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

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

June 18, 2018

MEMORANDUM

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

FROM: Helen Hull-Sanders, Ph.D., Entomologist
Registration Division
Office of Pesticide Programs

Matt Aubuchon, Ph.D., Entomologist
Registration Division
Office of Pesticide Programs

Michelle Arling, Human Research Ethics Review Officer
Office of the Director
Office of Pesticide Programs

TO: Catherine Aubee, Chief, Invertebrate-Vertebrate Branch 1
Registration Division
Office of Pesticide Programs

REF: Laboratory evaluation of mosquito bite protection from permethrin-treated clothing after 0, 50, 75, and 100 washings. Unpublished document prepared by *i2L*Research USA, Inc. and sponsored by Pulcra Chemicals, January 17, 2018. 47 p. (MRID 50555701)

OPP Decision Numbers: 540138
DP: 446737
EPA File Symbols: 86110-2
Product Names: SKINTEX MR III APPAREL
EPA Receipt Date: 26 March 2018
EPA Company Number: 86110

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 0.52% permethrin after 0, 50, 75, and 100 washings. Ethical aspects of the proposed research are assessed in terms of the standards defined by 40 CFR 26 subparts K and L. 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.

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 Schulman Institutional Review Board (IRB).

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 via a padding process with 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, 50 times washed, 75 times washed, and 100 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 whether fabrics treated via the chemical padding process for transferring permethrin to the fabric uniformly will provide protection against mosquitoes for up to 100 washings. The data resulting from this proposed study are intended to support registration of specific treated fabrics.

2. **Subject Selection:** Subjects will be recruited from the Baltimore, Maryland area. Advertisements will be posted on digital and social media platforms, including Facebook, Yahoo/Bing, Google, and Craigslist. The advertisements will provide an overview of the study, as well as how to get more information about the study and participation.

   Interested candidates will be directed to a secure website, where they will complete a preliminary screening questionnaire. Those who are qualified based on their responses will be contacted by a member of the study team, who will provide basic information about the study and verify that the subject is between 18 and 55 years old. If the person expresses an interest in participating in the study, they will have a second conversation with a member of the study team. This call will include a more detailed description of the study and a screening of the interested person against a subset of the eligibility criteria. A person who is qualified and interested in enrolling in the study will be invited to the test location, *i2L*Research's lab, for an in-person training and consent session.

According to the protocol, the eligibility criteria are as follows:

- Subjects must be between 18-55 years old and willing to provide a government-issued photo identification to verify age
- Subjects must be able to read and speak English fluently
- Subjects must not be an immediate employee of Pulcra Chemicals or of i2L, or be immediately related to employees or owners of either company
- Subjects must have a reliable form of transportation to get to and from the test location
- Subjects must feel they are healthy enough to participate in the study, including not having health conditions that would affect the study or be worsened by the wearing of insecticide-treated fabrics
- Subjects must be willing to be exposed to and bitten by mosquitoes
- Subjects must not be phobic of mosquito bites
- Subjects must not have allergies or sensitivities to mosquito bites, insect repellents, or insecticide-treated fabrics
- Subjects must be users of treated fabrics, insect repellent products, and/or other products used to repel biting mosquitoes
- Subjects must not be hypersensitive or allergic to latex or skin care products
- Subjects must be free from open cuts, scrapes, skin disease, and skin problems such as eczema, psoriasis, or atopic dermatitis
- Subjects must be willing to wear short sleeves on their scheduled test day(s)
- Subjects must be willing to refrain from alcohol, nicotine, and fragrance products for 24 hours before the test
- Subjects must agree to inform the study staff if they have violated any study-related restrictions in the previous 24 hours
- Subjects must be able to sit in a chair for at least 15-minute mosquito exposure intervals
- Subjects must be attractive to mosquitoes (evaluated at the initiation of each test day)
- Subjects must be willing to follow study procedures and sign informed consent document
- Female subjects must not be pregnant or breastfeeding, and must be willing to take a pregnancy test at the initiation of each test day in which they participate

If the candidate meets the criteria during the phone screening, they will be invited to the study location for an in-person training session. The study team member conducting the training will provide a detailed description of the research. In addition, candidates will be provided with a step-by-step demonstration of a 15-minute testing interval, as well as of how the fabric will be applied to their arm. Candidates will review the informed consent document and have the opportunity to ask questions throughout the training session. In addition, they will be informed that they may speak in private with the study director if necessary. Those who are interested and qualified will respond to questions asked by the person conducting the training session to gauge their comprehension of the materials presented and discussed. If a candidate does not answer correctly, the study

team member will review the topic on which the candidate provided an incorrect response to ensure that the candidate understands the material. 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.

The final study report will include demographic information about the subjects, including gender, age, and ethnic background.

The protocol calls for compiling a list of potential subjects that is at least two times the number required for the specific type of treated fabric being tested (e.g., a test of a FRACU-like fabric requiring 10 subjects plus 8 alternates would need a list of potential subjects that includes at least 36 individuals). For each test day, 5 test subjects and 4 alternates will be selected from the list, with approximately half of each gender. 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. EPA developed an analysis to support the sample size of 10 subjects for flame-resistant army combat uniforms (FRACU; true bite-through rate in control of 75% and true bite protection of 80%) and the sample size of 15 subjects for army combat uniforms (ACU; true bite-through rate in control of 10%, true bite protection of at least 80%).[1]

The bite protection against each mosquito species (*Aedes aegypti* and *Anopheles albimanus*) will be determined individually, i.e., only one species per test cage. A subject will be recruited to test all washing levels of a single type of treated fabric (e.g., permethrin-treated knit fabric washed 0, 50, 75, and 100 times times), as well as the untreated control, against both species of mosquitoes. As a result, a subject completing testing of a treated fabric against both mosquito species may participate in up to two test days to evaluate the efficacy a single type of treated fabric against both species of mosquitoes.

3. **Risks to Subjects:** The protocol discusses five potential hazards associated with this research: 1) adverse reaction to test substances, 2) exposure to mosquitoes, 3) physical discomfort of multiple mosquito bites, 4) unanticipated loss of confidential information, and 5) psychological risks related to pregnancy testing. Risks are minimized in the protocol by excluding candidates known to be sensitive to insect repellents or insecticide-treated fabrics and subjects with open cuts, scrapes, skin disease and skin problems; excluding candidates known to be hypersensitive to or phobic of mosquito

[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.

bites; using disease-free colony-raised mosquitoes that have never received a blood meal; taking steps to maintain the confidentiality of subjects' identities; 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. Subsets of each colony used in this research will be screened for pathogens, although the likelihood of the mosquitoes being infected is non-existent. The mosquito supplier will provide documentation that the mosquitoes are disease free and have not received a blood meal. 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, immobilized, 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, 50 times washed, 75 times washed, and 100 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 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 result in transmission of 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:** Schulman IRB conditionally approved the protocol, informed consent form, and recruitment materials. Documentation regarding Schulman IRB's approval of the protocol has been provided to the HSRB members with the background materials for this protocol. Schulman IRB is merging another IRB to form Advarra IRB. Depending on the schedule for review and approval of the final protocol, either Schulman or Advarra will issue the approval and conduct oversight. Schulman IRBs is independent of the investigators and sponsors. Satisfactory documentation of the IRB procedures and membership for Schulman IRB is on file with the Agency. Advarra will continue to follow the written practices and procedures of Schulman IRB in its oversight of research originally submitted to and reviewed by Schulman IRB, including this research. Advarra IRB will provide the necessary documentation related to membership to EPA if it assumes oversight for this research.

7. **Informed Consent:** With EPA's comments incorporated, the protocol will contain a complete and satisfactory description of the process by which potential subjects will be recruited, informed, and trained in preparation for the test day, as well as the process for

seeking subjects' consent to participate. A copy of the IRB-approved consent document 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 $30 for attending a training session/consent meeting. On a test day, subjects will be compensated $104 for any length of participation up to 8 hours, and $19.50 per hour for any participation beyond 8 hours. Alternates who are present at the test center at the start of a test day will be compensated $50; they will spend no more than 2 hours at the test center unless chosen to participate as a test subject. 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

The study sponsor and research team 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. They 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 protocol will be revised to address EPA and HSRB recommendations, and the revised protocol and supporting documents will be resubmitted for review and approval by 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.

Study Objective, Standards, and Rationale (1.1)

1. Regarding the fabric treatment process, EPA's understanding is that the fabrics tested will be treated via the "padding process". Delete discussion of other fabric treatment methods if they will not be used during this study.

IRB Review and Ethical Study Conduct (1.3)

2. Schulman IRB has become part of Advarra IRB. Ensure all references to the IRB with oversight for the research are accurate.
3. Revise section 1.3.1 as follows: "Written approval from the overseeing IRB (Schulman or Advarra) will be obtained prior to study initiation. Following approval by the overseeing IRB, the study protocol, approved informed consent document (ICD) 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 IRB approval of the revised final protocol has been granted."
4. Revise section 1.3.2 as follows: "All protocol changes (amendments and deviations) must be reported to the overseeing IRB as specified by the overseeing IRB's written policies and procedures. All amendments must be reviewed and approved by the overseeing IRB 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 the overseeing IRB as outlined in its policies and procedures. All other amendments must be reviewed and approved by overseeing IRB prior to implementation in the study."
5. Add a section following 1.3.2 that contains the following language: "The final study report will contain a summary of all protocol changes and the associated documentation as specified in 40 CFR 26.1303. 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 the overseeing IRB as soon as possible following the change. A dated acknowledgment from overseeing IRB of receipt of the deviations must be received and included in the final study report as specified in 40 CFR 26.1303."
6. Revise section 1.3.3. to reference 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 document, and other required documentation for this study will be approved by an independent institutional review board (Schulman IRB or Advarra IRB) 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."
7. Revise section 1.3.4 to reference the IRB(s) that reviewed and approved the protocol.

Human Test Subjects (Section 2)

8. Throughout this section and the entire protocol, use consistent terminology to refer to the treated fabrics and mosquitoes. EPA suggests using "treated fabric" whenever discussing the permethrin-treated fabric and mosquitoes (or the specific species) when referencing the mosquitoes used in the testing. Clarify what is meant when terms such as variables,

test systems, fabric variables, test substance, etc. are used. Simplify the protocol to the extent possible by clearly describing what is being discussed (fabric and/or mosquitoes).

9. Regarding a statistically-supported sample size, EPA has conducted an analysis to support the statistical validity of a sample size of 10 when the fabric being treated has a true bite-through rate in the control of at least 75% and a true bite protection of 80% (similar to FRACU fabrics). Similarly, EPA's analysis supports a sample size of 15 for testing fabrics when the true bite-through rate in the control is 10-20% and the true bite protection is 80% (ACU type fabrics). Revise the protocol to note that when testing fabrics that do not align with FRACU or ACU levels of protection, Pulcra will develop and consult with EPA on a statistically-supported sample size prior to initiating testing.
10. In 2.2.5, the protocol states "Prior to testing, the test substance will be submitted and approved by IRB for review and approval." EPA does not expect that the IRB will be review the actual test substance for each trial. Please revise to indicate that prior to testing each type of treated fabric (e.g., cotton knit), the IRB will be informed about the specific fabric being tested and that the protocol will be amended to include a reference to the specific type of fabric being tested.
11. Following 2.2.6, the subsection numbering is incorrect – correct for accuracy. In section 2.2.5 that follows 2.2.6, ensure that references to other sections within the protocol are accurate.
12. During the training meeting, include a description of the pregnancy testing and verification process (#9) – see, e.g., section 2.6.16.
13. In 2.2.8, revise to note that subjects will only be exposed to one test substance (permethrin-treated fabric). In addition, clarify why subjects may be exposed to 3-4 wash cycles; the protocol calls for testing the treated fabric unwashed, after 50 washes, after 75 washes, and after 100 washes. This would be 4 wash cycles. Subjects will be exposed to 4-8 permethrin-treated fabrics, depending on whether they participate in testing with one or two species of mosquitoes.
14. In sections 2.3.3 and 2.3.4, clarify whether the recruitment pool will represent members of the general public or members of the military. Specify that the number of individuals recruited will be at least two times greater than the <u>total</u> number (subjects and alternates) required for the study.
15. In 2.3.8, explain how you derive the number needed to test a treated fabric against both species of mosquitoes (20-30); be sure the total count needed includes the alternates that will show up for each test day.
16. In section 2.4, ensure the references to other sections are accurate.
17. Move the description of the pregnancy testing procedure out of the eligibility criteria in section 2.6.16.
18. In section 2.7 and the consent materials, indicate that i2L research will provide the subjects with unscented products to while they are on site for the testing.

<u>Application and Treatment Order (Section 5)</u>

19. In section 5.8.2, indicate whether the fabric will be washed beyond 50 cycles and how the washing will be performed.

Data and Statistical Analysis (Section 7)

20. To rely on EPA's previous analyses of sample sizes for treated fabric efficacy testing, the fabrics must be substantially similar to either FRACU or ACU in terms of true bite-through rate in the control and true bite protection. Revise the protocol to indicate that these parameters will be reported for each type of fabric tested. Also revise to include a provision that if a fabric does not meet the bite-through standards established for FRACU or ACU, Pulcra will conduct a power analysis to determine an appropriate sample size for the fabric, considering true bite-through rate in the control and true bite protection, based on EPA's model. Further, Pulcra should consult with EPA on the analysis and proposed sample size prior to initiating any testing.

Study Conduct and Oversight (Section 9)

21. Clarify how the data from a subject who withdraws after testing has begun will be handled. Specifically, if a subject does not complete testing with all wash levels of a treated fabric, will his or her data be used?
22. In sections 9.3.6 and 9.3.7, replace "plus any overtime worked" with "plus $19.50/hour, rounded up to the next hour, for any time beyond the first 8 hours."

Amendments and Deviations to the Protocol (Section 10)

23. Include a reference to the IRB oversight section (1.3) and specify that all amendments and deviations will be made in accordance with Schulman IRB's written policies and procedures. In addition, include this language or refer back to section 1.3: "The final study report will contain a summary of all protocol amendments and deviations, and the associated documentation indicating submission to and approval by the overseeing IRB as specified in 40 CFR 26.1303."

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 0.52% w/w permethrin after 0X (unwashed), 50X, 75X and 100X 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). (p. 5 of 47).

This protocol, submitted for the re-registration of EPA Reg. No. 86110-2, SKINTEX MR III APPAREL, is based on 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 (HSRB) 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 for FRACU and similar fabrics (15 for ACU and similar fabrics); testing is not restricted to military fabrics (FRACU, ACU, BCU); 0.52% permethrin-treated fabrics are used; and treated knit, woven and "non-woven" fabrics will

be included to test fabrics representative of consumer clothing.

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 human subject exposing a fabric-sleeved arm into a cage (18" X 14" X 14") containing 200 ± 25 individual female mosquitoes of one species for 15 minutes. Each subject may be exposed to up to two test substances (fabric types), at four wash cycles each and up to two mosquito species on each day of participation (p. 13 of 47). Both arms may be exposed at 15 minute intervals with breaks up to 10 minutes between exposures. Two mosquito species will be tested, *Anopheles quadrimaculatus* and *Aedes aegypti*. 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 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 (100X) FRACU fabric.
- 1 test with treated washed (75X) FRACU fabric.
- 1 test with treated washed (50X) FRACU fabric.
- 1 test with treated unwashed (0X) FRACU fabric.

Table 1 below provides an example of how a fabric *type* (similar to FRACU) should be tested. 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 D.2) of this review. Total replicates per fabric type would be higher for a ACU type fabric.

Table 1: Experimental Design

Fabric and Treatment Condition[1]	Number of Fabric Specimens	Number of Subjects[2]	Number of Species[3]	Total Replicates per Fabric Type
Untreated Unwashed Control[4]	1	10	2	20
Treated Washed 100X	1	10	2	20
Treated Washed 75X	1	10	2	20
Treated Washed 50X	1	10	2	20
Treated Unwashed 0X	1	10	2	20

[1] Fabric treatment conditions are either untreated and unwashed (Control) or treated and unwashed (0X), treated and washed 50 times (50X), treated and washed 75 times (75X), or treated and washed 100 times (100X).
[2] The minimum number of human subjects is based on the bite through rate of the fabric (see FRACU and ACU differences)
[3] The test species are *Aedes aegypti* or *Anopheles quadrimaculatus.*
[4] Each subject serves as their own control for the bite protection calculation.

Because repeated wash cycles will progressively remove some of the impregnated permethrin, the 100X washed fabric samples will be tested first followed by the 75X washed samples, the 50X washed samples, and finally the 0X (unwashed) samples. This order will reduce the possibility of any "carryover" contamination effects. Although EPA does not expect that such carryover effects to occur, this testing regime safeguards the subjects from permethrin accumulation. Subjects will wash their forearms with soap between each exposure period to further reduce the potential for carryover of permethrin 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 (AATCC) laundering protocol (§ 5.8). Testing will be conducted with treated and untreated clothing prior to laundering 0X (unwashed) and at the 50X, 75X, and 100X 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 to mosquitoes, whereas mosquitoes exposed to treated clothing must remain in contact with the treated cloth for a longer 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% § 1.1.2 (p. 4 of 47).

As in previous proposed protocols, 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 percent bite protection of treated fabrics from two mosquito species, *Anopheles quadrimaculatus* (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 response in laboratory assays, prior studies with human exposures have shown little difference between these species when evaluating product bite protection results. Therefore, the addition of *Culex* spp. would not be expected to contribute sufficiently distinct data to offset the burden to subjects from participation in this type of study. *Culex* mosquitoes were also not proposed for testing in any of the three previous protocols reviewed and accepted by EPA and the HSRB in April 2014, October 2016 and January 2018.

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 (knits, weaves, FRACU, etc.) and fiber types (cotton, silk, rayon, wool, blends, etc.), number of washes, and mosquito species].

1. **Study design:**

Replicate subjects will be used in this study to evaluate bite protection for fabrics treated with permethrin. The number of replicate subjects will be determined in advance by the fabric's previously determined "bite protection." 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 § 2.2.5 (p. 9 of 47) of the protocol, given a true bite-through rate in the control of 10% - 20% for ACU and similar fabrics and a true percent bite protection of 80%, the study requires a sample size of 15 subjects in order to obtain 80% power to determine that the half width of the 95% CI of an estimated percent bite protection is less than 6%. Given a true bite-through rate in the control of 75% for FRACU and similar fabrics, and a true percent bite protection of 80%, the study requires a sample size of 10 subjects in order to obtain 80% power to determine that the half width of the 95% CI of an estimated percent bite protection is less than 3%. 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: 0X (unwashed), 50X, 75X, and 100X washes. Separate fabric specimens for each wash interval are tested, similar to that described in U.S. military GL/PD specifications (Document GL/PD 07-13A, 10 June 2009). Two species of mosquitoes, *Aedes aegypti* and *Anopheles quadrimaculatus***,** will be tested separately. Five test subjects (2 males, 2 females, 5th subject of either sex) will be selected for each test day. Four additional subjects (2 males and 2 females) will be chosen on each test day to serve as alternates and replace any individuals that are withdrawn. Each subject will test only one mosquito species on a given day. 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 with a break of at least 10 minutes between each test period. A detailed summary of the experimental design is described in § 5 (pp. 29-31 of 47).

Laboratory-reared 5-9 day old adult female mosquitoes obtained from colonies maintained at Benzon Research (Carlisle, PA) will be used for the bite protection assay (pp. 31 of 47, § 6.1.2).

Female mosquitoes will be preselected from stock cages by placing an ungloved hand near the screened cage to attract host-seeking females and collecting the mosquitoes with a motorized vacuum pump to aspirate them into a 1.7 L plastic container. The container will then be transferred to the test cage for subsequent testing by subjects (p. 32 of 47, § 6.1.). 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 of 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 7.3 (p. 39 of 47). These values will be averaged across all subjects to obtain mean observed bite protection values to confirm any model-based bite protection estimates.

For a previous protocol reviewed by EPA and HSRB (see Appendix 1, October 2016), EPA conducted a power analysis for FRACU fabric treated with permethrin based on a similar study assessing bite protection of etofenprox- and permethrin-treated uniforms previously reviewed by HSRB in October 2015. 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 2015 (Table 2). 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 fabrics similar to FRACU and 15 individuals for fabrics similar to ACU. 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 2). 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 2). 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 2). 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 2: 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

**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 §7.3.2. 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-impregnated fabric) and one of two species of caged mosquitoes in the laboratory. Each subject will have permethrin-treated sleeves placed on both forearms. A subject may test multiple fabrics/test materials in 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 §6.4, pp. 33-34 of 47]. 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 §7.3 based on exposure to mosquitoes during a 15-minute bioassay with a 10-minute break between exposures for up to 8 hours per day. 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.

E. 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

EPA Science Comments

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

- Revise the description of the types of fabrics to be tested. The protocol should describe some examples of the types of fabrics that could fall under each category (e.g., "knit fabrics, using fibers such as …."). The description of the fabric should include the parameters for classifying which fabric/fiber combinations are FRACU-like or ACU-like. (p. 7 of 47, §2.2.1)
- Describe the drying cycle, both after the permethrin fabric impregnating padding process (p. 4 of 47, §1.1.1.1) and between washings (p. 30 of 47 § 5.8).
- Please include a description of the permethrin retention analysis. How will it be determined that treated fabrics washed 100X still retain permethrin?
- The maximum number of treatment combinations (i.e., fabric type x mosquito species) per subject per day should be described. (see Table 1)
- The number of subjects and alternates should be described for testing of ACU-like fabrics.
- Section 5.4 states that two substances may be tested at the same time – one on each subject's forearm during one test day. Describe the sequence of tests be for each arm.

- Describe the handling of the fabrics (more details on the washing and drying cycle, surfactant used, etc. p. 30 of 47 § 5.8)
- Describe in detail what, if any, help technicians will provide the test subjects with double gloving, inserting subject's arms into cages, and removing arms from cages (p. 33 of 47 § 6.4)
- Not required, but consider including the option to test fabrics at 25X washes.

cc: Rick Keigwin

Attachment 1: EPA Protocol Review
Attachment 2: EPA Completeness Checklists
Attachment 3: Sample Size Estimation for Design of Mosquito Laboratory Studies, dated 9/26/2016

Attachment 1 - EPA Protocol Review

Title: Laboratory evaluation of mosquito bite protection from permethrin-treated clothing after 0, 50, 75, and 100 washings

Date: January 27, 2018

Principal Investigator and any sub-investigators: Timothy Foard

Participating Laboratory:
i2LResearch USA, Inc.
1430 Joh Avenue
Suite L-M
Baltimore, MD 21227

Sponsor:
Pulcra Chemicals
474 Bryant Boulevard
Rock Hill, SC 29732

IRB:
Schulman IRB

1. Societal Value of Proposed Research

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

In this protocol, military uniform and/or consumer fabrics will be treated with permethrin. 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 quadrimaculatus*) the bite protection provided by U.S. Military Fire Resistant Army Combat Uniforms (FRACUs) and other fabrics impregnated with 0.52% permethrin after 0X, 50X, 75X, and 100X washes (p. 4 of 47, § 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 with permethrin that repel or prevent mosquito bites.

The rationale for testing is to collect data to show that military uniforms or consumer fabrics impregnated with 0.52% permethrin will provide ≥90% mean bite protection against mosquitoes for up to 100 washings. There are currently no adequate data demonstrating efficacy of permethrin-impregnated 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 on military and consumer fabric(s) with an open construction.

(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.

(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 0.52% w/w permethrin treated fabric products and to assess their bite protection performance after 0X, 50X, 75X, and 100X 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 10-15 individuals will serve as subjects for each fabric/treatment combination tested.

EPA has conducted a power analysis for a similar study previously reviewed by

HSRB.[2] 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 consumer 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% (§ 7.2.3). 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 (see Table 2 above). 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. 32 of 47 in §6.2. 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. Testing will be conducted in decreasing order of wash cycles. Untreated fabric sleeves will be tested first, followed by treated fabric sleeves washed 100X, 75X, 50X and 0X (unwashed).

(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 for FRACU-like fabrics and a minimum of 15 subjects for each combination for ACU-like fabrics. Five test subjects and four

[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 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

alternates will be selected per day. Individual test subjects will serve as their own control.

(e) Can the data be statistically analyzed?

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. 39 of 47, §7.3.4).

(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 *i2L* Research USA, Inc. indicates that 10 individuals will serve as test subjects for each type of FRACU-like treated fabric tested. EPA's power analysis demonstrated that a sample size of 10 provides enough power to the statistical analysis for fabrics similar to FRACU and a sample size of 15 for fabrics similar to ACU. See section (a) above for detailed rationale.

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. 29 of 47, §5.1). The active ingredient in the test material, permethrin, has a low acute and chronic risk profile (see section 4 below). Subjects with known allergic reactions (§2.6.9) are excluded from participation in the test.

Subjects will be exposed to laboratory reared populations of mosquitoes free of mosquito-borne pathogens (p. 25 of 47, §2.10.2.5). Subjects with known allergic reactions to mosquito bites will be excluded from research participation (p. 19 of 47, §2.6.7).

(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 will provide ≥ 90% mean bite protection against mosquitoes for up to 100 washings. There are no adequate data supporting currently registered fabric impregnated with 0.52% permethrin showing ≥ 90% efficacy through 100 washes using human subjects.

(c) What duration of exposure is proposed?

The exposure period is five 15-minute periods with 10-minute breaks between exposures for up to 8 hours.

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, 50X, 75X, and 100X 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, 50X, 75X, and 100X 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 pages 44-46 of 47.

(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.
- 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.

(c) What QA methods are proposed?

As explained in §1.2 on p. 3 of 47, a representative of i2LResearch Inc.'s independent Quality Assurance Unit (QAU) will inspect the study: Good Laboratory Practice (GLP) Standards 40 CFR 160 will be followed (§1.2.1). "The QA representative [Jennifer Hostetler] will conduct critical phase inspections at intervals adequate to ensure study integrity, and maintain written and signed records of each inspection. Records shall identify the study and include the date of the inspection, the phase inspected, the individual conducting the inspection, positive and negative findings, actions recommended and taken to resolve negative findings, the scheduled date for re-inspection (if any), and the date(s) the findings are reported. All inspection findings will be reported to management and the Study Director."

(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 §7. 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. 37 of 47, § 7.2.3).

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 fabrics treated with permethrin.

(b) From what populations will subjects be recruited?

Subjects will be recruited from the Baltimore area through digital and social media advertising. 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 employees' family members) are excluded from becoming subjects.

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

Recruitment is not targeting subjects from a vulnerable population.

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

Recruiting Subjects:

"Subjects will be recruited from the Baltimore, Maryland area, via advertising through digital and social media. Advertisements will be posted in digital and social media mediums, such as Facebook, Yahoo/Bing, Google and Craigslist. The advertisements will contain a link to a study-specific secure website where interested respondents can learn more about the study as well as complete a pre-screening qualification form. The forms that are filled out on the website will be automatically uploaded into a secure and encrypted portal, to which i2L employees will have access. Every effort will be made to achieve the appropriate demographic composition, via a stratified random sample of the pool of recruited subjects, based on the availability of test subjects on each test day. The final study will specify the demographics of subjects who participated in the study, based on gender, age, and ethnic background.

"Individuals from the pool will be contacted by telephone or e-mail (in which case a

follow up telephone call will be made) to determine whether they meet the basic inclusion criteria. They will be given a brief outline of the study. If they are interested in enrolling in the study, they will be given a time, date and location to meet with i2L staff for a training session to learn more about the study and their potential role in it, go over the inclusion/exclusion criteria (see 'Individual inclusion/exclusion criteria', below), listen to the other information to be provided by researchers during training as described in section 2.2.6 of the protocol, and receive answers to any questions they 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.

"Individuals will continue to be contacted until a pool of potential subjects exists that is at least two times that required for the study to assess one test substance against both species (20-30 depending on subjects required), and in which all potential subjects fulfill the inclusion/exclusion criteria. These individuals will be given a time, date and location to meet with the Study Director (or other designated i2L staff member) for the consenting process (see 'Consenting', below). If desired, interested individuals may provide an email or mailing address to which the ICD can be sent for advance review. For additional test substances, the recruitment campaign will repeat the above process." (pp. 14-15)

Informing Subjects:

"All subjects must attend a training session prior to their participation. Within 4 weeks prior to the first test day for any given subject, the subject will attend a training session that will last approximately two hours. If a subject chooses to participate in two testing days, the subject will be required to attend another training session prior to their participation in the subsequent test day if their last training occurred more than 4 weeks prior to the second test day.

"During the training session, the following aspects of the study will be discussed and the following activities completed:
1. Upon arrival, subjects will be asked to provide proof of age with a driver's license, passport, or other valid identification.
2. Subjects will be given the Informed Consent Document (ICD), time to read the ICD, and the opportunity to ask questions about it. The trainer will provide a brief outline of the study including its purpose, the subjects' potential role in the study, the potential length of the study on any given test day, the identity and function of the pesticide to which they will be exposed, the potential hazards associated with the study and steps being taken to mitigate each hazard as addressed in the protocol, and the inclusion/exclusion criteria. The procedures involved with a 15-minute exposure interval will be explained and demonstrated step-by-step to all subjects who participate in the training. The subjects will be shown how the fabric will be applied to their arm for the future testing as per section 5.1-5.3 of the protocol, will be informed that they will wear gloves to protect their hands, and will be shown how to position their arm for testing inside the test cage per section

7.4.2 of the protocol. i2L will also explain that the subjects will wash and dry their forearms at the beginning of each test day and after each test period and take up to a 10-minute break between each test period. If a subject needs to take a longer break, that will be allowed. One 10-minute break will overlap with a 30-minute lunch break.
3. Any questions or concerns about the study will be discussed and answered.
4. The employee conducting the training session with test subjects will let all training attendees know that if a test subject needs to speak to the study director in private about any aspect of the study, time will be made for this discussion once the general training session is over.
5. To confirm understanding of the consent form, the following questions will be asked of each potential subject:

a. What will your arms be in the test cages with?
b. What will you be wearing on your arm during the exposure period?
c. How long will your arms be in the test cages for each exposure?
d. What are the potential discomforts or hazards from this study?
e. Do you have the freedom to quit or withdraw from the study at any time?
f. If you quit or withdraw from the study, for how many hours will you be paid?

6. All subjects who meet the requirements for participation and agree to participate in the study will sign the ICD and will receive a copy of the signed ICD. They will also receive a copy of the testing schedule.
7. The trainer will recommend that subjects bring their own form of entertainment (book, DVD player, computer, etc.) to minimize participant anxiety and potential boredom during testing procedures. The researcher will have drinks (e.g., bottled water, soft drinks, etc.) and snacks available for subjects during the study day. Researchers will ask subjects if they have any food allergies and make snacks available taking into account the responses. Subjects will be told that they can bring their own lunch to consume during a 30-minute lunch break which will overlap with one of the 10-minute breaks between test periods, assuming a subject wishes to eat lunch.
8. The trainer will provide test subjects with the study director's contact information (name, email, and phone number) to field any follow up questions. This information will be on the first page of the provided ICD.
9. Female participants will be notified that they will be required to undergo pregnancy testing at the beginning of each testing day." (pp. 10-12)

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

Employees of the study director or sponsor, as well as the employees' family members, 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 $30 for attending a training and consent session, whether or not they enroll in the study. Subjects who are selected to enroll as test subjects will be paid

$104 ($13/hour) for their participation, for any amount of time up to 8 hours, plus $19.50/hour rounded up to the next hour for participation beyond the first 8 hours. Alternates who show up to the test site but who are not selected to replace a test subject will be released within 2 hours of the start of the test day and compensated $50.

(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 check, either mailed to the address indicated by the subject or delivered in person at the test facility. The test facility issues payments on the 15th and last day of the month.

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 x 10^{-6} and 3.6 x 10^{-6} for military personnel and garment workers, respectively.

Results from toxicity testing:

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.

(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, 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. In addition, a subset of each colony used will be screened prior to the testing to ensure that they are free of dengue and malaria.

(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 eight treated sleeves in one test day will potentially be exposed to up to 716 mg permethrin. Of this 716 mg, it is estimated that 0.5% will be transferred to the skin, so each subject can receive up to 3.58 mg permethrin in one day. Assuming an average subject weight of 70 kg, the estimated human exposure is 0.05 mg/kg/subject. The MOE can then be calculated by dividing the dermal NOAEL by the estimated human exposure. This MOE of 10,000 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?

"2.10.2. These potential hazards will be addressed as follows:
2.10.2.1. Safety Data Sheets (SDS) for each test substance tested under this protocol will be available for subjects to review during the training session if they wish to do so. These SDSs attest to the safety of these products for use on human skin when used as directed.
2.10.2.2. No subjects with known allergies or sensitivities to insect repellents or insecticide-treated fabrics will be allowed to take part in this study.
2.10.2.3. No subjects with known allergies or sensitivities to mosquito bites will be allowed to take part in this study. The forearm is usually less sensitive to bites and the subjects' hands and wrists will be protected by gloves to restrict bites to the forearm.
2.10.2.4. Mosquitoes can transmit various disease-causing organisms to humans, notably Plasmodium spp. (the cause of malaria), dengue, chikungunya, Zika, and yellow fever viruses."

"2.10.2.5. To eliminate the risk of contracting any mosquito-borne diseases, the study will be conducted only with laboratory-reared mosquitoes, which are known not to harbor any pathogens. In order to ensure the mosquitoes used in the study are not carrying any diseases, a subset of the colony will be screened for pathogens. Ae aegypti will be screened for all four serotypes of dengue. An. quadrimaculatus will be screened for malaria pathogens. These screens will be conducted using VecTOR test kits available from www.vectortest.com. Each test will consist of a pooled set of 10 mosquitoes removed from stock cages. Tests will be replicated two additional times (in triplicate) to verify that the colony mosquitoes are free of all four serotypes of dengue (Ae. aegypti) and malaria pathogens (An. quadrimaculatus). In addition, Benzon Research or any other lab supplying the mosquitos used in this study will provide documentation to be included in the final study report that these laboratory-reared mosquitoes are disease free, and that they have never received a blood meal.
2.10.2.6. The final study report will include chain of custody documentation to confirm that at no point prior to the test were the mosquitoes used in the study exposed to disease-causing pathogens.
2.10.2.7. Subjects will be told that if anyone experiences any skin reaction, experiences an injury, or simply feels unwell, he or she should inform i2L staff right away. Such

subjects will immediately be given appropriate care, may be withdrawn from testing, and may be transported to a local hospital if necessary. The closest hospital to the laboratory test site and directions will be identified prior to the test date. At least one study staff member will remain with the other subjects if other staff members have to depart with an injured or ill subject. If a subject is injured as a result of wearing the insecticide-treated fabrics or from procedures used during the study, the study sponsor will directly pay for those medical expenses necessary to treat the subject's injury that are not covered by medical insurance or other third-party coverage. All adverse effects will be followed until resolution is reached; this means that the study director and/or study sponsor will follow-up with subjects who are injured as a result of the study and check on the status of their injuries until the medical issues resulting from the study are resolved. There are no plans to provide other compensation beyond what is listed in this protocol and informed consent document.
2.10.2.8. If requested by the subject, standard over-the-counter first aid items such as bandages, antiseptics, and hydrocortisone cream, will be provided immediately upon completion of the test at no cost to the subject. They may also request First Aid assistance at any time. A nurse/Research Monitor will be contacted prior to the test date and will be on call during each test day for non-emergency queries or problems.
2.10.2.9. Subjects will have been advised on several occasions that they can withdraw from the study for any reason, without penalty.
2.10.2.10. All efforts will be taken to maintain the subjects' confidentiality (see 'Confidentiality of Human Subjects' in section 2 above).
2.10.2.11. 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, a female member of the study team will verify the test result, and the study director will ensure confidentiality of any test result. The results of the test will not be discussed with or released to anyone besides the subject. The confidentiality of the pregnancy testing will be discussed during the consent process." pp. 23-27

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

"9.3.1 This study may be terminated early if adverse events occur among the subjects, by Pulcra's decision, or for other reasons. The decision to terminate will be made by Pulcra Chemicals in conjunction with the Study Director.
9.3.2 If a test subject decides to withdraw because of any adverse reactions or sensitivity, such as redness, edema or itching, or if pain from the test substance is observed or reported, and/or medical management is needed, the test subjects will be removed from the test immediately. This is discussed in detail in section 2.10.10. The test subject will be instructed to gently wash the treated skin with soap and water and, where warranted, appropriate medical attention will be sought.
9.3.3 Subjects may decline to participate in this study at any time without penalty. Subjects must tell i2L staff if they want to withdraw; this can be done orally.
9.3.4 i2L may ask any subject who does not follow instructions to withdraw from the study." p. 43

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

*"9.2 **Medical monitoring and reporting unanticipated problems***
9.2.1 The Research Monitor, [redacted], is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.
9.2.2 Prior to the first test day, i2L will ensure that at least 2 staff members (one male, one female) will renew CPR/AED and First Aid Training certification.
9.2.3 The i2L staff will watch for unanticipated problems or adverse effects to the subjects. Subjects will be told that if anyone experiences any skin reaction, experiences an injury, or simply feels unwell, he or she should inform i2L staff right away. Such subjects will immediately be given appropriate care, may be withdrawn from testing, and may be transported to a local hospital if necessary. If a subject is injured as a result of wearing the study's insecticide-treated fabrics or from procedures used during the study, the study sponsor will directly pay for those medical expenses necessary to treat the subject's injury that are not covered by medical insurance or other third-party coverage.
9.2.4 The on-call nurse/Research Monitor will be familiar with the study and available for any non-emergency related queries or questions that subjects may have. The nurse's telephone number is included on the consent form which the subject will receive." pp. 41-42

"9.2.8 The nearest local hospital to i2L's laboratory will be located and directions identified prior to any study-related procedures taking place.
9.2.9 The risk of a Type 1 allergic reaction (i.e., anaphylaxis) occurring on the test day as a result of participation in the study is negligible. However, should such an event, or any other serious injury or medical issue occur, the i2L staff will call 911 and follow the instructions given by the emergency dispatchers. If instructed to transport the subject to a hospital, one study staff member and one other i2L staff member (one to drive and one to observe and take care of subject) will perform this task. If there are not sufficient study staff present to both carry on the study and transport the affected subject(s), the Study Director will abort the test day.
9.2.10 Subjects will be instructed that if they experience continued swelling or other severe irritation on their forearms after 48 hours following the end of the most recent test day, or have a serious adverse reaction before 48 hours, they should inform i2L staff and seek medical advice." p. 42

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

See Section 4.2(c) above. A registered nurse will serve as the Research Monitor, and will be responsible for overseeing the safety of the research and monitoring subjects

throughout their participation.

(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 protocol notes that "*[w]hen one or more test subjects participate in more than one test day, their two test days will be spaced apart a minimum of 72 hours in order to minimize any possible discomfort or complications such as an allergic response.*" *p. 8*

The consent form notes that the study staff and on-call nurse are available to subjects 24 hours a day in the event of a study-related injury: "*If you have any questions about this study or suffer a research-related reaction, call i2L at [number], or call [number] after office hours. There will also be an on-call nurse for non-emergency related questions related to your participation in the study [number].*" *Consent Form*

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

"*If you are injured as a result of wearing the permethrin-treated fabric or from procedures done for the purpose of this study, Pulcra Chemicals will pay for those medical expenses necessary to treat your injury that are not covered by your medical insurance or any other third party coverage." Consent Form*

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 various types of fabrics treated with permethrin. The treated materials will be tested unwashed, 50 times washed, 75 times washed, and 100 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, Pulcra Chemicals, who is seeking EPA-registration for permethrin-treated fabrics. 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?

Schulman 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?

Schulman IRB is accredited by the Association for the Accreditation of Human Research Protection Programs.

(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, and questions will be asked following the training session to ensure participants' comprehension of the material covered. 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:

"*Participation in this study is voluntary. You may refuse to take part in this study or quit at any time without penalty or loss of benefits to which you may be otherwise entitled." Consent Form*

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

"*2.5.* ***Subject compensation***
2.5.1. Each subject will be paid $30 for taking part in each training session.
2.5.2. For each test day, test subjects will be paid $104.00 ($13 per hour) for any length of participation up to 8 hours (see 'Study termination, individual participation, and withdrawal' in Section 9 for exceptions). In the unlikely event that a test day exceeds 8 hours, subjects will be paid $19.50 (time and a half) for each additional hour, rounded up to the nearest hour.
2.5.3. An alternate who is not needed to replace a test subject will be able to leave and will be paid $50. The decision as to whether an alternate is needed will occur within the first 2 hours of the test, during the preparation time, control exposure, but before all the treatment exposures. If an alternate is asked to replace a test subject, he or she will be paid at the same rate as other test subjects, as described above.
2.5.4. Subjects who have participated in the training session, but then choose to withdraw or are asked to withdraw from or during the training session, will still be paid $30.00 for attending all or part of this session.

2.5.5. Subjects may decline to participate at any time during the training session or test day without penalty. Subjects who withdraw or choose not to participate will be compensated for the amount of time they participated as outlined in this section (2.5).
2.5.6. If the Study Director or other i2L staff ask a subject to withdraw from the test and the subject has complied with all of their requests, or if a test subject needs to withdraw early because of a health or emergency reason, full payment will still be made even if the test subject has participated for less than eight hours. This will not affect payment for any previous test days that had been completed.
2.5.7. The Study Director or other designated i2L staff may end a particular subject's participation in a training session or on a test day, at any time, for any reason. If a test subject is asked to withdraw from the test because they have refused to follow given directions or if they choose to withdraw from testing early on a test day for a non-health related or non-emergency reason, full payment will not be made if the test subject participates in less than eight hours. Instead, they will be paid for the number of hours worked (rounded to the next hour) at a rate of $13.00 per hour. This will not affect payment for any previous test days that had been completed." pp. 17-18

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

Conclusion

- Given a true bite-through rate in the control of 10% - 20% for the **ACU** and a true percent bite protection of 80%, the study requires a sample size of 15 subjects in order to obtain 80% power to determine that the half width of the 95% CI of an estimated percent bite protection is less than 6%.
- Given a true bite-through rate in the control of 75% for the **FRACU** and a true percent bite protection of 80%, the study requires a sample size of 10 subjects in order to obtain 80% power to determine that the half width of the 95% CI of an estimated percent bite protection is less than 3%.

Detailed information for simulation

The required number of subjects for the proposed mosquito repellency study design is determined by a number of study characteristics and desired performance criteria including:

- the true percent protection;
- the true bite-through rate of the control group;
- the variation between subjects;
- the (desired) degree of precision of the estimated percent protection; and
- the (desired) power (to determine that degree of precision)

Here, we used a simulation approach to compute the power/sample size of the study, with 1000 iterations performed (i.e., generation of 1000 datasets). In order to simplify the approach, the simulation assumes each subject experiences and is exposed to two test box conditions, one being the control cloth material condition and one being the treated cloth material condition. For each iteration/dataset, the percent bite protection of the treated cloth material vs. control cloth material was estimated using a generalized linear model for binomial distribution using a log link function, using subject as random effect (SAS PROC GLIMMIX). The inputs to this simulation are described below:

- True percent protection: the true percent protection was initially set as 80%. As the true bite protection increases, the estimated bite protection becomes more precise (i.e. narrower 95% CI) given all other factors are fixed. As a sensitivity analysis to determine how sample size might change with a true bite protection of 95%, further runs were performed with 95% true bite protection (a true percent bite protection as

80% requires a larger sample size than a true percent protection as 95%, given all other factors are the same).

- The true bite-through rate in the control: The results from a previous study reviewed by the HSRB in April 2014 using etofenprox suggested a bite-through rate in the control of about 75% or greater, and that (earlier) sample size was determined -- in part, by this assumed 75% control bite-through rate. However, the bite-through rate of the control group depends on the type of fabric. For these simulations, the bite-through rates were set to be 10%, 20%, 50%, 75% and 90% to develop a range of potential sample sizes. Smaller bite-through rates result in larger required sample sizes, given all other factors remain the same such that control bite-through rates of 10% will require larger sample sizes than those of 90%.

- Variation between subjects: Subjects may have a distribution of subject-specific propensities to be bitten and the simulation needed to account for the fact that there thus may be a correlation between the (repeated) measurements from the same subject. The number of subject-specific mosquitoes and number of subject-specific blood-fed mosquitoes in a control cloth test cage (or treated cloth test cage) of a subject were derived in the 5 following steps:

    - Step 1: compute the group logit value for each condition = natural log of (proportion of blood-fed mosquitoes/ (1-proportion of blood-fed mosquitoes));
    - Step 2: create a random subject logit value = the control group logit value + a random value, where the random value is randomly generated from a normal distribution with mean = 0 and SD = 1[3]
    - Step 3: compute the true proportion of blood-fed mosquitoes of the subject = exp(subject logit value)/(1+ exp(subject logit value));
    - Step 4: randomly generate the number of mosquitoes in the subject's box from a Poisson distribution with mean = 200.
    - Step 5: randomly generate (1000 times) the number of blood-fed mosquitoes for the subject from binomial distributions with the rate of blood-fed mosquitoes derived in step 3 and the total number of mosquitoes randomly generated in Step 4.

  The correlation between the measurements from the same subject was generated by using the same random value (that was randomly generated from the normal distribution N(0,1) in step 2) in the derivation of the blood-fed mosquito rates of both control cloth and treated cloth per subject. A study requires a larger sample size if the

[3] Simulations with SD =1 were done for control bite through rates of 20%, 50%, 75%, and 90%. The simulation was also done when the bite-through rates of the control were 50%, 75%, and 90%.for SD = 0 (no variation between subjects) and SD = 0.3. A study requires a larger sample size if the SD increases, given all other factors are the same.

SD increases, given all other factors are the same. To be conservative, the results of simulation where SD = 1 would be recommended for use. (Note that the logit value will range from -2.94 to 2.94 when the proportion of blood-fed mosquitoes ranges from 5% to 95%. When SD = 1, 95% limits of the random value (that is added to the common population logit) ranges from -2 to 2).

- The precision of estimated percent protection: The precision of the estimated percent protection is expressed by the half-width of the 95% CI of the estimated percent protection. The precision of estimated percent protection was estimated from the 80th percentile of the 1000 half widths of the 95% CIs of the estimated percent protection (1000 iterations/datasets for each combination of all factors indicated above). This 80th percentile value is the value that is associated with 80% power. As a sensitivity analysis, the 90th %-tile and 95th %-tile values were also investigated (i.e., those associated with 90% and 95% power, respectively).

- Sample size of the study: the simulation evaluated the precision of estimated percent protection of a study at sample sizes = 5 through 20 if the bite-through rates of control group = 10% and 20%, (more typical of the ACU) and sample sizes = 5, 6, 7, 8, 9, 10, 15, and 20 if the bite-through rates = 50%, 75%, and 90% (more typical of the FRACU). As the sample size increases, the precision of estimated percent protection increases, given all other factors remain the same.

Given the above, the results of the simulation are as follows:

  - For the **ACU**, the simulation results for the sample size calculation indicate that if a true bite-through rate in the control is 10%-20% and the true percent bite protection is 80%, a study with 15 subjects would have 80% power to generate a half width of a 95% CI of 6% or less.

  - For the **FRACU**, the simulation results for the sample size calculation indicate that if a true bite-through rate in the control is 75% and the true percent bite protection is 80%, a study with 10 subjects would have 80% power that the half width of 95% CI would be 3% or less.

Note that the desired precision (expressed as the 95% CI half-width) differs between the FRACU and the ACU, with the FRACU half-width criterion being set at 3% and the ACU half-width at 6%. This is due in part to the Agency's desire to minimize the number of subjects required (consistent with required power) and the fact that increases in the number of test subjects beyond 15 for the ACU and 10 for the FRACU produce only marginal decreases in the half-width of the confidence interval at the simulated inputs. That is, increases in the number of subjects beyond these numbers do not substantially narrow half-

width the 95% CI, or –equivalently– a large increase in the number of subjects would be required in order to achieve substantive decreases in the half-width of the CI. In addition, the Agency believes that it is more important to have higher confidence in the precision associated with higher bite rates. Specifically: at a 75% bite-through rate in the control and a percent bite protection of 80% (for the FRACU) the bite rate in the treated cloth material is 15% and it is not desirable to have a large uncertainty in this high rate so we selected a half-width 95% CI of 3% for this high rate. When the bite-through rate in the control is 10% (as is the case for the ACU) and the desired percent bite protection is 80%, the bite rate in the treated cloth material is only 2% and the Agency believes that a larger uncertainty (half width as 6%) at this low rate is acceptable.

The above results are shown in Tables 1 - 5 below. SAS code is provided in the Appendix.

Results of simulation

Table 1: Results of Simulation when Variation between logit values between subjects SD = 1 and True Bite Rate of Control group = 10% and 20%.

True bite-through Rate in control	True Percent Protection	Nr Subs	subject as fixed effect					GEE: subject NOT 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	N*	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile
10	80	5	1000	9.3	10.9	12.4	14.2	489	390.0	15.1	39.1	131.0	998	13.6	16.0	18.1	21.2
		6	1000	8.4	9.7	10.7	12.1	605	25.6	10.8	15.0	23.2	999	11.2	13.0	14.4	16.3
		7	1000	7.6	8.7	9.5	10.4	702	29.2	9.1	10.9	13.9	1000	9.6	11.0	12.0	13.1
		8	1000	7.2	8.1	8.7	9.4	759	13.7	8.6	10.8	14.1	999	8.7	9.9	10.5	11.4
		9	1000	6.7	7.6	8.2	8.7	818	8.7	7.8	9.3	11.3	997	7.9	9.0	9.7	10.2
		10	1000	6.4	7.1	7.6	8.1	862	11.3	7.5	9.1	10.6	999	7.4	8.2	8.8	9.4
		11	1000	6.0	6.6	7.1	7.7	877	6.9	7.2	8.1	9.5	997	6.8	7.5	8.0	8.8
		12	999	5.7	6.3	6.7	7.2	913	11.4	6.7	7.8	8.8	995	6.4	7.1	7.5	8.1
		13	1000	5.5	6.1	6.5	6.8	922	5.6	6.7	7.7	8.5	997	6.1	6.7	7.2	7.6
		14	999	5.3	5.9	6.2	6.5	949	5.5	6.7	7.7	8.7	995	5.9	6.5	6.8	7.2
		15	999	5.1	5.6	5.9	6.2	951	5.6	6.3	7.4	8.4	996	5.6	6.1	6.5	6.8
		16	999	4.9	5.4	5.7	6.1	957	8.4	6.3	7.1	7.8	995	5.4	5.8	6.2	6.6
		17	1000	4.8	5.3	5.6	5.8	980	5.1	6.1	7.0	8.0	990	5.2	5.7	6.0	6.3
		18	1000	4.6	5.0	5.3	5.6	978	5.1	5.9	7.0	7.8	994	5.0	5.4	5.7	6.0
		19	1000	4.5	4.9	5.2	5.4	980	109.8	5.9	6.9	7.8	992	4.9	5.3	5.5	5.8
		20	1000	4.4	4.8	5.0	5.3	986	4.9	5.7	6.7	7.6	990	4.7	5.1	5.3	5.6
10	95	5	999	4.7	5.7	6.6	7.4	905	5.4	4.9	6.1	7.5	987	7.7	9.2	10.8	12.1
		6	999	4.3	5.1	5.7	6.3	970	3.5	4.5	5.6	6.6	998	6.0	7.1	8.0	9.0
		7	999	3.9	4.5	4.9	5.5	980	3.3	4.4	5.2	6.1	997	5.0	5.9	6.5	7.4
		8	998	3.5	4.1	4.5	4.9	997	3.4	4.0	4.9	5.5	997	4.4	5.1	5.6	6.1
		9	999	3.4	3.9	4.3	4.7	997	3.0	3.9	4.6	5.1	999	4.1	4.7	5.2	5.6
		10	1000	3.1	3.6	3.9	4.1	997	2.9	3.6	4.3	4.8	999	3.7	4.2	4.5	4.8
		11	1000	3.0	3.4	3.7	3.9	999	2.8	3.4	4.1	4.7	997	3.4	3.9	4.2	4.5
		12	1000	2.9	3.2	3.5	3.7	998	2.8	3.5	4.0	4.6	996	3.2	3.7	3.9	4.2
		13	1000	2.7	3.1	3.3	3.5	1000	2.6	3.2	3.7	4.2	997	3.0	3.4	3.7	3.9
		14	1000	2.6	3.0	3.1	3.3	1000	2.6	3.1	3.5	4.0	993	2.9	3.3	3.5	3.7
		15	1000	2.5	2.8	3.0	3.2	1000	2.5	3.1	3.5	4.0	997	2.8	3.1	3.3	3.5
		16	999	2.4	2.7	2.9	3.1	1000	2.5	3.0	3.4	3.9	995	2.7	2.9	3.1	3.3
		17	999	2.4	2.6	2.8	3.0	1000	2.4	2.9	3.4	3.8	994	2.6	2.8	3.0	3.2
		18	1000	2.3	2.5	2.7	2.9	1000	2.3	2.8	3.2	3.6	993	2.5	2.7	2.9	3.1

True bite-through Rate in control	True Percent Protection	Nr Subs	subject as fixed effect					GEE: subject NOT 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	N*	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile
		19	999	2.2	2.5	2.6	2.8	999	2.3	2.7	3.2	3.6	998	2.4	2.6	2.8	3.0
		20	997	2.2	2.4	2.5	2.7	1000	2.3	2.7	3.1	3.5	996	2.3	2.6	2.7	2.9
20	80	5	1000	6.8	7.5	8.2	8.8	370	274.7	23.1	111.6	517.4	998	9.7	10.8	11.8	12.8
		6	1000	6.2	6.9	7.3	7.7	458	474.1	9.4	63.4	143.1	998	8.1	9.0	9.7	10.2
		7	1000	5.7	6.3	6.8	7.1	553	50.9	7.8	11.4	58.1	998	7.1	7.8	8.4	8.9
		8	1000	5.3	5.8	6.1	6.5	605	1725.2	6.8	8.7	31.3	998	6.3	7.0	7.4	7.8
		9	1000	5.0	5.5	5.8	6.1	709	19.2	6.6	8.5	14.7	999	5.9	6.5	6.8	7.2
		10	999	4.7	5.1	5.4	5.7	754	16.0	6.6	7.8	10.3	999	5.4	5.9	6.2	6.6
		11	1000	4.5	4.9	5.1	5.4	790	12.0	6.5	7.7	9.4	999	5.1	5.5	5.8	6.1
		12	999	4.3	4.7	4.9	5.1	841	5158.0	6.5	8.1	10.3	996	4.8	5.2	5.4	5.7
		13	1000	4.2	4.5	4.7	4.9	868	6.3	6.4	7.8	9.7	995	4.6	5.0	5.2	5.4
		14	1000	4.0	4.3	4.5	4.7	903	6.1	6.3	7.8	9.2	998	4.4	4.7	4.9	5.2
		15	1000	3.9	4.1	4.3	4.5	937	5.3	6.2	7.6	9.4	996	4.2	4.5	4.7	4.9
		16	1000	3.8	4.0	4.1	4.3	956	5.2	6.3	7.8	9.4	997	4.0	4.3	4.5	4.7
		17	1000	3.7	3.9	4.0	4.2	961	5.8	6.3	7.8	9.4	996	3.9	4.2	4.3	4.5
		18	1000	3.6	3.8	4.0	4.1	972	5.1	6.1	7.7	9.0	997	3.8	4.0	4.2	4.4
		19	1000	3.4	3.7	3.8	3.9	979	5.0	6.1	7.2	8.4	992	3.7	3.9	4.0	4.1
		20	1000	3.4	3.6	3.7	3.8	984	5.0	6.2	7.6	9.1	999	3.6	3.8	3.9	4.0
20	95	5	1000	3.5	4.0	4.4	4.7	887	181.3	4.0	4.9	5.8	991	5.2	6.0	6.7	7.2
		6	999	3.1	3.6	3.9	4.2	944	2.9	3.7	4.6	5.5	999	4.2	4.8	5.2	5.7
		7	999	2.9	3.2	3.5	3.7	960	2.9	3.6	4.6	5.2	999	3.6	4.1	4.4	4.7
		8	1000	2.7	3.0	3.2	3.4	990	2.8	3.5	4.1	5.1	997	3.3	3.6	3.9	4.2
		9	999	2.5	2.8	3.0	3.2	983	2.7	3.4	4.2	5.2	998	3.0	3.3	3.5	3.8
		10	998	2.4	2.6	2.8	3.0	994	2.6	3.2	3.9	4.7	999	2.8	3.0	3.2	3.4
		11	1000	2.3	2.5	2.7	2.8	996	2.6	3.3	4.0	5.0	996	2.6	2.9	3.0	3.2
		12	1000	2.2	2.4	2.5	2.7	995	2.6	3.2	4.0	4.9	997	2.5	2.7	2.8	3.0
		13	1000	2.1	2.3	2.5	2.6	997	2.5	2.9	3.8	5.1	999	2.3	2.6	2.7	2.9
		14	1000	2.0	2.2	2.3	2.4	998	2.4	2.9	3.5	4.4	999	2.2	2.4	2.5	2.6
		15	999	1.9	2.1	2.2	2.3	998	2.3	2.7	3.4	4.4	996	2.1	2.3	2.4	2.5
		16	1000	1.9	2.0	2.1	2.3	999	2.3	2.8	3.5	4.3	999	2.0	2.2	2.3	2.4
		17	999	1.8	2.0	2.1	2.2	1000	2.3	2.8	3.4	4.0	999	2.0	2.1	2.2	2.3
		18	999	1.8	1.9	2.0	2.1	1000	2.2	2.6	3.3	4.1	999	1.9	2.1	2.2	2.3

True bite-through Rate in control	True Percent Protection	Nr Subs	subject as fixed effect					GEE: subject NOT 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	N*	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile
		19	999	1.7	1.9	2.0	2.0	1000	2.2	2.5	3.3	4.0	996	1.8	2.0	2.1	2.2
		20	998	1.7	1.8	1.9	2.0	1000	2.2	2.6	3.3	4.2	999	1.8	1.9	2.0	2.1

True bite-through Rate in control	True Percent Protection	Nr Subs	subject as fixed effect					GEE: subject NOT 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	N*	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile
10	80	5	1000	9.8	11.2	12.1	12.7	880	23.8	12.0	15.0	18.7	991	14.4	16.4	17.8	18.8
		6	1000	8.9	10.0	10.7	11.2	925	10.1	10.4	12.2	14.5	990	11.9	13.4	14.3	14.9
		7	1000	8.1	9.1	9.5	10.1	962	7.7	9.4	10.8	12.5	993	10.3	11.4	12.1	12.8
		8	1000	7.6	8.4	9.0	9.5	980	7.7	8.5	9.9	11.3	990	9.3	10.3	11.0	11.6
		9	1000	7.1	7.9	8.2	8.7	992	6.6	8.0	9.1	10.0	983	8.5	9.3	9.7	10.3
		10	1000	6.8	7.4	7.9	8.2	997	6.3	7.7	8.6	9.4	983	7.9	8.6	9.1	9.5
		11	1000	6.4	7.0	7.4	7.8	998	5.9	7.1	7.9	8.8	977	7.3	7.9	8.4	8.9
		12	1000	6.1	6.7	7.0	7.3	1000	5.7	6.8	7.5	8.2	980	6.9	7.5	7.9	8.2
		13	1000	5.9	6.4	6.7	7.0	1000	5.5	6.6	7.2	7.9	980	6.6	7.1	7.5	7.7
		14	1000	5.7	6.2	6.5	6.7	1000	5.4	6.5	7.1	7.6	978	6.3	6.8	7.1	7.4
		15	1000	5.4	5.9	6.2	6.4	1000	5.2	6.1	6.7	7.2	976	6.0	6.5	6.8	7.0
		16	1000	5.3	5.7	5.9	6.1	1000	5.0	5.9	6.5	6.9	988	5.7	6.2	6.5	6.7
		17	1000	5.1	5.5	5.8	5.9	1000	4.9	5.7	6.2	6.6	962	5.5	6.0	6.2	6.4
		18	1000	5.0	5.3	5.6	5.8	1000	4.8	5.5	6.1	6.5	971	5.3	5.7	6.0	6.2
		19	1000	4.8	5.2	5.4	5.6	1000	4.7	5.4	5.9	6.3	963	5.2	5.6	5.8	6.0
		20	1000	4.7	5.0	5.3	5.4	1000	4.5	5.3	5.6	6.1	975	5.0	5.4	5.6	5.8
10	95	5	1000	5.0	5.9	6.4	6.9	903	4.4	5.6	7.0	8.1	990	8.2	9.4	10.2	10.8
		6	1000	4.5	5.2	5.7	6.1	957	4.7	5.1	6.2	7.1	988	6.4	7.3	7.9	8.5
		7	1000	4.1	4.7	5.1	5.4	984	4.2	4.7	5.5	6.3	991	5.4	6.2	6.6	7.0
		8	1000	3.8	4.3	4.7	4.9	992	3.4	4.3	5.0	5.7	994	4.7	5.4	5.8	6.1
		9	1000	3.6	4.1	4.4	4.7	995	3.3	4.0	4.7	5.3	985	4.3	4.9	5.2	5.6
		10	1000	3.3	3.8	4.0	4.2	999	3.0	3.8	4.2	4.7	988	3.9	4.4	4.7	5.0
		11	1000	3.2	3.6	3.8	4.0	1000	2.9	3.6	4.0	4.4	985	3.7	4.2	4.4	4.6
		12	1000	3.0	3.4	3.7	3.9	1000	2.9	3.5	3.9	4.3	985	3.4	3.9	4.1	4.3
		13	1000	2.9	3.2	3.4	3.6	1000	2.7	3.3	3.7	3.9	970	3.2	3.6	3.9	4.0
		14	1000	2.8	3.1	3.3	3.4	1000	2.6	3.1	3.5	3.8	977	3.1	3.4	3.6	3.8
		15	1000	2.7	2.9	3.1	3.3	1000	2.5	3.0	3.4	3.6	979	2.9	3.2	3.5	3.6
		16	1000	2.6	2.8	3.0	3.2	1000	2.4	2.9	3.2	3.4	968	2.8	3.1	3.3	3.4
		17	1000	2.5	2.7	2.9	3.0	1000	2.4	2.8	3.1	3.3	970	2.7	3.0	3.1	3.3
		18	1000	2.4	2.7	2.8	2.9	1000	2.3	2.7	2.9	3.1	966	2.6	2.9	3.0	3.2
		19	1000	2.4	2.6	2.7	2.8	1000	2.2	2.6	2.9	3.1	961	2.5	2.8	2.9	3.1

		20	1000	2.3	2.5	2.7	2.8	1000	2.2	2.6	2.8	3.1	956	2.4	2.7	2.8	3.0
20	80	5	1000	6.7	7.4	7.8	8.3	812	8.1	8.2	10.1	18.8	993	9.7	10.6	11.3	11.9
		6	1000	6.1	6.7	7.0	7.3	891	789E55	7.2	8.9	13.5	990	8.1	8.8	9.2	9.6
		7	1000	5.6	6.1	6.4	6.6	947	7.5	6.7	7.9	10.2	991	7.1	7.6	8.0	8.3
		8	1000	5.2	5.6	5.8	6.1	969	5.4	6.1	6.8	7.6	989	6.3	6.8	7.1	7.3
		9	1000	4.9	5.3	5.5	5.7	989	3E7	5.6	6.3	7.1	991	5.8	6.3	6.5	6.8
		10	1000	4.7	5.0	5.2	5.4	996	4.5	5.3	5.8	6.3	988	5.4	5.8	6.0	6.2
		11	1000	4.5	4.7	4.9	5.1	994	4.2	5.0	5.6	6.0	986	5.1	5.4	5.6	5.7
		12	1000	4.3	4.5	4.7	4.8	999	4.1	4.9	5.3	5.7	982	4.8	5.1	5.3	5.4
		13	1000	4.1	4.3	4.5	4.6	999	3.9	4.6	5.0	5.4	974	4.6	4.8	5.0	5.1
		14	1000	3.9	4.2	4.3	4.5	1000	3.7	4.4	4.9	5.2	982	4.3	4.6	4.7	4.9
		15	1000	3.8	4.0	4.1	4.2	1000	3.6	4.2	4.5	4.8	982	4.2	4.4	4.5	4.6
		16	1000	3.7	3.9	4.0	4.1	1000	3.5	4.1	4.5	4.8	983	4.0	4.2	4.3	4.4
		17	1000	3.6	3.8	3.9	4.0	999	3.5	4.0	4.3	4.5	979	3.9	4.1	4.2	4.3
		18	1000	3.5	3.7	3.8	3.9	1000	3.3	3.9	4.2	4.4	975	3.7	3.9	4.0	4.1
		19	1000	3.4	3.5	3.6	3.7	1000	3.3	3.8	4.0	4.3	971	3.6	3.8	3.9	4.0
		20	1000	3.3	3.4	3.5	3.6	1000	3.2	3.7	4.0	4.2	980	3.5	3.7	3.8	3.9
20	95	5	1000	3.4	3.8	4.1	4.3	896	445271	3.8	4.6	5.5	994	5.1	5.7	6.1	6.4
		6	1000	3.0	3.4	3.7	3.8	963	242.3	3.3	3.9	4.4	990	4.1	4.6	4.9	5.2
		7	1000	2.7	3.1	3.3	3.4	986	2.7	3.2	3.7	4.2	986	3.5	3.9	4.1	4.4
		8	1000	2.6	2.9	3.0	3.2	990	2.3	2.9	3.3	3.6	990	3.2	3.5	3.7	3.9
		9	1000	2.4	2.7	2.8	2.9	999	2.3	2.7	3.1	3.4	986	2.9	3.2	3.4	3.4
		10	1000	2.3	2.5	2.6	2.8	1000	2.2	2.5	2.9	3.1	984	2.7	2.9	3.1	3.2
		11	1000	2.2	2.4	2.5	2.6	999	2.0	2.5	2.8	3.1	984	2.5	2.7	2.9	3.0
		12	1000	2.1	2.3	2.4	2.4	1000	2.0	2.4	2.6	2.9	980	2.3	2.5	2.7	2.8
		13	1000	2.0	2.2	2.3	2.4	1000	1.9	2.2	2.5	2.7	981	2.2	2.4	2.6	2.7
		14	1000	1.9	2.1	2.2	2.3	1000	1.8	2.2	2.4	2.6	977	2.1	2.3	2.4	2.5
		15	1000	1.9	2.0	2.1	2.2	1000	1.8	2.1	2.3	2.5	980	2.0	2.2	2.3	2.4
		16	1000	1.8	1.9	2.0	2.1	1000	1.7	2.0	2.2	2.4	988	2.0	2.1	2.2	2.3
		17	1000	1.7	1.9	1.9	2.0	1000	1.7	2.0	2.1	2.3	983	1.9	2.0	2.1	2.2
		18	1000	1.7	1.8	1.9	1.9	1000	1.6	1.9	2.1	2.2	972	1.8	2.0	2.0	2.1
		19	1000	1.6	1.8	1.8	1.9	1000	1.6	1.8	2.0	2.1	981	1.8	1.9	2.0	2.0
		20	1000	1.6	1.7	1.8	1.8	1000	1.5	1.8	1.9	2.0	978	1.7	1.8	1.9	1.9

*: the number of datasets were analyzable by the model.

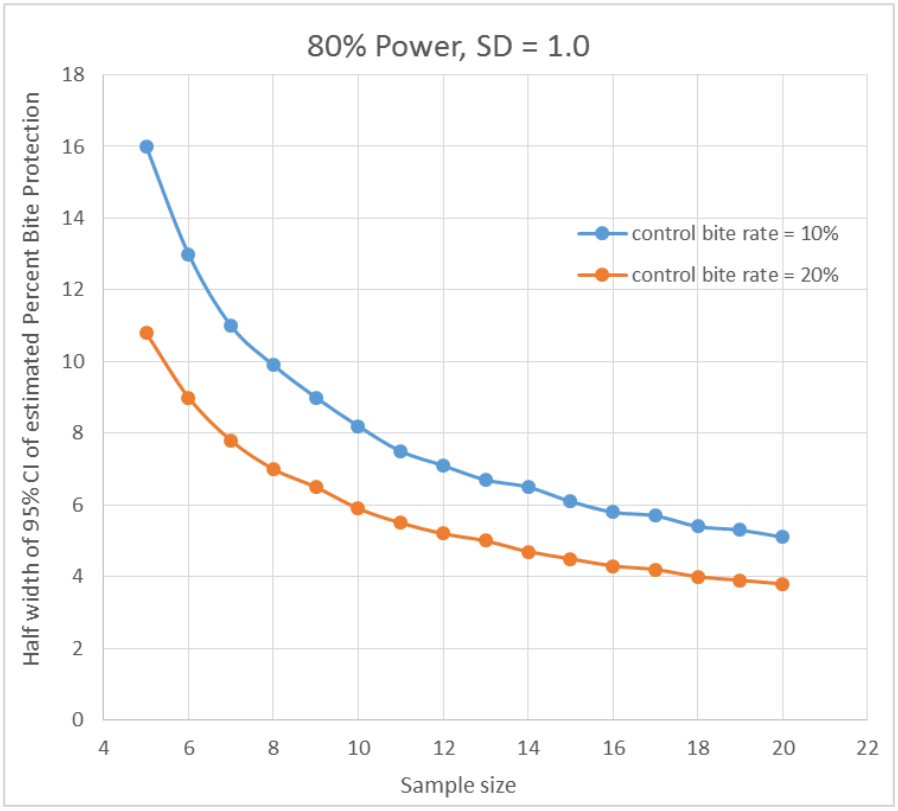

Table 2: Results of Simulation when Variation between logit values between subjects SD = 0.3 and True Bite Rate of Control group = 10% and 20%.

*: the number of datasets were analyzable by the model.

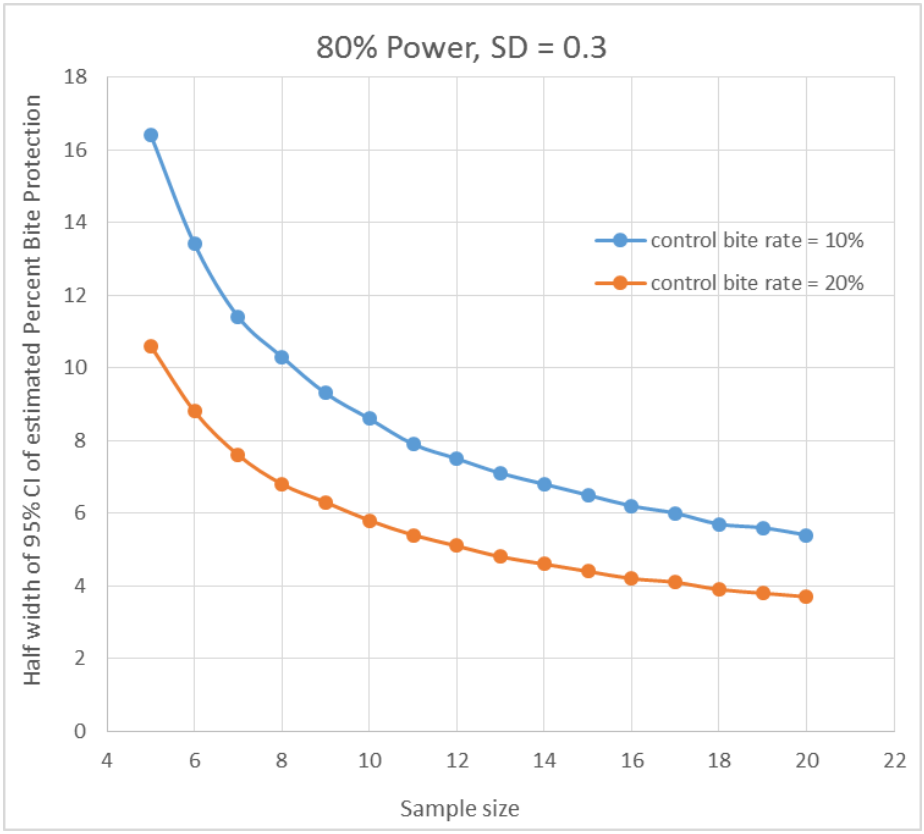
80% Power, SD = 0.3
Half width of 95% CI of estimated Percent Bite Protection
Sample size
control bite rate = 10%
control bite rate = 20%
0
2
4
6
8
10
12
14
16
18
4
6
8
10
12
14
16
18
20
22

Table 3: Results of Simulation when Variation between logit values between subjects SD = 1 and True Bite Rate of Control group = 50%, 75%, and 90%.

True bite-through Rate in control			GENMOD: subject as fixed effect					GENMOD: subject NOT as fixed effect					GLIMMIX: subject as random effect				
	True Percent Protection	Nr Subs	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	N*	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile
50	80	5	1000	4.5	4.9	5.2	5.4	178	341576	119.1	375.3	1003.7	995	6.4	6.8	7.2	7.5
		6	1000	4.1	4.4	4.7	4.9	255	551.0	49.6	228.4	445.3	988	5.3	5.7	6.0	6.2
		7	1000	3.9	4.1	4.3	4.6	297	54.9	9.8	74.3	258.1	984	4.7	5.0	5.3	5.5
		8	1000	3.6	3.9	4.0	4.3	426	1.48E6	10.2	55.4	203.5	983	4.3	4.6	4.7	4.9
		9	1000	3.4	3.6	3.8	4.0	512	2126E9	9.2	15.8	86.6	980	3.9	4.2	4.3	4.5
		10	998	3.2	3.4	3.6	3.7	626	5526.3	9.2	13.0	24.0	973	3.7	3.9	4.0	4.1
		15	982	2.7	2.8	2.9	3.0	931	7.0	8.3	9.9	11.5	938	2.8	3.0	3.1	3.1
		20	964	2.3	2.4	2.5	2.5	993	5.7	7.0	8.3	9.3	921	2.4	2.5	2.6	2.6
	95	5	1000	2.4	2.7	2.9	3.1	745	4.6	3.9	4.9	6.3	995	3.5	3.9	4.1	4.4
		6	1000	2.2	2.4	2.6	2.8	856	454.5	4.0	5.2	6.4	996	2.9	3.2	3.4	3.6
		7	1000	2.0	2.2	2.4	2.5	916	4.5	4.2	5.5	6.8	995	2.5	2.8	3.0	3.1
		8	1000	1.9	2.1	2.2	2.4	969	3.4	4.4	5.8	7.3	989	2.3	2.5	2.7	2.8
		9	1000	1.8	2.0	2.1	2.3	978	3.5	4.4	6.2	8.0	994	2.1	2.3	2.5	2.6
		10	1000	1.7	1.9	2.0	2.1	985	3.1	4.1	5.3	6.8	990	2.0	2.1	2.2	2.4
		15	1000	1.4	1.5	1.6	1.6	999	2.8	3.6	4.7	5.7	984	1.5	1.6	1.7	1.8
		20	999	1.2	1.3	1.3	1.4	1000	2.5	3.2	4.0	4.8	976	1.3	1.4	1.4	1.5
75	80	5	998	3.6	3.9	4.1	4.3	125	191E11	26.1	173.1	1466.0	955	5.0	5.3	5.5	5.7
		6	995	3.3	3.5	3.7	3.9	233	347E8	21.2	100.9	421.2	945	4.2	4.5	4.6	4.8
		7	990	3.1	3.3	3.4	3.6	364	220.6	17.5	23.4	141.7	912	3.7	3.9	4.1	4.2
		8	985	2.8	3.0	3.1	3.3	495	9.97E6	14.4	17.5	26.9	898	3.3	3.5	3.6	3.7
		9	982	2.7	2.9	3.0	3.1	612	12.3	12.9	15.0	18.2	894	3.1	3.3	3.4	3.4
		10	967	2.6	2.7	2.8	2.9	750	3782.7	12.2	14.4	17.0	885	2.9	3.0	3.1	3.2
		15	915	2.1	2.2	2.3	2.3	979	7.5	9.1	10.1	11.3	817	2.2	2.3	2.4	2.4
		20	831	1.8	1.9	1.9	2.0	999	6.3	7.6	8.3	8.9	717	1.9	2.0	2.0	2.0
	95	5	1000	2.0	2.3	2.4	2.6	616	80.4	4.7	6.7	10.2	982	2.8	3.2	3.4	3.7
		6	1000	1.8	2.0	2.2	2.3	739	104E8	5.1	7.6	10.2	984	2.4	2.7	2.9	3.0
		7	1000	1.7	1.9	2.1	2.2	861	4.4	5.8	8.5	10.5	980	2.1	2.4	2.5	2.7
		8	1000	1.6	1.8	1.9	2.0	937	4.7	5.3	7.4	9.2	965	1.9	2.1	2.2	2.4
		9	1000	1.5	1.7	1.8	1.9	970	5.0	5.4	6.9	8.6	978	1.8	1.9	2.1	2.2
		10	1000	1.4	1.6	1.7	1.7	989	3.7	5.0	6.4	7.5	982	1.6	1.8	1.9	2.0
		15	999	1.2	1.3	1.3	1.4	1000	3.2	4.2	5.1	5.9	951	1.3	1.4	1.4	1.5
		20	987	1.0	1.1	1.1	1.2	1000	2.9	3.7	4.5	5.3	946	1.1	1.1	1.2	1.2
90	80	5	995	3.1	3.3	3.5	3.6	295	401E12	20.8	24.1	43.0	834	4.2	4.5	4.7	4.9
		6	986	2.8	3.0	3.1	3.3	469	34657	17.8	21.3	24.6	802	3.5	3.8	3.9	4.1
		7	967	2.6	2.8	2.9	3.0	628	1.98E7	15.6	18.0	19.8	767	3.1	3.3	3.4	3.5
		8	947	2.4	2.6	2.7	2.8	765	15.3	13.9	15.9	18.0	723	2.8	3.0	3.1	3.2
		9	911	2.3	2.4	2.5	2.6	854	10.0	12.7	14.5	16.1	698	2.6	2.7	2.8	2.9
		10	896	2.2	2.3	2.4	2.4	912	9.6	11.7	13.4	14.8	677	2.4	2.6	2.6	2.7
		15	766	1.8	1.9	1.9	2.0	993	7.7	9.5	10.3	11.1	517	1.9	2.0	2.0	2.0
		20	683	1.5	1.6	1.6	1.7	1000	6.8	8.0	8.8	9.3	411	1.6	1.7	1.7	1.7
	95	5	1000	1.8	2.0	2.2	2.4	544	122E15	7.8	11.8	16.4	888	2.5	2.8	3.0	3.2
		6	1000	1.6	1.9	2.0	2.1	715	6.4	7.5	10.1	13.1	877	2.1	2.4	2.6	2.7
		7	1000	1.5	1.7	1.8	2.0	844	4.6	6.5	8.5	10.5	853	1.8	2.1	2.2	2.3
		8	1000	1.4	1.6	1.7	1.8	910	32.4	6.3	7.7	9.2	836	1.7	1.9	2.0	2.1
		9	1000	1.3	1.5	1.6	1.7	961	4.2	5.6	7.3	8.9	846	1.5	1.7	1.8	1.9
		10	998	1.3	1.4	1.5	1.6	982	4.0	5.5	6.7	7.9	810	1.4	1.6	1.7	1.7
		15	989	1.0	1.1	1.2	1.3	1000	3.4	4.4	5.3	6.3	730	1.1	1.2	1.3	1.3
		20	983	0.9	1.0	1.0	1.1	1000	3.0	3.8	4.4	5.1	684	0.9	1.0	1.0	1.1

*: the number of datasets were analyzable by the model.

Table 4: Results of Simulation when Variation between logit values between subjects SD = 0.3 and True Bite Rate of Control group = 50%, 75%, and 90%.

True bite-through Rate in control	True Percent Protection	Nr Subs	GENMOD: subject as fixed effect					GENMOD: subject NOT 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	N*	Half Width Mean	Half Width 80th %-tile	Half Width 90th %-tile	Half Width 95th %-tile
50	80	5	1000	4.0	4.2	4.3	4.4	657	2.15E7	5.6	17.9	61.1	984	5.7	6.0	6.2	6.3
		6	1000	3.7	3.8	3.9	4.0	773	81149	4.9	6.8	38.7	976	4.8	5.0	5.1	5.2
		7	1000	3.4	3.5	3.6	3.7	842	5.5	4.4	5.2	11.8	978	4.2	4.4	4.5	4.6
		8	1000	3.2	3.3	3.4	3.4	901	5.3	4.1	4.7	5.6	974	3.8	4.0	4.1	4.1
		9	1000	3.0	3.1	3.2	3.2	947	4.2	3.7	4.2	4.6	972	3.5	3.6	3.7	3.8
		10	1000	2.8	2.9	3.0	3.0	962	37433	3.6	4.0	4.5	982	3.3	3.4	3.4	3.5
		15	1000	2.3	2.4	2.4	2.5	998	2.5	2.9	3.2	3.5	975	2.5	2.6	2.6	2.7
		20	1000	2.0	2.1	2.1	2.1	1000	2.2	2.5	2.7	2.9	975	2.1	2.2	2.2	2.2
	95	5	1000	2.0	2.2	2.3	2.4	860	13.4	2.4	2.9	3.4	981	3.0	3.2	3.3	3.5
		6	1000	1.9	2.0	2.1	2.2	941	645E11	2.1	2.4	2.7	987	2.5	2.7	2.8	2.9
		7	1000	1.7	1.8	1.9	2.0	979	13.9	2.0	2.3	2.5	978	2.2	2.3	2.4	2.5
		8	1000	1.6	1.7	1.8	1.8	985	1.7	1.9	2.1	2.3	979	1.9	2.1	2.1	2.2
		9	1000	1.5	1.6	1.7	1.7	996	1.5	1.8	2.0	2.2	974	1.8	1.9	2.0	2.0
		10	1000	1.4	1.5	1.6	1.6	999	1.4	1.7	1.8	2.0	970	1.6	1.7	1.8	1.8
		15	1000	1.2	1.2	1.3	1.3	1000	1.2	1.4	1.5	1.6	967	1.3	1.3	1.4	1.4
		20	1000	1.0	1.0	1.1	1.1	1000	1.0	1.2	1.3	1.4	961	1.1	1.1	1.1	1.2
75	80	5	1000	3.1	3.2	3.3	3.3	554	12E55	5.2	19.2	69.5	915	4.4	4.5	4.6	4.7
		6	1000	2.8	2.9	3.0	3.0	683	2.15E8	4.7	9.4	38.9	899	3.7	3.8	3.9	4.0
		7	1000	2.6	2.7	2.7	2.8	776	6.9	4.4	5.2	20.2	907	3.3	3.4	3.4	3.5
		8	1000	2.5	2.5	2.6	2.6	843	4984.8	4.0	5.0	19.8	887	3.0	3.0	3.1	3.1
		9	1000	2.3	2.4	2.4	2.4	892	4.6	3.8	4.3	5.0	893	2.7	2.8	2.8	2.9
		10	1000	2.2	2.3	2.3	2.3	940	116.9	3.6	4.0	4.6	878	2.5	2.6	2.6	2.7
		15	1000	1.8	1.8	1.9	1.9	998	2.5	2.9	3.2	3.4	850	2.0	2.0	2.0	2.0
		20	1000	1.6	1.6	1.6	1.6	1000	2.2	2.5	2.7	2.9	785	1.6	1.7	1.7	1.7
	95	5	1000	1.6	1.7	1.8	1.9	779	5633.9	2.0	2.6	3.1	949	2.4	2.5	2.6	2.7
		6	1000	1.5	1.6	1.6	1.7	907	3.8	1.9	2.2	2.4	936	2.0	2.1	2.2	2.2
		7	1000	1.4	1.5	1.5	1.5	942	54.3	1.8	2.1	2.2	934	1.7	1.8	1.9	1.9
		8	1000	1.3	1.4	1.4	1.4	975	2.17E7	1.7	1.9	2.1	912	1.6	1.6	1.7	1.7
		9	1000	1.2	1.3	1.3	1.3	985	1.4	1.6	1.8	2.0	911	1.4	1.5	1.5	1.6
		10	1000	1.2	1.2	1.2	1.3	995	1.3	1.5	1.7	1.8	912	1.3	1.4	1.4	1.5
		15	1000	0.9	1.0	1.0	1.0	1000	1.0	1.2	1.4	1.4	854	1.0	1.1	1.1	1.1
		20	1000	0.8	0.8	0.9	0.9	1000	0.9	1.1	1.1	1.2	879	0.9	0.9	0.9	0.9
90	80	5	1000	2.7	2.8	2.9	2.9	653	4.03E9	5.7	6.7	12.2	728	3.9	4.0	4.1	4.1
		6	1000	2.5	2.6	2.6	2.6	767	5.66E8	4.9	5.7	6.7	677	3.2	3.4	3.4	3.5
		7	1000	2.3	2.4	2.4	2.4	834	1.81E8	4.5	5.3	6.2	641	2.9	3.0	3.0	3.0
		8	1000	2.1	2.2	2.2	2.3	914	2.55E9	4.2	4.7	5.2	623	2.6	2.7	2.7	2.7
		9	1000	2.0	2.1	2.1	2.1	934	86.8	3.9	4.5	4.9	579	2.4	2.5	2.5	2.5
		10	1000	1.9	2.0	2.0	2.0	975	6.6	3.7	4.2	4.5	559	2.2	2.3	2.3	2.3
		15	1000	1.6	1.6	1.6	1.6	999	2.6	3.1	3.3	3.6	424	1.7	1.8	1.8	1.8
		20	1000	1.4	1.4	1.4	1.4	1000	2.3	2.6	2.8	2.9	350	1.4	1.5	1.5	1.5
	95	5	1000	1.5	1.6	1.6	1.7	768	3.6	2.1	2.4	2.7	669	2.1	2.3	2.3	2.4
		6	1000	1.4	1.4	1.5	1.5	866	3.1	2.0	2.2	2.6	616	1.8	1.9	2.0	2.0
		7	1000	1.2	1.3	1.4	1.4	926	6841.3	1.8	2.1	2.3	543	1.6	1.7	1.7	1.7
		8	1000	1.2	1.2	1.3	1.3	958	18.5	1.7	1.9	2.1	474	1.4	1.5	1.5	1.6
		9	1000	1.1	1.2	1.2	1.2	984	2.3	1.6	1.8	1.9	428	1.3	1.4	1.4	1.4
		10	1000	1.0	1.1	1.1	1.1	992	1.2	1.5	1.7	1.8	424	1.2	1.3	1.3	1.3
		15	1000	0.8	0.9	0.9	0.9	1000	1.0	1.2	1.3	1.4	259	0.9	1.0	1.0	1.0
		20	1000	0.7	0.8	0.8	0.8	1000	0.9	1.1	1.1	1.2	233	0.8	0.8	0.8	0.8

*: the number of datasets that were analyzable by the model.

Table 5: Results of Simulation when Variation between logit values between subjects SD = 0 and True Bite Rate of Control group = 50%, 75%, and 90%.

True bite-through Rate in control	True Percent Protection	Nr Subs	GENMOD: subject as fixed effect				GENMOD: subject NOT as fixed effect				GLIMMIX: subject as random effect			
			N*	Half Width Mean	Half Width 90th %-tile	Half Width 95th %-tile	N*	Half Width Mean	Half Width 90th %-tile	Half Width 95th %-tile	N*	Half Width Mean	Half Width 90th %-tile	Half Width 95th %-tile
50	80	5	1000	4.0	4.3	4.4	952	25.8	5.6	12.7	982	5.6	6.1	6.2
		6	1000	3.6	3.8	3.9	978	4.3	4.6	5.3	954	4.7	5.1	5.2
		7	1000	3.3	3.5	3.6	993	3.2	4.2	4.5	892	4.2	4.4	4.5
		8	1000	3.1	3.3	3.4	997	4.0	3.8	4.1	853	3.8	4.0	4.1
		9	1000	2.9	3.1	3.2	1000	2.7	3.6	3.9	806	3.5	3.6	3.7
		10	1000	2.8	2.9	3.0	1000	2.6	3.4	3.6	788	3.2	3.4	3.4
		15	1000	2.3	2.4	2.4	1000	2.1	2.7	2.8	848	2.5	2.6	2.6
		20	1000	2.0	2.0	2.1	1000	1.9	2.3	2.4	905	2.1	2.2	2.2
	95	5	1000	2.0	2.3	2.4	911	58.2	2.7	3.4	993	2.9	3.3	3.4
		6	1000	1.8	2.0	2.1	969	2.6	2.4	2.6	991	2.4	2.7	2.8
		7	1000	1.7	1.9	1.9	992	2.1	2.2	2.4	968	2.1	2.4	2.4
		8	1000	1.6	1.7	1.8	998	1.4	1.9	2.2	928	1.9	2.1	2.1
		9	1000	1.5	1.6	1.7	1000	1.4	1.8	2.0	912	1.8	1.9	2.0
		10	1000	1.4	1.5	1.6	1000	3.0	1.7	1.9	890	1.6	1.8	1.8
		15	1000	1.1	1.2	1.3	1000	1.1	1.4	1.5	907	1.2	1.3	1.4
		20	1000	1.0	1.1	1.1	1000	0.9	1.2	1.2	930	1.0	1.1	1.1
75	80	5	1000	3.0	3.2	3.2	952	7479.1	4.3	12.1	828	4.3	4.6	4.6
		6	1000	2.8	2.9	3.0	989	3.0	3.6	3.9	799	3.6	3.8	3.9
		7	1000	2.6	2.7	2.7	991	2.9	3.3	3.6	771	3.2	3.3	3.4
		8	1000	2.4	2.5	2.5	999	2.3	3.0	3.3	707	2.9	3.0	3.1
		9	1000	2.3	2.4	2.4	998	2.1	2.8	3.0	707	2.7	2.8	2.8
		10	1000	2.2	2.2	2.3	999	2.0	2.6	2.8	667	2.5	2.6	2.6
		15	1000	1.8	1.8	1.8	1000	1.7	2.1	2.2	645	1.9	2.0	2.0
		20	1000	1.5	1.6	1.6	1000	1.5	1.8	1.9	634	1.6	1.7	1.7
	95	5	1000	1.6	1.8	1.8	930	31.2	2.2	2.7	897	2.3	2.5	2.6
		6	1000	1.5	1.6	1.6	972	1.7	1.9	2.1	848	1.9	2.1	2.2
		7	1000	1.3	1.5	1.5	988	1.3	1.7	1.9	795	1.7	1.8	1.9
		8	1000	1.3	1.4	1.4	998	1.2	1.6	1.7	784	1.5	1.7	1.7
		9	1000	1.2	1.3	1.3	999	1.1	1.5	1.6	759	1.4	1.5	1.5
		10	1000	1.1	1.2	1.2	999	1.0	1.4	1.5	733	1.3	1.4	1.4
		15	1000	0.9	1.0	1.0	1000	0.9	1.1	1.2	692	1.0	1.1	1.1
		20	1000	0.8	0.8	0.8	1000	0.8	0.9	1.0	663	0.8	0.9	0.9
90	80	5	1000	2.7	2.8	2.8	950	4.5	3.8	8.3	707	3.8	4.0	4.0
		6	1000	2.4	2.6	2.6	983	5.0	3.0	3.4	652	3.2	3.4	3.4
		7	1000	2.3	2.3	2.4	994	2.2	2.8	3.0	637	2.8	2.9	3.0
		8	1000	2.1	2.2	2.2	997	2.0	2.7	2.9	608	2.6	2.7	2.7
		9	1000	2.0	2.1	2.1	999	1.8	2.4	2.6	605	2.4	2.4	2.5
		10	1000	1.9	2.0	2.0	1000	1.8	2.3	2.5	611	2.2	2.3	2.3
		15	1000	1.5	1.6	1.6	1000	1.5	1.8	2.0	564	1.7	1.7	1.8
		20	1000	1.3	1.4	1.4	1000	1.3	1.6	1.6	566	1.4	1.5	1.5
	95	5	1000	1.4	1.6	1.6	928	2.0	1.9	2.2	714	2.1	2.3	2.3
		6	1000	1.3	1.4	1.5	974	1.5	1.7	1.9	669	1.7	1.9	1.9
		7	1000	1.2	1.3	1.3	985	1.3	1.5	1.7	642	1.5	1.6	1.7
		8	1000	1.1	1.2	1.2	996	1.0	1.4	1.6	629	1.4	1.5	1.5
		9	1000	1.1	1.2	1.2	999	1.0	1.3	1.4	602	1.3	1.4	1.4
		10	1000	1.0	1.1	1.1	1000	1.0	1.3	1.4	634	1.2	1.2	1.3
		15	1000	0.8	0.9	0.9	1000	0.8	1.0	1.1	526	0.9	1.0	1.0
		20	1000	0.7	0.8	0.8	1000	0.7	0.9	0.9	579	0.8	0.8	0.8

*: the number of datasets that were analyzable by the model.

APPENDIX

SAS codes

```
*================================================================================*
* Programmers:                                                                   *
*             - Dr. Larry R. Holden originally developed the SAS program to      *
*               calculate the sample size for Etofenprox study for the 4/2015 HSRB   *
*               meeting.  In his program, for generating the random data,        *
*               Dr. Holden set the SD = 0.3 for the variation of logits          *
*               between subjects.  Dr. Holden assumed subject                    *
*               as fixed effect in the SAS PROC GENMOD and used the average of the   *
*               half widths of 95% CIs to derive the sample size.                *
*                                                                                *
*             - James Nguyen/EPA modified Dr. Holden's program by adding the     *
*               variation in logits between subjects SD = 1.  James also added the   *
*               SAS PROC GLIMMIX model where subject was considered as random       *
*               effect and used the 80th %-tile (instead the average as Dr.      *
*               Holden did) of the half widths of 95% CIs to derive the sample   *
*               size in addition to 90th & 95th percentiles as a sensitivity     *
*               analysis.                                                        *
*================================================================================*;

Options ls=90 ps=57 Nonumber Nodate;

%Let NrSims = 1000;   * Number of iterations/datasets;

%Let mPop = 200;      * Mean mosquito pop size;

%Let NrProts=2;  %Let Prots = 80 95; * True percent Protection;

%Let Seed = 98135187; * Fixed random number seed for reproducibility;

*--------------------------------------------------------------------------------;

%Macro GenTest(Group,TruPct);
              ** Generate and random population size and binomial NrBites for a single test;
       Group = "&Group";
       TruPea = &TruPct/100;
       Pop = RanPoi(Seed,&mPop);                              ** population size;
       Q = Log( TruPea / (1 - TruPea) ) + SubEff;   ** convert to logit scale & add Sub RE;
       SubPea = exp(Q)/(1 + exp(Q) );               ** convert logit back to probability;
       NrBites = RanBin(Seed,Pop,SubPea);           ** generate Nr 'bites';
       Output;
%Mend GenTest;

%Macro MainRun;
       Data Simmer;
              Retain Seed &Seed;
              Length Group $10;
              Array Rates(&NrRates) _Temporary_ (&Rates);
              Array Prots(&NrProts) _Temporary_ (&Prots);

              Do ka = 1 to &NrRates;
                     Rate_C = Rates(ka);
                     Do kb = 1 to &NrProts;
                            Prot = Prots(kb);
                            Rate_T = Rate_C*(1 - Prot/100);

                            Do NrSubs = &nmin to &nmax;
                                   Do Sim = 1 to &NrSims;
                                          Do Sub = 1 to NrSubs;

                                                 SubEff = RanNor(Seed)*&sdSub ;
                                                 %GenTest(C,Rate_C);
                                                 %GenTest(T,Rate_T);

                                          End; *Sub;
```

```
                                        End; *Sim;
                                End; *NrSubs;
                        End; *Prot;
                End; *Rate_C;

                Keep Rate_C Prot Rate_T NrSubs Sim Sub Group Pop NrBites;
        Run;

        dm log 'clear';

        Data EstOut; set _NULL_; run;
        Data EstOut1; set _NULL_; run;
        Data EstOut2; set _NULL_; run;

        %do i = 1 %to &NrRates;
                %do j = &nmin %to &nmax;
                        Data Simmer_&i._&j;
                                set Simmer;
                                if Rate_C = %nrbquote(%scan(&Rates,&i, %str( ))) and NrSubs = &j;
                        run;

                        Proc Sort Data=Simmer_&i._&j;
                                By Rate_C Prot NrSubs Sim Sub Group;
                        Run;

                        ODS Listing Close;
                        proc genmod data=Simmer_&i._&j;
                                By Rate_C Prot NrSubs Sim;
                          Class Sub Group;
                          Model NrBites/Pop = Sub Group  / Dist=binomial Link=Log;
                          Estimate 'T/C' Group -1 1;
                          ODS Output Estimates=EstOut_&i._&j;
                         Run;
                        ODS Listing;
                        dm log 'clear';

                        Data EstOut;
                                set EstOut EstOut_&i._&j;
                        run;

                        proc genmod data=Simmer_&i._&j;
                                By Rate_C Prot NrSubs Sim;
                          Class Sub Group;
                          Model NrBites/Pop = Group  / Dist=binomial Link=Log;
                          repeated subject=Sub/type=exch;
                          Estimate 'T/C' Group -1 1;
                          ODS Output Estimates=EstOut1_&i._&j;
                         Run;
                        ODS Listing;
                        dm log 'clear';

                        Data EstOut1;
                                set EstOut1 EstOut1_&i._&j;
                        run;

                         Proc GLIMMIX data = Simmer_&i._&j;
                                By Rate_C Prot NrSubs Sim;
                                Class Sub Group;
                                Model NrBites/Pop = Group  / Dist=binomial Link=Log;
                                random Sub;
                                Estimate 'T/C' Group -1 1/exp cl;
                                ODS Output Estimates=EstOut2_&i._&j;
                         run;
                        ODS Listing;

                        dm log 'clear';

                        Data EstOut2;
                                set EstOut2 EstOut2_&i._&j;
                        run;

                        Proc datasets nolist; delete Simmer_&i._&j  EstOut_&i._&j
EstOut1_&i._&j   EstOut2_&i._&j; quit;
```

```
		%end;
	%end;

	Proc sort data = EstOut; By Rate_C Prot NrSubs; run;
	Proc sort data = EstOut1; By Rate_C Prot NrSubs; run;
	Proc sort data = EstOut2; By Rate_C Prot NrSubs; run;

	Data EstOut;
		Set EstOut;
		PctProt = 100*(1 - MeanEstimate);
		LoCL = 100*(1-MeanUpperCL);
		HiCL = 100*(1-MeanLowerCL);
		HalfWidth = (HiCL - LoCL)/2;
	Run;

	Proc Univariate NoPrint Data=EstOut;
		By Rate_C Prot NrSubs;
		Var PctProt HalfWidth;
		Output Out=Statz Mean=PctProt_Mean HalfWidth_Mean  N=N
			pctlpts  = 80 90 95 pctlpre = PctProt_ HalfWidth_;
	Run;


	Data EstOut1;
		Set EstOut1;
		PctProt = 100*(1 - MeanEstimate);
		LoCL = 100*(1-MeanUpperCL);
		HiCL = 100*(1-MeanLowerCL);
		HalfWidth = (HiCL - LoCL)/2;
	Run;

	Proc Univariate NoPrint Data=EstOut1;
		By Rate_C Prot NrSubs;
		Var PctProt HalfWidth;
		Output Out=Statz1 Mean=PctProt_Mean HalfWidth_Mean  N=N
			pctlpts  = 80 90 95 pctlpre = PctProt_ HalfWidth_;
	Run;


	Data EstOut2;
		Set EstOut2;
		PctProt = 100*(1 - ExpEstimate);
		LoCL = 100*(1-ExpUpper);
		HiCL = 100*(1-ExpLower);
		HalfWidth = (HiCL - LoCL)/2;
	Run;

	Proc Univariate NoPrint Data=EstOut2;
		By Rate_C Prot NrSubs;
		Var PctProt HalfWidth;
		Output Out=Statz2 Mean=PctProt_Mean HalfWidth_Mean N=N
			pctlpts  = 80 90 95 pctlpre = PctProt_ HalfWidth_;
	Run;

	Title1 "Examination of effect of Nr Subjects";
	Title2 "Subject-Subject logit SD = &sdSub";
	Title3 "NrSims = &NrSims, Seed = &Seed";

	option orientation=landscape;
	ods rtf file = "C:\Users\JNguyen\Desktop\Junks\sdSub = &sdSub..rtf" bodytitle
startpage=no style=JamesStyle1;
	title4 "GEE: Subject as fixed effect";
	Proc Print NoObs Data=Statz(drop=PctProt_80 PctProt_90 PctProt_95);
		var Rate_C Prot NrSubs N PctProt_Mean HalfWidth_Mean HalfWidth_80 HalfWidth_90
HalfWidth_95;
		format PctProt_: HalfWidth_: 6.1;
	Run;

	title4 "GEE: Subject NOT as fixed effect";
	Proc Print NoObs Data=Statz1(drop=PctProt_80 PctProt_90 PctProt_95);
		var Rate_C Prot NrSubs N PctProt_Mean HalfWidth_Mean HalfWidth_80 HalfWidth_90
HalfWidth_95;
		format PctProt_: HalfWidth_: 6.1;
```

```
        Run;

        title4 "GLIMMIX";
        Proc Print NoObs Data=Statz2(drop=PctProt_80 PctProt_90 PctProt_95);
                var Rate_C Prot NrSubs N PctProt_Mean HalfWidth_Mean HalfWidth_80 HalfWidth_90
HalfWidth_95;
                format PctProt_: HalfWidth_: 6.1;
        Run;
        ods rtf close;
        option orientation=portrait;

        Proc datasets nolist; save sasmacr; quit;

%Mend; *MainRun;
/*
%let nmin=5; %let nmax=20;
%Let sdSub = 1;     * Sub-Sub SD on logit scale;
%Let NrRates=3;         %Let Rates = 50 75 90; * Specify true % fed females;
%MainRun;

%let nmin=5; %let nmax=20;
%Let sdSub = 0.3;     * Sub-Sub SD on logit scale;
%Let NrRates=3;         %Let Rates = 50 75 90; * Specify true % fed females;
%MainRun;

%let nmin=5; %let nmax=20;
%Let sdSub = 0;     * Sub-Sub SD on logit scale;
%Let NrRates=3;         %Let Rates = 50 75 90; * Specify true % fed females;
%MainRun;
*/

%let nmin=5; %let nmax=20;
%Let sdSub = 1;     * Sub-Sub SD on logit scale;
%Let NrRates=2;         %Let Rates = 10 20; * Specify true % fed females;
%MainRun;

%let nmin=5; %let nmax=20;
%Let sdSub = 0.3;     * Sub-Sub SD on logit scale;
%Let NrRates=2;         %Let Rates = 10 20; * Specify true % fed females;
%MainRun;

%let nmin=5; %let nmax=20;
%Let sdSub = 0;     * Sub-Sub SD on logit scale;
%Let NrRates=2;         %Let Rates = 10 20; * Specify true % fed females;
%MainRun;
```