraw for any reason, the subject will be compensated for testing completed prior to withdrawal, at a rate of $20 per sleeve tested.

(b) Is proposed remuneration so high as to be an undue inducement?

No.

(c) Is proposed remuneration so low that it will only be attractive to economically disadvantaged subjects?

No.

(d) How and when would subjects be paid?

Subjects will be paid by the sponsor after completing testing. The protocol will be revised to include more information about how payment will be provided.

4. Risks to Subjects

4.1 Risk characterization

(a) Have all appropriate prerequisite studies been performed? What do they show about the hazards of the test material?

Permethrin is an EPA-registered pesticide with an essentially complete supporting toxicity database. It has been tested extensively in animals and is of low toxicity by all routes of exposure. The acute dermal LD_{50} of permethrin is greater than 2,000 mg/kg body weight. Permethrin is not a skin sensitizer.

All non-cancer post-application exposure scenarios for permethrin-impregnated clothing do not exceed the Agency's level of concern. The margins of exposure (MOE) are 6,700 and 26,000 for military personnel and garment workers, respectively [level of concern (LOC) = 300]. Further, all of the post-application cancer risk estimates for both populations are in the 10^{-6} range. The cancer risk estimates are 1.2×10^{-6} and 3.6×10^{-6} for military personnel and garment workers, respectively.

Etofenprox is an EPA-registered pesticide with a complete supporting toxicity database. It has been tested extensively in animals and is of low toxicity by all routes of exposure. The acute dermal LD_{50} of etofenprox is greater than 2,100 mg/kg body weight. Etofenprox is not a skin sensitizer.

The Agency has assessed the use of etofenprox impregnated fabric uses to make military uniforms. Short-, intermediate-, and long-term dermal exposures to etofenprox treated fabric result in MOEs of 1,700 for military personnel and 7,500 for garment workers, which are not of concern (LOC = 1000). In addition, etofenprox is classified as "not likely to be carcinogenic to humans at doses that do not alter rat thyroid hormone."

<u>Results from toxicity testing</u>:

Permethrin

- A primary eye irritation study on rabbits showed that permethrin is a low irritant to the eyes. Irritation was observed for 24-48 hours but was all cleared by 72 hours.
- A dermal sensitization study in Guinea pigs showed that permethrin is not a contact sensitizer.
- A primary skin irritation study with rabbits showed that permethrin is minimally irritating to the skin. All irritation was cleared by 48 hours.
- The single dose acute dermal LD_{50} of the permethrin is >2,000 mg/kg in rabbits.
- The acute oral LD_{50} of permethrin is 3,580 mg/kg and 2,280 mg/kg in male and female rats, respectively.

Etofenprox

- A primary eye irritation study on rabbits showed that etofenprox is a low irritant to the eyes. Conjunctival redness was observed at 24 hours but was cleared by 72 hours.
- A dermal sensitization study in Guinea pigs showed that etofenprox is not a contact sensitizer.
- A primary skin irritation study in rabbits study showed that etofenprox is minimally irritating to the skin.
- The single dose acute dermal LD_{50} of the etofenprox is >2,100 mg/kg in rabbits.
- The acute oral LD_{50} of etofenprox is >5000 mg/kg in dogs.

(b) What is the nature of the risk to the subjects of the proposed research?

The protocol discusses five potential hazards associated with these tests including adverse reaction to the test substances, exposure to mosquitoes and mosquito-borne diseases, physical discomfort of enduring multiple mosquito bites, unanticipated loss of confidential information, and psychological risks related to pregnancy testing.

Risks are minimized in the proposed research by excluding candidates known to be hypersensitive to or phobic of mosquito bites; using disease-free colony-raised mosquitoes; excluding candidates known to be sensitive to insect repellents or insecticide-treated fabrics and subjects with open cuts, scrapes, skin disease and skin problems; including medical monitoring procedures; incorporating procedures to keep the subjects' identities and results of pregnancy testing private, and to permit discrete withdrawal. Practical steps to minimize subject risks have been described in the protocol, and the remaining risks have a low probability of occurrence.

To eliminate the risk of contracting any mosquito-borne diseases, the study will be conducted only with laboratory-reared mosquitoes (*Aedes albopictus; Anopheles albimanus*), which are not known to harbor any pathogens.

(c) How do the proposed dose/exposure levels compare with the established NOAELs for the test material?

A 2017 Draft Risk Assessment for permethrin identified a dermal NOAEL of 500 mg/kg/day, based on a 21-day dermal toxicity study in rats. Given the size of the fabric samples proposed in this study design (716 cm^2) and the amount of permethrin applied during the impregnation process (0.125 mg/cm^2), the amount of permethrin per fabric sleeve, without consideration of potential loss during wash cycles, is calculated as 89.5 mg/sleeve. Subjects who wear the maximum of four treated sleeves in one test day will potentially be exposed to up to 358 mg permethrin. Of this 358 mg, it is estimated that 0.5% will be transferred to the skin, so each subject can receive up to 1.79 mg permethrin in one day. Assuming an average subject weight of 70 kg, the estimated human exposure is 0.026 mg/kg/subject. The MOE can then be calculated by dividing the dermal NOAEL by the estimated human exposure. This MOE of 19,553 is well above the Agency's LOC of 300.

(d) What is the probability of each risk associated with the research? How was this probability measured?

No numerical probability is estimated, but risks have a low probability of occurrence. Practical steps to minimize subject risks have been described in the protocol; risks are minimized by excluding candidates known to be hypersensitive to or phobic of mosquito bites; using disease-free colony-raised mosquitoes; excluding candidates known to be sensitive to insect repellents or insecticide-treated fabrics; excluding subjects with open cuts, scrapes, skin disease and skin problems; including medical monitoring procedures; incorporating procedures to keep the subjects' identities and results of pregnancy testing private, and to permit discrete withdrawal.

4.2 Risk minimization

(a) What specific steps are proposed to minimize risks to subjects?

"Risks of Exposure to Arthropods

"Laboratory-reared mosquitoes that are not infected with disease will bite participants. Candidates who are phobic or sensitive to bites will be excluded from the study. There are risks from exposure to biting arthropods and subsequent itching. Because this protocol uses only colony mosquitoes, disease risk is negligible. There is a risk of secondary infection from scratching due itching from bites. There is also a risk of an allergic response consisting of inflammation and warmth in the areas where bites were received. To reduce the possibility of these risks, participants will be informed during the consent process that the itching will most likely be the most significant discomfort to them from participation in this study. If requested, Over-the-Counter (OTC) topical steroid cream to relieve itching will be provided immediately upon completion of the tests. If at any point in the study the participant becomes uncomfortable due to itching they will be permitted to stop the study. In some rare cases, individuals may be allergic to mosquito bites and need to seek medical attention. There will be a nurse on call who has read the protocol and discussed the research with the study director to assist if needed. UF Health Shands Hospital is located 0.5 miles from the testing facility. Medical care for research-related injuries will be provided at no cost to the subjects.

"Risks from Exposure to Disease Vectors

"There are risks from exposure to disease vectors when participants are subjected to biting arthropods collected from the wild. However, because the test mosquitoes for this study have been reared in colony on sterilized bovine blood and have never been exposed to organisms that are infected with pathogens, there is no disease risk from using colony mosquitoes in this study. Participants will be informed of the risks from exposure to disease vectors during the consent process and the reasons that these mosquitoes do not contain pathogens will be explained.

"Risks from Exposure to the Test Material

"There are some risks from exposure to the test material. There is always a remote possibility of allergic reactions to chemical repellents; although, compounds to be tested will only be those for which there is no clear association with allergic reactions. Because the forearm is usually less sensitive to bites, the hand and wrist will be protected by 2 gloves to restrict bites to the forearm. The outer glove is latex therefore there is a risk of irritation from the outer glove. Alternatively, nitrile gloves will be available for use by subjects. In order to minimize the exposure to the test chemical, people who are allergic to the test chemical will be excluded from the study and the individuals who participate will be instructed to wash their arms after the testing has been completed. If at any time during the testing process a subject expresses a wish to discontinue, the test will immediately be stopped. There will be a nurse on call who has read the protocol and discussed the research with the study director to assist if needed. UF Health (Shands) Hospital is located 0.5 miles from the testing facility. Medical care for research- related injuries will be provided at no cost to the subjects.

"<u>Psychological Risks Related to Pregnancy Testing</u>

"There can be psychological stress relating to pregnancy testing. In order to minimize the psychological stress, women will be given a private place to take the test and the study director will ensure confidentiality of any test result. The results of the test will be recorded in the raw data, but the results will not be discussed or released to anyone besides the test subject. The confidentiality of the pregnancy testing will be discussed during the consent process." pp. 36-37

(b) What stopping rules are proposed in the protocol?

"*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.*" p. 38

(c) How does the protocol provide for medical management of potential illness or injury to subjects?

"*If at any point in the study the participant becomes uncomfortable due to itching they will be permitted to stop the study. In some rare cases, individuals may be allergic to mosquito bites and need to seek medical attention. There will be a nurse on call who has read the protocol and discussed the research with the study director to assist if needed. UF Health Shands Hospital is located 0.5 miles from the testing facility. Medical care for research-related injuries will be provided at no cost to the subjects.*" p. 36

(d) How does the protocol provide for safety monitoring?

Subjects are clearly and repeatedly informed that they may remove themselves for any reason from the study at any time. All subjects are asked to immediately tell the study director or study staff if they believe they are experiencing a reaction or feel ill during the study. The consent form also states that if, after participating in the study, a subject believes he or she has become ill as a result of their participation in the study, they should contact the study director or nurse anytime, 24-hours a day.

On the day of testing, a nurse who has read the protocol and discussed the research with the study staff will be on call for medical advice and/or assistance as necessary.

(e) How does the protocol provide for post-exposure monitoring or follow-up? Is it of long enough duration to discover adverse events which might occur?

The protocol does not provide an end date for post-exposure monitoring or follow-up. The consent form states:

"COMPENSATION FOR INJURY

If you are injured or become ill during the study, tell the study director, laboratory technician, or on call nurse immediately. The study director will obtain emergency medical treatment for you, if necessary. If your illness or injury is a direct result of being in this study, the sponsor of this research will cover the costs of any necessary medical treatment that is not covered by your insurance or the insurance of a third party under which you are covered.

"If, after participating in the study, you believe you have become ill as a direct result of your participation in the study, please call the study director, Ulrich Bernier Ph.D., at [phone number] (24 hours) or Wendy L. Morrison, RN, BSN, at [phone number] (24 hours)." p. 38, IRB Correspondence Report

"Questions

Contact Ulrich Bernier Ph.D., at [phone number] (24 hours) or Wendy L. Morrison, RN, BSN, at [phone number] (24 hours) for any of the following reasons:

- *If you have any questions about your participation in the study,*
- *If you feel you have had a research-related injury or a reaction to the test substance or any severe reaction to any mosquito bites you received,*
- *If you have question, concerns, or complaints about the research"* p. 38, IRB Correspondence Report

(f) How and by whom will medical care for research-related injuries to subjects be paid for?

"*If you are injured or become ill during the study, tell the study director, laboratory technician, or on call nurse immediately. The study director will obtain emergency medical treatment for you, if necessary. If your illness or injury is a direct result of being in this study, the sponsor of this research will cover the costs of any necessary medical treatment that is not covered by your insurance or the insurance of a third party under which you are covered."* p. 38, IRB Correspondence Report

5. Benefits

(a) What benefits of the proposed research, if any, would accrue to individual subjects?

There are no direct benefits to subjects.

(b) What benefits to society are anticipated from the information likely to be gained through the research?

This study is designed to determine the bite protection level of knit and woven fabrics treated with permethrin or etofenprox. The treated materials will be tested unwashed, 20 times washed, and 50 times washed, and 75 times washed for protection against bites by mosquitoes. The data collected in the study will be used to support product registration. The research has societal value because U.S. military personnel and civilians, both domestically and abroad, are at risk of contracting mosquito-borne diseases.

(c) How would societal benefits be distributed? Who would benefit from the proposed research?

One beneficiary will likely be the sponsor who is seeking EPA-registration for etofenprox and permethrin-treated clothing. Indirect beneficiaries would include the U.S. military soldiers and civilians who may benefit from wearing clothing made from these treated fabrics.

(d) What is the likelihood that each identified societal benefits would be realized?

EPA cannot predict the outcome of the testing results; the testing could demonstrate that the formulation is effective at providing the target level of mosquito bite protection. The purpose of the study is to determine the level of mosquito bite protection.

6. Risk/Benefit Balance

(a) How do the risks to subjects weigh against the anticipated benefits of the research, to subjects or to society?

The risk mitigation measures proposed in the protocol reduce risks to subjects without reducing the robustness of the scientific design. No reasonable opportunities to further reduce subject risk have been overlooked. The resulting residual risk to subjects is very low. The potential benefits from availability of a wider variety of effective insecticide-treated clothing for the US military and civilians are likely to be realized, and make the residual risks to subjects in this proposed research reasonable.

7. Independent Ethics Review

(a) What IRB reviewed the proposed research?

Western Institutional Review Board

(b) Is this IRB independent of the investigators and sponsors of the research? Yes

(c) Is this IRB registered with OHRP? Yes

(d) Is this IRB accredited? If so, by whom?

WIRB has full AAHRPP accreditation.

(e) Does this IRB hold a Federal-Wide Assurance from OHRP?

Yes.

(f) Are complete records of the IRB review as required by 40 CFR 26.1125 provided?

Yes.

(e) What standard(s) of ethical conduct would govern the work?

This is a protocol for third-party research involving what EPA has interpreted to be intentional exposure of human subjects to a pesticide. The study is being conducted with the intention of submitting the resulting data to EPA under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). Thus, the primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply.

8. Informed Consent

(a) Will informed consent be obtained from each prospective subject?

Yes.

(b) Will informed consent be appropriately documented, consistent with the requirements of 40 CFR 26.1117?

Yes.

(c) Do the informed consent materials meet the requirements of 40 CFR 26.1116, including adequate characterization of the risks and discomforts to subjects from participation in the research, the potential benefits to the subject or others, and the right to withdraw from the research?

Yes.

(d) What is the literacy rate in English or other languages among the intended research subjects?

Ability to speak and read English is a requirement for participation.

(e) What measures are proposed to overcome language differences, if any, between investigators and subjects?

N/A

(f) What measures are proposed to ensure subject comprehension of risks and discomforts?

The training session will cover risks and discomforts. The consent form addresses risks and discomforts. In addition, there will be frequent opportunities to ask questions during the consent process.

(g) What specific procedure will be followed to inform prospective subjects and to seek and obtain their consent?

See section 3.2(d).

(h) What measures are proposed to ensure fully voluntary participation and to avoid coercion or undue influence?

See section 3.2(e).

9. Respect for Subjects

(a) How will information about prospective and enrolled subjects be managed to ensure their privacy?

The subjects' identities will be protected as follows: each subject will be assigned a code number, and only subjects' code numbers will appear on data sheets. The subjects' names will not appear anywhere on the data sheet, or in the reports. Provision is made for discrete handling of the pregnancy testing that is required of female subjects on the day of testing. The test results will not be disclosed to anyone other than the test subject, the verifying female employee, and/or the Study Director.

(b) How will subjects be informed of their freedom to withdraw from the research at any time without penalty?

Subjects will be informed about this during the training session and the informed consent meeting. In addition, the informed consent form states:

"*Your participation in this study is voluntary. You may decide not to be in the study or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled."* p. 38, IRB Correspondence Report

(c) How will subjects who decline to participate or who withdraw from the research be dealt with?

Each candidate will be paid $20 for taking part in the consent meeting, whether or not they enroll. Enrolled subjects will be paid at a rate of $20 per sleeve tested, whether they complete all test periods as scheduled or their participation terminates early. A subject who shows up on the test day but does not complete any training will be paid $20.

Data from subjects who withdraw will not be used in the study.

Attachment 2
§ 26.1111 Criteria for IRB approval of research
Protocol for Laboratory Evaluation of Bite Protection from Repellent-Impregnated Fabric

Criterion	Y/N	Comment/Page Reference
(a)(1)(i) Risks to subjects are minimized by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk.	Y	
(a)(1)(ii) Risks to subjects are minimized, whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.	N/A	
(a)(2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (for example, the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.	Y	
(a)(3) Selection of subjects is equitable, taking into account the purposes of the research and the setting in which it will be conducted, and being particularly cognizant of the special problems of research involving vulnerable populations, such as prisoners, mentally disabled persons, or economically or educationally disadvantaged persons.	Y	
(a)(4) Informed consent will be sought from each prospective subject or the subject's legally authorized representative, in accordance with, and to the extent required by §26.1116.	Y	
(a)(5) Informed consent will be appropriately documented, in accordance with, and to the extent required by §26.1117.	Y	
(a)(6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.	Y	
(a)(7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.	Y	
(b) When some or all of the subjects are likely to be vulnerable to coercion or undue influence, additional safeguards have been included in the study to protect the rights and welfare of these subjects.	N/A	

§26.1116 General requirements for informed consent
Protocol for Laboratory Evaluation of Bite Protection from Repellent-Impregnated Fabric

Criterion		Y/N	Comment/Page Reference
No investigator may involve a human being as a subject in research covered by this subpart unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative		Y	
An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence		Y	
The information that is given to the subject or the representative shall be in language understandable to the subject or the representative		Y	
No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence		Y	
(a) In seeking informed consent the following information shall be provided to each subject	(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental	Y	
	(2) A description of any reasonably foreseeable risks or discomforts to the subject	Y	
	(3) A description of any benefits to the subject or to others which may reasonably be expected from the research	Y	
	(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject	Y	
	(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained	Y	
	(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained	Y	
	(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject	Y	
	(8) A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled	Y	
(b) When appropriate, one or more of the following elements of information shall also be provided to each subject	(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject may become pregnant) which are currently unforeseeable	Y	
	(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent	Y	
	(3) Any additional costs to the subject that may result from participation in the research	Y	
	(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject	Y	
	(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject	Y	
	(6) The approximate number of subjects involved in the study	Y	
(e) If the research involves intentional exposure of subjects to a pesticide, the subjects of the research must be informed of the identity of the pesticide and the nature of its pesticidal function.		Y	

§26.1117 Documentation of informed consent
Protocol for Laboratory Evaluation of Bite Protection from Repellent-Impregnated Fabric

Criterion	Y/N	Comment/Page Reference
(a) Informed consent shall be documented by the use of a written consent form approved by the IRB and signed by the subject or the subject's legally authorized representative. A copy shall be given to the person signing the form.	Y	
(b)(1) The consent form may be a written consent document that embodies the elements of informed consent required by §26.1116. This form may be read to the subject or the subject's legally authorized representative, but in any event, the investigator shall give either the subject or the representative adequate opportunity to read it before it is signed; or	Y	Consent form meets requirements of §26.1116; procedure described in protocol provides adequate opportunity to read the consent form before it is signed.
(b)(2) The consent form may be a short form written consent document stating that the elements of informed consent required by §26.1116 have been presented orally to the subject or the subject's legally authorized representative. When this method is used, there shall be a witness to the oral presentation. Also, the IRB shall approve a written summary of what is to be said to the subject or the representative. Only the short form itself is to be signed by the subject or the representative. However, the witness shall sign both the short form and a copy of the summary, and the person actually obtaining consent shall sign a copy of the summary. A copy of the summary shall be given to the subject or the representative, in addition to a copy of the short form.	N/A	

§26.1125 Submission of proposed human research for EPA review

Protocol for Laboratory Evaluation of Bite Protection from Repellent-Impregnated Fabric

Any person or institution who intends to conduct or sponsor human research covered by §26.1101(a) shall, after receiving approval from all appropriate IRBs, submit to EPA prior to initiating such research all information relevant to the proposed research specified by §26.1115(a), and the following additional information, to the extent not already included:

	Requirement		Y/N	Comments/Page Refs
The following Information, to the Extent not already included:	§1125(a) a discussion of:	(1) The potential risks to human subjects	Y	
		(2) The measures proposed to minimize risks to the human subjects;	Y	
		(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	
		(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	
		(5) The balance of risks and benefits of the proposed research.	Y	
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.		Y	
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.		Y	
	§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	
	§1125(e): All correspondence between the IRB and the investigators or sponsors.		Y	
	§1125(f): Official notification to the sponsor or investigator. . . that research involving human subjects has been reviewed and approved by an IRB.		Y	
all information relevant to the proposed research specified by § 26.1115(a)	(1) Copies of • all research proposals reviewed by the IRB, • scientific evaluations, if any, that accompanied the proposals reviewed by the IRB, • approved sample consent documents, • progress reports submitted by investigators, and reports of injuries to subjects.		Y n/a Y n/a	
	(2) Minutes of IRB meetings . . . 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 Y Y n/a n/a	Separately provided to HSRB members
	(3) Records of continuing review activities.		n/a	
	(4) Copies of all correspondence between the IRB and the investigators.		Y	
	(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, for example, full-time employee, a member of governing panel or board, stockholder, paid or unpaid consultant.		Y Y	Previously provided to EPA.
	(6) Written procedures for the IRB in the same detail as described in §26.1108(a) and §26.1108(b).		N	Previously provided to EPA.
	(7) Statements of significant new findings provided to subjects, as required by §26.1116(b)(5).		n/a	n/a for protocols