For assistance in accessing this document, please contact:

Jim Downing
downing.jim@epa.gov

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

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

September 29, 2016

MEMORANDUM

SUBJECT: 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

FROM: Timothy Ciarlo**,** Entomologist
Invertebrate-Vertebrate Branch I, Registration Division
Office of Pesticide Programs

Eric W. Bohnenblust, Ph.D., Entomologist
Invertebrate-Vertebrate Branch 2, Registration Division
Office of Pesticide Programs

Maureen Lydon, Human Research Ethics Review Officer
Office of the Director
Office of Pesticide Programs

TO: Marietta Echeverria, Chief,
Invertebrate-Vertebrate Branch I, Registration Division
Office of Pesticide Programs

REF: Foard, Timothy, Study Director (2016) Laboratory bioassay to assess the Efficacy of permethrin applied to military uniform fabric for protection from mosquito bites (*Aedes aegypti and Anopheles quadrimaculatus*), after 0, 20 and/or 50 washings. Unpublished document prepared by i2LResearch USA, Inc. for Study Sponsor LaunchBay LLC, September 13, 2016.

We have reviewed the referenced protocol for a laboratory test of permethrin-treated clothing for the United States Army from both scientific and ethics perspectives. This EPA review evaluates the scientific aspects of the proposed research for an efficacy study to assess permethrin-treated U.S. Army uniforms after 0, 20 and/or 50 washings. Ethical aspects of the proposed research are assessed in terms of the standards defined by 40 CFR 26 subparts K and L.

A. Completeness of Protocol Submission

The submitted protocol was reviewed for completeness against the required elements listed in 40 CFR §26.1125. EPA's checklist is appended to this review as Attachment 3. All

elements of required documentation are addressed in the submitted protocol package and supplementary documentation to be provided by Schulman Institutional Review Board (IRB).

As part of the completeness review, EPA noted that the study sponsor has changed from Triton Systems, Inc. to LaunchBay LLC. LaunchBay acquired from Triton the Invexus technology and all products and intelligence related to this insecticide treatment. For that reason, EPA's review references the current study sponsor, LaunchBay.

B. Summary Assessment of Ethical Aspects of the Proposed Research

Here is a summary of our 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 up to two permethrin-treated military uniforms, specifically U.S. Army Combat Uniforms (ACU) and U.S. Army Flame Resistant Army Combat Uniforms (FRACUs). The permethrin-treated materials will be tested unwashed, 20 times washed, and 50 times washed, for protection against bites by mosquitoes. The fabric is treated with permethrin via the Invexus™ process. The data collected in the study will be used to support product registration. Invexus™ Insecticide Treatment is a process by which the fabrics are treated with permethrin in a continuous, roll to roll treatment process in factory. The permethrin formulation is applied and adhered to fabric via a proprietary process that minimizes energy usage and process waste. The target levels of mean bite protection are ≥90% for the unwashed, 20 times washed, and 50 times washed permethrin-treated fabrics. The research has societal value because U.S. military personnel serving domestically and abroad are at risk of contracting mosquito-borne diseases, but the data supporting currently registered military uniforms impregnated with permethrin do not show ≥90% efficacy through 50 washes in human studies. The rationale for this testing is to collect data to show that military uniforms impregnated with permethrin through the Invexus™ process will provide ≥90% mean bite protection against mosquitoes for up to 50 washings. As intended, the data resulting from this proposed study will be used to support registration of either or both of LaunchBay's Invexus™ treated ACU and FRACU.

2. **Subject Selection:** With regard to the number of subjects who will participate in the testing of the permethrin-treated fabric, as further explained in EPA's comments, in order to generate statistically-sound data, the preferred sample size is 15 test subjects for testing the Army Combat Uniforms (ACU) fabric. The preferred sample size is 10 test subjects for testing the Flame Resistant Army Combat Uniform (FRACU) fabric.

   To determine the bite protection against each mosquito species individually, and limit the discomfort associated with mosquito bites, one mosquito species will be tested at a time, on different test days, against all permethrin-treated and untreated uniform fabrics; the only exception is identified in revised protocol section 5.4.5. As a result, up to 5 test days with 5 test subjects on each day will be required to conduct the study. Although the study will be conducted over 5 separate days, this will not increase the length of the testing time for each subject participating in the study.

(Conducting the study over 5 days is due in part to the number of *Anopheles* mosquitoes that would be required for testing on any one of the test days.) Subjects may choose to participate in up to 2 test days, if they desire and are eligible. When 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. Testing for control attractiveness will be conducted on each test day. Four additional subjects (2 males and 2 females) will serve as alternates for each test day, and will be available to replace any individuals who choose to withdraw before or at the start of a test day. Alternates will be randomly selected. Therefore a total of nine subjects (five subjects and four alternates) will be selected for each test day, with up to forty-five subjects (with approximately half of each gender) participating over five test days. The decision as to whether an alternate is needed will occur within the first 2 hours of the test, during the preparation time and control exposure, but before all the treatment exposures. An alternate who is not needed to replace a test subject will be able to leave. (i2LResearch provided a chart depicting how the testing could occur over 5 test days; this is provided in Attachment 4 to this memorandum.)

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. A Spanish language advertisement will also be posted online using the same media, plus an online Spanish language newspaper that advertises within the recruitment area. The advertisement 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. The results of testing the permethrin-treated fabric should be as generalizable as possible to the target population of treated-fabric users. Researchers will try to ensure that the ethnic groups represented in the demographics of the members of the military who are the intended users of the treated clothing, have the opportunity to volunteer for the study. Every effort will be made to achieve the appropriate demographic composition, via a stratified random sample of the pool of recruited subjects. The final study will specify the demographics of subjects who participated in the study, based on gender, age, and ethnic background, due to availability of test subjects on each test day.

3. **Risks to Subjects:** The protocol discusses five potential hazards associated with these tests including adverse reaction to the test substances, exposure to mosquitoes and disease vectors, physical discomfort of enduring multiple mosquito bites, unanticipated loss of confidential information, and psychological risks related to pregnancy testing. Risks are minimized in the protocol by excluding candidates known to be hypersensitive to or phobic of mosquito bites; using disease-free colony-raised mosquitoes; excluding candidates known to be sensitive to insect repellents or insecticide-treated fabrics and subjects with open cuts, scrapes, skin disease and skin problems; spacing test days at least 72 hours apart when the same subject is participating in up to two test days; 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, which are not known 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 (*Aedes aegypti*) and malaria pathogens (*An. quadrimaculatus*). In addition, the supplier will document that these laboratory-reared mosquitoes are disease free, and that they have never received a blood meal.

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

5. **Risk/Benefit Balance:** The protocol describes measures to further reduce risk to subjects while maintaining the robustness of the scientific design. Due to the risk mitigation measures put in place, the residual risk to subjects is low and reasonable in light of the potential benefits of the data to society, including U.S. Army soldiers who will wear the permethrin-treated uniforms.

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

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

8. **Respect for Subjects**: The subjects' identities will be protected as follows: each subject will be assigned a code number, and only subjects' code numbers will appear on data sheets. The subjects' names will not appear anywhere on the data sheet, or in the reports. The study records will be maintained at the testing facility in locked cabinets and electronic files kept on a password-protected computer server.

Provision is made for discrete handling of the pregnancy testing that is required of female subjects on the day of testing. Candidates and subjects will be informed that they are free to decline to participate or to withdraw at any time for any reason. Subjects will be compensated as described in the protocol. Breaks for subjects between exposures and provision of snacks and drinks for interested subjects 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

i2LResearch USA, Inc. and the study sponsor were notified that, before the research is conducted, the protocol and supporting documents should be revised to address EPA's comments and recommendations resulting from the review by the Human Studies Review Board (HSRB). i2LResearch and the study sponsor have already agreed to address EPA's comments. To facilitate the HSRB's review of the latest protocol, which incorporates the EPA's comments, the EPA is providing a separate file for the HSRB entitled "Revised Protocol with EPA Comments Incorporated." After the HSRB completes its review of the protocol and relays its recommendations to the EPA, the EPA and i2LResearch should reach agreement on implementation of the HSRB's recommendations; the revised protocol and supporting documents should be resubmitted for review and approval to the overseeing IRB prior to initiating the research. It should also be noted that i2LResearch simplified the title of the revised protocol to read, "Laboratory evaluation of mosquito bite protection from permethrin-treated clothing for the U.S. Army after 0, 20 and/or 50 washings."

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

Objective – Section 1.1

1. Does the "Invexus process" involve factory treatment of fabrics, as opposed to "self-treatment" meaning individuals treating the fabric? If so, in the first section, when this process is first referenced, please state that this. Please provide other brief information which can be provided to describe the Invexus process without providing confidential information.
2. Use the proper abbreviation for Flame Resistant Army Combat Uniforms.
3. For purposes of accuracy, please change all references to "HSRB-approved" to "HSRB-reviewed" consistent with the language used in EPA's federal rule for protecting human subjects.

IRB review and ethical study conduct – Section 1.3

4. For purposes of accuracy, please rewrite the section on "IRB and ethical study conduct," to clarify that the IRB has reviewed and approved the protocol. For clarity, in section 1.3.3, please state that "the following IRB is overseeing the research" instead of "the following IRB will be used."
5. In the IRB review section, in section 1.3.2, you state, "the final report was reviewed by the HSRB during the October 2015 meeting." Please change "report" to "study" in this phrase so there's no confusion as to the document to which you're referring.

Rationale for use of human test subjects – Section 2.1

6. Section 2.1.3 - In order for EPA to rely on a study from an ethics standpoint, in addition to ensuring that the study is ethically conducted, it must also have scientific integrity, which includes statistically-sound data. The EPA's Office of Pesticide Programs (OPP) does not believe that two extra subjects are sufficient as alternates. What if some subjects do not appear on the test day or choose to withdraw which is their right to do so? OPP is concerned about the proposed number of subjects and number of alternates and has addressed this topic in revised section 2.2.5. Please revise section 2.1.3 as follows:

   "In order to generate statistically-sound data, human test subjects will be required to assess each fabric and treatment condition, against each mosquito species. There will be alternates, extra subjects, of each gender, who will be ready to replace any other subjects who choose to withdraw before or at the start of a test day. The number of subjects and alternates is discussed in the next section."
7. Section 2.1.4 currently reads, "In the unlikely event that a subject withdraws after testing has started, that study will continue with the remaining subjects." Again, to ensure the scientific and ethical integrity of the study, please revise section 2.1.4 to read as follows: "Should a subject withdraw from the study, their data will not be used in the study. An alternate will be selected to replace the withdrawn subject. This process will continue until the required number of subjects have completed the study."

Number of Subjects and Duration of Participation – Section 2.2

8. Section 2.2.1 - In the first sentence, for purposes of clarity, please replace "test substances/wash cycles" with the word "fabrics."
9. Section 2.2.2 – To further minimize any discomfort of subjects participating in up to two test days, please space apart the two test days a minimum of 72 hours instead of 48 hours if a subject is participating in more than one test day. Please reflect this change in all areas of the protocol where this timeframe is mentioned. Please expand section 2.2.2 to clearly state the following given that this is the intent: "Subjects may choose to participate in up to two test days, if they desire and are eligible." Also, in discussions with EPA, i2LResearch agreed to revisions to the number of subjects who will complete testing. Section 2.2.2 should be revised to reflect the results of that discussion as follows:

   "To determine the bite protection against each species individually, and limit the discomfort associated with mosquito bite, one mosquito species will be tested at a time

against all permethrin-treated and untreated uniform fabrics (with one exception identified in section 5.4.5). Therefore, up to 5 test days with 5 test subjects on each day will be required to conduct the study. Although the study will be conducted over 5 separate days, this will not increase the length of the testing time for each subject participating in the study. (Conducting the study over 5 days is due in part to the number of *Anopheles* mosquitoes that would be required for testing on any one of the test days.) Subjects may choose to participate in up to 2 test days, if they desire and are eligible. When 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. Testing for control attractiveness will be conducted on each test day."

10. Section 2.2.3 – Please revise and expand this section to reflect a break of up to 10 minutes for subjects between each exposure and a 30 minute lunch break. Suggested language is included in red below:
"Each test day will potentially last up to 8 hours. This includes up to one hour of preparation time prior to testing, and time in between testing of each test substance for subjects to remove the previous test fabric, wash their forearms, take a break of up to 10 minutes between exposures, and put the subsequent test fabric on. During the break between exposures, subjects can relax, get a drink, or use restroom facilities. One of the 10 minute breaks will overlap with a 30 minute lunch period. If a subject needs to take a longer break, that will be allowed. The length of the test day also includes time for technicians to remove all mosquitoes from the test cages, place the mosquitoes in the freezer for counts, and place a new batch of mosquitoes into test cages prior to the next 15-minute exposure interval."
11. Section 2.2.4 – Section 2.2.4 should be revised to reflect the increase in the proposed number of test subjects:
"Five test subjects (two subjects of each gender, plus a fifth subject) will be required for each of 5 test days. 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 to test ACU fabric in order to obtain 80% power to determine that the half width of the 95% confidence interval (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 to test FRACU fabric 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%. This is explained further in the sample size section, section 7."
12. Section 2.2.5 – Section 2.2.5 should be revised as follows to reflect the increase in the proposed number of alternates to 4 subjects (2 males and 2 females) per test day:
"Four additional subjects (2 males and 2 females) will serve as 'alternates' for each test day, and will be available to replace any individuals who choose to withdraw before or at the start of a test day. Alternates will be randomly selected. Therefore a total of nine subjects (five subjects and four alternates) will be selected for each test day, with up to forty-five subjects (with approximately half of each gender) participating over five test days. The decision as to whether an alternate is needed will occur within the first 2 hours of the test, during the preparation time and control exposure, but before all the treatment exposures. An alternate who is not needed to replace a test subject will be able to leave."

13. Section 2.2.6 – During the training session, it's important that subjects clearly understand up-front what they will need to do during the study. It's important for i2LResearch to demonstrate for the subjects during the training session each individual step involved in the study as part of the process for fully informed consent. i2LResearch already references this, in part, on page 9, **step # 9** which is positive. However, OPP has some comments on this section. We recommend that current step # 9 in the IRB-approved protocol be moved up to become part of step #2 given that they address the same topic. After steps # 2 and 9 are combined, OPP recommends the re-ordering of some sentences and the following language changes in red to help ensure that every step is demonstrated to potential subjects. In cases where EPA recommends re-ordering of sentences, we have highlighted those sentences in red as well to draw attention to the suggested new order:

    Revised Step # 2 from section 2.2.6 with step # 9 incorporated
    "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, discuss 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 of 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 6.1 - 6.3 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. i2LResearch will also explain that the subjects will wash and dry their forearms after each exposure and take up to a 10 minute break between each exposure. 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."

    Also, per i2LResearch's suggestion, if a subject chooses to participate in up to two test days, the subject will be required to attend a training session prior to their participation in the subsequent test day only if their last training occurred more than four weeks prior to the second test day. This is acceptable to EPA and can be reflected in all applicable sections of the revised protocol.

14. Section 2.2.6 – step # 5 – Regarding the comprehension questions, please replace question b ("What type of product will be applied to your [sic] during each exposure period of the study?") with the following new question: "What will you be wearing on your arm during the exposure period?"
15. Section 2.2.6 – step # 7 – Please add the following sentences at the end of step # 7: "The researcher will have drinks (i.e. 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 exposures, assuming a subject wishes to each lunch."

16. Section 2.2.7 – Please update the number of subjects per test day and alternates referenced in this section so it read as follows: "Five test days will be required for the study. Each test day will include five subjects (plus four alternates)."
17. In section 2.2.8, please reflect that subjects may choose to participate in up to two test days, if they desire, are eligible and selected.
18. Section 2.2.10 and 2.2.11 – In section 2.2.10, please reflect that up to 5 test days will be involved. In section 2.2.11, please reflect the revised total number of test subjects and the increased number of alternates. The updated sentence would read, "Depending on the number of days of each test subject's participation, the total number of subjects who will participate will include 15 to 25 being scheduled as test participants and 12 to 16 serving as alternates."

Subject Recruitment and selection - Section 2.3

19. For purposes of accuracy, please update section 2.3.1 to read as follows with suggested changes highlighted in red:
"Recruitment will not begin until the IRB has approved and the EPA and HSRB have reviewed the protocol and the associated ICD, comments have been incorporated as appropriate, and i2L has received an official letter that approves the protocol and an IRB stamped approved copy of the ICD. The IRB will approve any revisions to the protocol in response to EPA and HSRB comments prior to implementation."
20. Please update section 2.3.2 to reflect the updated number of test subjects and alternates as follows: "Five test subjects (two subjects of each gender, plus a fifth subject) will be selected for each of 5 test days. Four additional subjects (2 males and 2 females) will be selected as 'alternates' for each test day, and will be available to replace any individuals who choose to withdraw before or at the start of a test day. All subjects will be 18 to 55 years of age."
21. Please update section 2.3.3 as discussed in this section. Although ethnicity and native language do not affect subjects' attractiveness to mosquitoes, the study sponsor must still take actions to help ensure that the recruitment pool and selected subjects represent the demographics of members of the military who are the intended users of the treated clothing and, secondly, that from an ethics standpoint, the study sponsor is providing fair and equitable access. EPA cannot support a protocol that does not provide fair and equitable access. With that as the background, EPA requests the following changes to this section of the protocol:
"~~The ethnicity and the native language of any given test subject does not affect its attractiveness to mosquitoes; rather,~~ Each individual's physical (such as heat and moisture levels) and chemical composition (i.e. body odor and associated skin bacteria) determines varying levels of attractiveness for mosquitoes (Citation: Verhulst NO, Qiu YT, Beijleveld H, Maliepaard C, Knights D, Schulz S, et al. (2011) Composition of Human Skin Microbiota Affects Attractiveness to Malaria Mosquitoes. PLoS ONE 6(12): e28991. doi:10.1371/journal.pone.0028991). ~~Therefore, targeting specific ethnic demographics for this study is not necessary.~~ Potential subjects will be recruited from a pool of individuals who will have expressed interest in testing with i2L. This pool will

generally represent the demographics of members of the military who are the intended users of the treated clothing. ~~US repellent and/or treated fabric users~~."

22. Please update the first sentence of section 2.3.4 to state that, "Subjects will be recruited from a group from the general public at least two times greater than the number required for the study." The last sentence should state that this pool will generally represent the demographics of members of the military who are intended users of the treated clothing.
23. Please update section 2.3.5 to incorporate the following language in red type:
"Current treated fabric product labels are in English and the language that someone speaks does not directly affect attractiveness to mosquitoes. To target users familiar with and that understand the product labels, we will be recruiting English-speaking subjects. This research does not offer benefits to the subjects. Given that current labels are available only in English, ~~so~~ limiting recruitment to English speakers will not result in equity-of-access issues; the researchers will still ensure that the ethnic groups represented in the demographics of members of the military, who are the intended users of the treated clothing, have the opportunity to volunteer for the study."
24. Consistent with the aforementioned background explanation and expanded language, please expand section 2.3.6 to include the language provided below in red: "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. A Spanish language advertisement will also be posted online using the same media, plus an online Spanish language newspaper that advertises within the recruitment area. 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. The final study will specify the demographics of subjects who participated in the study, based on gender, age, and ethnic background, due to availability of test subjects on each test day." Please note that i2LResearch will need to submit the proposed advertisement to EPA and the overseeing IRB for review and approval prior to implementation.
25. In section 2.3.7, in order to be comprehensive, please expand the third sentence to include the language in red:
"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."
26. In section 2.3.8, for purposes of accuracy, please update the size of the pool in the first sentence so that it reads as follows:
"Individuals will continue to be contacted until a pool of potential subjects exists that is at least two times that required for the study (82), in which all potential subjects fulfill the inclusion/exclusion criteria."

27. Please update section 2.3.9 to include the language in red which reflects the increased number of alternates:

    "For each test day, five subjects and four alternates (with approximately half of each gender) will be selected from the pool of subjects that fulfill the inclusion/exclusion criteria and have signed the ICD for the study, by a subject allocation table via appropriate software (such as Excel or Minitab). This selection will be achieved by randomly selecting the test subjects' assigned code numbers. The first two females and two males will be assigned as the test subjects, in addition to a fifth subject. The sixth and seventh female and the sixth and seventh male will be assigned to act as alternates."
28. Please clarify section 2.3.10 as follows consistent with changes previously discussed:
    "At any time during the recruitment/selection process, if one or more individuals decline to participate, or if one or more test subjects chooses to withdraw from the study or is asked to withdraw, subjects for the study will be randomly selected from the alternates taking into account the gender of the withdrawn subject. If the unexpected occurs and none of the alternates can participate, then an equal number of new individuals (of the same gender as those who declined/withdraw) may be randomly selected from the recruitment pool and contacted following the same criteria as that outlined in the protocol. The study will continue until 15 subjects have completed the ACU testing process and 10 subjects have completed the FRACU testing process, in both cases per mosquito species as outlined in this protocol."

Consenting – Section 2.4

EPA's comments on the draft consent form and telephone screening script are provided in separate files to the Human Studies Review Board.

29. For completeness, please expand the second sentence in section 2.4.1 to read as follows: "Logistics for the training session and details to be shared with subjects during the training are listed in section 2.2.6."
30. For completeness, please add a sentence at the end of section 2.4.3 which reads as follows: "Each subject will be asked comprehension questions listed in section 2.2.6 to help ensure their understanding of the consent form."

Individual Inclusion/Exclusion Criteria – Section 2.6

31. In section 2.6, for clarity purposes, please revise 2.6.5 to read as follows, with a minor edit identified in red:
    "Subjects must feel they are healthy enough to participate in the study and do not have any health conditions that may affect the study or be worsened by ~~the application of~~ wearing insecticide-treated fabrics."
32. To be comprehensive, please expand 2.6.6 to read as follows, with the new proposed language identified in red:
    "The subjects must be willing to be exposed to and bitten by mosquitoes and cannot be phobic of mosquito bites."
33. Please expand 2.6.7 to read as follows, with the new proposed language identified in red:
    "The subjects must have no known allergies or sensitivities to mosquito bites, insect repellents or insecticide-treated fabrics."

34. Please expand 2.6.9 to read as follows:
    "The subjects must not be hypersensitive or allergic to latex or skin care products. The subjects must be free from open cuts, scrapes, skin disease, and skin problems such as eczema, psoriasis or atopic dermatitis."
35. In section 2.6.13, please replace the last sentence (which reads, "*The study will continue with the remaining subjects.*") with the following language so the number of subjects who must be tested is clear: "The subject will be replaced by an alternate and the study will not be considered concluded until fifteen subjects have been tested as described in the protocol."

Monitoring of human subjects – Section 2.8

36. Please add the following new sentences at the end of section 2.8.2: "The on-call nurse will be given a copy of the final approved protocol and will be briefed by telephone on the study process and test substances. i2LResearch will contact the nurse at the initiation of each test day to confirm that testing has begun for that day and i2LResearch will call the nurse for medical advice and/or assistance as necessary."

Hazards to the Human Subjects – Section 2.10

37. In section 2.10.1, please add a hazard "E" which reads, "E) Psychological risks related to pregnancy testing." As the last write-up in section 2.10 (following the last sentence on efforts to be taken to maintain subjects' confidentiality), please add the following new subsection:
    "There can be psychological stress relating to pregnancy testing. In order to minimize the psychological stress, women will be given a private place to take the test and the study director will ensure confidentiality of any test result. The results of the test will not be discussed or released to anyone besides the subject. The confidentiality of the pregnancy testing will be discussed during the consent process."
38. In section 2.10.2.1, please refer to Material Safety Data Sheets simply as "Safety Data Sheets (SDS)" because the title of these sheets has changed. This correction should also be made in 3.3.1.
39. To be comprehensive, please expand 2.10.2.2 to read as follows: "No subjects with known allergies or sensitivities to mosquito bites will be allowed to take part in the study."
40. By way of explanation, please add a new subsection after the aforementioned language which reads as follows:
    "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."
41. As part of risk mitigation, regarding **2.10.2.9,** as appropriate, please include the following language: "In order to ensure the mosquitoes used in the study are not carrying any disease vectors, 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 disease free." OPP would like the

same screening as described above conducted in this case. Please arrange to do so and include the aforementioned language at the end of 2.10.2.9.

42. In section 2.10.2.10, please revise the sentence, "The study sponsor will reimburse test subjects for the costs of medical care" so that it reads, **"**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." The reimbursement process can be lengthy which is why the study sponsor should cover applicable costs instead of using a reimbursement approach. This topic was also discussed in a previous HSRB meeting. The suggested language above is consistent with the language used in the consent form. In this same section, please clarify what you mean by "All adverse effects will be followed until resolution is reached." Perhaps you could explain that 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.
43. In section 2.10.2.11, in the last sentence, please replace the phrase, "made to be available" with "will be." Also, please revise the reference to provision of first aid items to subjects to read as follows: "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 will be contacted prior to the test date and will be on call during each test day for non-emergency queries or problems."

Benefits to Human Subjects – Section 2.11

44. Section 2.11.2 currently reads as follows and appears to be incomplete:
"The information collected may help people who will be exposed to mosquitoes; however, this cannot be guaranteed." OPP/EPA recommends that you replace this language in section 2.11.2 with the following: "There are indirect benefits to society. The data collected in this study will be used to establish the level at which insecticide treated uniforms prevent mosquito bites. Pending EPA review of the final study based on this protocol, data generated from this study may be used for U.S. EPA registration of insecticide treatment for military clothing. It is anticipated that laboratory data can be translated to bite protection of covered areas of the body for individuals in the field."

Application and Treatment Order – Section 6.0

45. In section 6.1, in the fourth sentence, please revise the sentence to read as follows so it's clear that different sized sleeves will be created:
"Different sized sleeves (small, medium, and large) ~~may need to~~ will be created to fit to the different test subjects' needs."

Methodology for Efficacy Testing

46. In the section on methodology for efficacy testing, for purposes of clarity, please replace the title "test systems" with "mosquito species".

Experimental Design

47. For purposes of clarity, please expand the section on experimental design (which was section 7.4.2 in the IRB-approved protocol and is now 6.4.2 in the revised protocol) to include the language in red so it reads as follows: "Each subject will then insert both forearms into two of the test cages (or one cage if only one fabric type is tested), placed side by side (i.e. one arm per cage), containing the day's test system, for 15 minutes. During this time the mosquitoes will land on the fabric, probe through it to the subjects' skin and feed. During each exposure period, subjects will be seated while their forearms are inserted into the case."
48. Please revise former section 7.4.4 (which is now section 6.4.5 in the revised protocol) so that it adds the following point and reads clearly:
    - Following each exposure, subjects will remove the gloves they wore to protect their hands and wash and dry their forearms. After this, subjects can take a break of up to 10 minutes after each exposure.
49. Please expand former section 7.4.6 (which is now 6.4.7 in the revised protocol) so that it reflects breaks and a lunch period for subjects, so it reads as follows:
    "Sections 7.4.4 – 7.4.5 will be repeated for the remaining two test substances of each fabric type. One of the subjects' 10 minute breaks will overlap with a 30 minute lunch break which each subject will receive."
50. For clarity, please revise former section 7.6.6 (which is now section 6.6.6) so that the first sentence reads as follows:
    "If ~~either~~ a subject has received less than 10% of bites in the control replicate (i.e. 20 bites out of 200 released mosquitoes), from either or both species, ~~the~~ an alternate test subject ~~may~~ will replace that subject."

Data and Statistical Analysis

51. In the sample size section (which was section 8.2.1 in the original protocol and is section 7.2.1 of the revised protocol), the second sentence should be revised to read as follows: "When testing the insecticide-treated fabrics, there are three goals, namely statistical robustness, minimizing exposure and associated potential hazard to ~~the~~ test subjects ~~(which conflicts with the first goal)~~ and consistency with previous repellent studies where appropriate." This revision takes into account the fact that you can have a statistically robust study which still minimizes exposure and hazards.
52. In the revised protocol, in section 7.2.6, please revise the language to read, "In summary, the proposed sample size represents a sound compromise between statistical principals, minimizing exposure and hazard to subjects and following accepted practice."
53. Please clarify former section 8.2.4 (which is section 7.2.4 in the revised protocol) so that it reads as follows:
    "The second goal (of minimizing exposure and potential hazard to ~~the~~ subjects) can be achieved by implementing risk mitigation measures as described in this protocol and reducing the number of subjects when appropriate, so that ~~their~~ exposure to mosquito bites and to the products being tested are also reduced. It should be noted that larger numbers of subjects make for more expensive studies; therefore there are also cost reasons for minimizing the numbers of subjects."
54. Please update section 7.2.7 in the revised protocol so that it reads as follows:

"Thus, in this study, five test subjects (two subjects of each gender, plus a fifth subject) will be selected for each of 5 test days. Four additional subjects (2 males and 2 females) will be selected as 'alternates' for each test day, and will be available to replace any individuals who choose to withdraw before or at the start of a test day. Alternate Subjects: There will also be four subjects, two of each gender, to serve as alternates in case any of the test subjects withdraw from the study, or in the case that a test subject did not receive the acceptable number of control bites. If an alternate subject withdraws after replacing one of the original subjects, he or she will be replaced by another alternate of the same gender."

Monitoring of Study Conduct

55. Please expand former section 10.1.4 (which is section 9.3.4 in the revised protocol) to read as follows:
"All amendments and deviations will be reported to the study sponsor in a timely manner. All amendments and deviations to the protocol will be reported to SAIRB consistent with their standard reporting guidance. Protocol amendments may not be initiated without prior IRB review and approval except where necessary to eliminate apparent immediate hazards to human subjects. SAIRB's website states in part that, 'under normal conditions, you must submit to the Board all amendments, including administrative letters, or changes to the protocol for review and approval prior to the implementation… Occasionally, safety concerns may require you to implement an amendment prior to Board approval. When changes to the protocol are implemented in order to eliminate an apparent immediate hazard to a research subject without prior Board approval, you must report changes to Schulman within **10 business days**.'"
56. Please add the following language at the end of former section 10.2.2 (which was renumbered 9.4.2 in the revised protocol) consistent the language from the consent form:
"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."
57. Please expand former section 10.2.3 (which is section 9.4.3 in the revised protocol) to read as follows: "The on-call nurse 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 will the subject will receive."
58. Please expand former section 10.2.4 (which was renumbered 9.4.4 in the revised protocol) to read:
"Any problems or adverse effects will be promptly reported to LaunchBay and the IRB consistent with IRB reporting procedures.
59. Please expand the last sentence in former section 10.2.5 (which is section 9.4.5 in the revised protocol) to read as follows:
"The Study Director and recruitment firm will keep on file the phone numbers, email addresses and street addresses for each study participant as a means to contact them if needed."
60. Please revise former section 10.2.6 (which is section 9.4.6 in the revised protocol) to read as follows:
"New findings will also be reported, in writing, to LaunchBay and the IRB in a timely manner consistent with IRB reporting procedures."

61. At the end of former section 10.2.9 (which is section 9.4.9 in the proposed revised protocol), please add the following language: "LaunchBay will also comply with FIFRA section 6(a)(2) adverse effects reporting requirements as applicable."
62. Please expand the last sentence of former section 10.2.10 (which is section 9.4.10 in the revised protocol) to read as follows:
"The IRB will also be advised consistent with IRB reporting procedures."

Amendments and deviations to the protocol

63. Please revise former section 11.3 (which is section 10.5 in the revised protocol) to read as follows:
"All amendments, deviations, and any adverse events will be documented in the final ~~reports~~ study and reported consistent with IRB reporting procedures. Documentation will include a description of the change, the reason for the change, ~~and~~ the effect of the change on the conduct and outcome of the study, and whether or not the IRB approved each amendment prior to implementation."

D. Summary Assessment of Scientific Aspects of the Proposed Research

The objective of this proposed study is *"To assess the efficacy of up to two permethrin treated Army Combat Uniforms: (ACU) and Flame Resistant Army Combat Uniforms (FRACU). The materials will be tested unwashed, 20 times washed, and 50 times washed, for protection against bites by mosquitoes. The fabric is treated with permethrin via the Invexus™ process. The data collected in the study will be used to support product registration." (p. 4 of 69, §1.1.1)*[1].

The basic experimental unit in this study is a sleeve test. Each test involves a subject exposing for 15 minutes a (unwashed treated, untreated, washed treated) fabric-sleeved arm into a cage containing 200 individual female mosquitoes of one species. Each arm will be exposed 4 times (8 total) over a period of up to 8 hours (p. 9 of 69, §2.2.3). 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 is the proportion of blood-fed female mosquitoes to the total number of mosquitoes in each test cage. Rates of bite-through for the permethrin-treated fabrics will be corrected using Abbott's formula for 'background' bite-through rates in the control (untreated fabric sleeve). To increase testing precision, each subject will serve as their own treatment and control. Therefore, the experiment consists of 4 exposures per fabric type (FRACU or ACU) 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 (50x) FRACU fabric.
- 1 test with treated washed (20x) FRACU fabric.
- 1 test with treated unwashed (0x) FRACU fabric.

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

[1] Section and page numbers referenced in this science section refer to the revised protocol with EPA comments incorporated unless otherwise noted.

- 1 test with treated unwashed (0x) ACU fabric.

FRACU and ACU fabric will be tested as described in Table 1 below. Subjects will test each FRACU fabric treatment level once per mosquito species for a total of 10 replicates per FRACU fabric treatment level per species, resulting in 20 replicates per fabric treatment level for this experiment. Subjects will test each ACU fabric treatment level once per mosquito species for a total of 15 replicates per ACU fabric treatment level per species, resulting in 30 replicates per fabric treatment level for this experiment. The rationale for the different number of human test subjects using the different fabrics is explored in Section 2 of this review.

Because repeated wash cycles will progressively remove some of the impregnated permethrin, the 50x washed fabric samples will be tested before the 20x washed samples, which will be tested before the 0x unwashed samples. This order will reduce possible "carryover" contamination effects. The Agency recommends subjects wash their forearms with unscented soap between each test 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 (AATC) laundering protocol (pp. 32-33 of 69, §5.5). Testing will be conducted with treated and untreated clothing prior to laundering (0x wash cycle) and at the 20x and 50x wash cycles.

The unit of measure for determining efficacy in this proposed experiment (% bite protection based on the proportion of blood-fed to total mosquitoes in a cage) differs from skin applied repellent evaluations where the "Landing with Intent to Bite" measure is used and efficacy is measured as Complete Protection Time. In brief, the repellent effect created by skin-applied repellents is instantaneous and non-toxic, whereas mosquitoes exposed to treated clothing must remain in contact with the treated cloth for a longer time period to elicit an effect. The resulting effect is usually 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% (p. 5 of 69, §1.1.2).

This protocol also proposes to evaluate the repellent effect (% bite protection) of treated clothing using only two mosquito species - unlike skin applied repellent studies conducted under field conditions where three species are evaluated. In the proposed study, representative species from the genus *Anopheles* (malaria vector) and genus *Aedes* (vector of dengue, yellow fever, chikungunya, and zika) will be evaluated. A mosquito species from the genus *Culex* (vector of West Nile virus or St. Louis encephalitis) will not be tested. Justification for exclusion of the third species is not mentioned. Reference is made to Dr. Bernier's protocol previously reviewed by the HSRB on which this protocol is based, which did not include *Culex* (p. 6 of 69, §1.3.2).

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

1. **Study design:**

Replicate subjects will be used in this study to evaluate bite protection for two U.S. Army clothing fabrics (FRACU and ACU) treated with insecticide/repellent (permethrin). 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 §7.3.2 (p. 44 of 69) of the protocol, the observed bite protection for a subject is calculated using the subject's bite-through rates for the treated fabric and a corresponding untreated/unwashed control fabric. Each subject serves as their own control. The purpose of the control is to compensate for the subject's individual attraction level, the general host-seeking response of the test mosquito population, and to correct for bite-through rate of the untreated fabric. The treatment and control values for a subject are then used in Abbott's formula to calculate the observed bite protection level of the fabric for that subject.

Treated fabric will be evaluated at the following wash intervals: unwashed (0x), 20x washes, and 50x washes. Separate fabric specimens for each wash interval are tested, similar to that described in U.S. military GL/PD specifications. Two species of mosquitoes, *Aedes aegypti* and *Anopheles quadrimaculatus***,** will be tested separately. Ten subjects will be used to test each FRACU fabric and mosquito species combination. Fifteen subjects will be used to test each ACU fabric and mosquito species combination. Subjects may be chosen for testing against both mosquito species, although using the same subjects is not necessary because each subject serves as his/her own control. EPA recommends 4 alternates be present on each of the 5 test days (2 males, 2 females). However, alternates would only need to be on site on the morning of the test day until the control exposures are completed. The exposure time to mosquitoes at each test interval for control and treated fabric is 15 minutes per arm. A summary of the experimental design is described in §6.4-6.6 (pp. 35-40 of 69) and the testing paradigm for each mosquito species shown below in Tables 2 and 3. The fabric type used on each arm (right vs. left) will be determined by using a random number generator (p. 32 of 69, §5.4.5). Because the ACU testing requires 5 additional test subjects, the Agency suggests that these test subjects simultaneously be exposed to both mosquito species (one per cage per arm). This allows the additional 5 test subjects to complete ACU testing against both species in one day, thereby minimizing inconvenience to the test subjects. Additionally, the additional 5 test subjects will be exposed to mosquitoes for the same period of time as subjects testing ACU and FRACU fabrics simultaneously on each arm.

Table 1: Experimental Design

Fabric and Treatment Condition[1]	Number of Fabric Specimens	Number of Subjects	Number of Species[2]	Total Replicates per Fabric Type
FRACU Untreated Unwashed Control[3]	1	10	2	20
FRACU Treated Washed 50x	1	10	2	20
FRACU Treated Washed 20x	1	10	2	20
FRACU Treated Unwashed (0x)	1	10	2	20
ACU Untreated Unwashed Control[3]	1	15	2	30
ACU Treated Washed 50x	1	15	2	30
ACU Treated Washed 20x	1	15	2	30
ACU Treated Unwashed (0x)	1	15	2	30

[1] Fabric treatment conditions are either untreated and unwashed (Control) or treated and unwashed (0x), treated and washed 20 times (20x) or treated and washed 50 times (50x).

[2]The test species are *Aedes aegypti* or *Anopheles quadrimaculatus.*
[3]Each subject serves as their own control for the bite protection calculation.

Table 2: Testing Paradigm using *Aedes aegypti**

	Subject Right Arm		Subject Left Arm	
Test Set[1]	**Treatment Condition**	**Specimen Designation**	**Treatment Condition**	**Specimen Designation**
1	FRACU Untreated Unwashed Control[2]	Sleeve 1	ACU Untreated Unwashed Control[2]	Sleeve 2
2	FRACU Treated Washed 50x	Sleeve 3	ACU Treated Washed 50x	Sleeve 4
3	FRACU Treated Washed 20x	Sleeve 5	ACU Treated Washed 20x	Sleeve 6
4	FRACU Treated Unwashed (0x)	Sleeve 7	ACU Treated Unwashed (0x)	Sleeve 8

*Each subject will have both their right arm and left arm tested simultaneously and complete Test Set 1-4 for *Aedes aegypti*. Each subject will have a break between test sets when new cages are being filled with mosquitoes.
[1] Each test set runs for 15 minutes.
[2]Each subject serves as their own control for the bite protection calculation.

Table 3: Testing Paradigm using *Anopheles quadrimaculatus**

	Subject Right Arm		Subject Left Arm	
Test Set[1]	**Treatment Condition**	**Specimen Designation**	**Treatment Condition**	**Specimen Designation**
5	FRACU Untreated Unwashed Control[2]	Sleeve 9	ACU Untreated Unwashed Control[2]	Sleeve 10
6	FRACU Treated Washed 50x	Sleeve 11	ACU Treated Washed 50x	Sleeve 12
7	FRACU Treated Washed 20x	Sleeve 13	ACU Treated Washed 20x	Sleeve 14
8	FRACU Treated Unwashed (0x)	Sleeve 15	ACU Treated Unwashed (0x)	Sleeve 16

*Each subject will have both their right arm and left arm tested simultaneously and complete Test Set 5-8 for *Anopheles quadrimaculatus*. Each subject will have a break between test sets when new cages are being filled with mosquitoes.
[1] Each test set runs for 15 minutes.
[2]Each subject serves as their own control for the bite protection calculation.

Laboratory-reared 5-9 day old adult mosquitoes from colonies maintained at Benzon Research (Carlisle, PA) will be used for the bite protection assay (p. 34 of 69, §6.1.2). Adult female mosquitoes of two aggressive and anthropophilic species will be tested. One of these selected species will be *Aedes aegypti*, a vector of yellow fever, dengue fever, zika, and chikungunya that is found heavily in tropical and subtropical regions of the world, including the southeastern US and parts of the southwestern US. The second species will be *Anopheles quadrimaculatus*, a mosquito that is an aggressive biter, is native to the eastern US, and is a competent vector for malaria transmission. Mosquitoes from a colony typically respond more aggressively to attractant stimuli than strains reared from freshly collected wild-types.

"To select host-seeking females only for testing, the technician collecting mosquitoes will place an ungloved hand near the screened cage to attract these mosquitoes, and will then use a motorized vacuum pump with adjustable pressure control to gently aspirate them into a 1.7L plastic container with screened lid just prior to their use in the test. (p. 34 of 69, §6.1.5)."

2. Statistical design:

The original protocol submitted by i2LResearch USA, Inc. proposed that 8 individuals serve as test subjects. However, the justification for the proposed sample size provided in the initial protocol appears to pertain to studies where Complete Protection Time

(time from application to a confirmed mosquito bite) is evaluated, which is not applicable for this study design where Percent Bite Protection will be evaluated. After consultation with EPA, i2LResearch USA, Inc. has agreed to EPA's proposed sample size described below.

EPA has done a power analysis for a similar study previously reviewed by HSRB. In this past HSRB study submission, the bite-through rate of the control group (non-treated FRACU fabric) was assumed to be set as 20% and 50%. In the proposed study design, i2LResearch USA, Inc. indicated that the bite-through rate of the control ACU fabric is expected to be about 10%. The aforementioned study previously reviewed by HSRB examined bite-through rates of FRACU fabric only – not ACU fabric. FRACU fabric is constructed with a lower fiber density in comparison to ACU fabric (p. 28 of 69, §3.0), and therefore presents less of a physical barrier to mosquitoes attempting to bite through it. Consequently, bite-through rates with control ACU fabrics are expected to be lower than with control FRACU fabric. This affects the number of test subjects required to achieve an acceptable level of statistical power to answer the charge question presented to the HSRB.

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 ACU fabric is 10% and the true percent bite protection of the ACU fabric is at least 80%. The Agency's 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 6%, the study requires a sample size of 15 subjects, given that a true bite-through rate in the control is 10% and the true percent bite protection is 80% (Table 4). To reach 80% power of achieving the width of the 95% confidence interval of the estimated percent bite protection of less than 3%, our simulations indicate that the study requires a sample size of 10 subjects, given that a true bite-through rate in the control is 75% and the true percent bite protection is 80% (Table 5). A detailed report of the Agency's power analysis is presented in Attachment 2. For each iteration/dataset, the percent bite protection of the treated fabric vs. untreated control fabric was estimated using a generalized linear model for binomial distribution using a log link function, using subject as random effect (SAS PROC GLIMMIX).

Note that the desired precision (expressed as the 95% confidence interval half-width) differs between the FRACU and the ACU, with the ACU half-width criterion being set at 6% and the FRACU at 3%. 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 assumed parameters. That is, increases in the number of subjects beyond these numbers do not substantially narrow half-width the 95% confidence interval, 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 confidence interval. 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% confidence interval 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.

Table 4: Impact of the Number of Replications on the Number of Subjects when Control Bite-Through is 10%.

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
10	80	5	1000	9.3	10.9	12.4	14.2	998	13.6	16.0	18.1	21.2
		6	1000	8.4	9.7	10.7	12.1	999	11.2	13.0	14.4	16.3
		7	1000	7.6	8.7	9.5	10.4	1000	9.6	11.0	12.0	13.1
		8	1000	7.2	8.1	8.7	9.4	999	8.7	9.9	10.5	11.4
		9	1000	6.7	7.6	8.2	8.7	997	7.9	9.0	9.7	10.2
		10	1000	6.4	7.1	7.6	8.1	999	7.4	8.2	8.8	9.4
		11	1000	6.0	6.6	7.1	7.7	997	6.8	7.5	8.0	8.8
		12	999	5.7	6.3	6.7	7.2	995	6.4	7.1	7.5	8.1
		13	1000	5.5	6.1	6.5	6.8	997	6.1	6.7	7.2	7.6
		14	999	5.3	5.9	6.2	6.5	995	5.9	6.5	6.8	7.2
		15	**999**	**5.1**	**5.6**	**5.9**	**6.2**	**996**	**5.6**	**6.1**	**6.5**	**6.8**
		16	999	4.9	5.4	5.7	6.1	995	5.4	5.8	6.2	6.6
		17	1000	4.8	5.3	5.6	5.8	990	5.2	5.7	6.0	6.3
		18	1000	4.6	5.0	5.3	5.6	994	5.0	5.4	5.7	6.0
		19	1000	4.5	4.9	5.2	5.4	992	4.9	5.3	5.5	5.8
		20	1000	4.4	4.8	5.0	5.3	990	4.7	5.1	5.3	5.6
	95	5	999	4.7	5.7	6.6	7.4	987	7.7	9.2	10.8	12.1
		6	999	4.3	5.1	5.7	6.3	998	6.0	7.1	8.0	9.0
		7	999	3.9	4.5	4.9	5.5	997	5.0	5.9	6.5	7.4
		8	998	3.5	4.1	4.5	4.9	997	4.4	5.1	5.6	6.1
		9	999	3.4	3.9	4.3	4.7	999	4.1	4.7	5.2	5.6
		10	1000	3.1	3.6	3.9	4.1	999	3.7	4.2	4.5	4.8
		11	1000	3.0	3.4	3.7	3.9	997	3.4	3.9	4.2	4.5
		12	1000	2.9	3.2	3.5	3.7	996	3.2	3.7	3.9	4.2
		13	1000	2.7	3.1	3.3	3.5	997	3.0	3.4	3.7	3.9
		14	1000	2.6	3.0	3.1	3.3	993	2.9	3.3	3.5	3.7
		15	1000	2.5	2.8	3.0	3.2	997	2.8	3.1	3.3	3.5
		16	999	2.4	2.7	2.9	3.1	995	2.7	2.9	3.1	3.3
		17	999	2.4	2.6	2.8	3.0	994	2.6	2.8	3.0	3.2
		18	1000	2.3	2.5	2.7	2.9	993	2.5	2.7	2.9	3.1
		19	999	2.2	2.5	2.6	2.8	998	2.4	2.6	2.8	3.0

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
		20	997	2.2	2.4	2.5	2.7	996	2.3	2.6	2.7	2.9

*Number of datasets analyzed by the model. Model used log link function. Variation between logit values between subjects SD = 1

Table 5: 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 and two species of caged mosquitoes in the laboratory. Each subject will have permethrin-treated sleeves placed on one or both forearms. Sleeved arms will be exposed to caged mosquitoes for 15 minutes [The step-wise procedure is described in detail in §6.4, pp. 30-32 of 69]. 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 §7.3.2 based on exposure to mosquitoes during a 15 minutes bioassay every hour for up to 8 hours. 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:

- Experimental design
- Pre-training of subjects.

EPA Science Comments

The following elements in the protocol require revision before the research goes forward. Section numbers and page numbers refer to the IRB-approved protocol unless otherwise noted.

1. The control uniforms should be listed on the first page (p. 1 of 54). It is inadequate to only have "Control substance(s): N/A" here.
2. Section 1.1.1 – Please revise the sentence, "The data collected in the study will be used to support **data** registration" to "The data collected in the study will be used to support **product** registration." (p. 4 of 54, §1.1.1)
3. Change "protection" to "mean bite protection." (p. 4 of 54, §1.1.2)
4. Revise §1.1.4 (pp. 5-6 of 54). The rationale for testing is to collect data to show that military uniforms impregnated with permethrin through the InvexusTM process will provide ≥90% mean bite protection against mosquitoes for up to 50 washings. The data supporting currently registered military uniforms impregnated with permethrin do not show ≥90% efficacy through 50 washes. Delete "better," "are intended to be more effective," and "with higher protection potential," as the Agency is not interested in comparative efficacy against similar products.
5. Please revise "repellent treated clothing…" to "insecticide-treated clothing…" Permethrin is not a repellent. It is a toxicant. (p. 5 of 54, §1.3.2)
6. Change "treated uniform" to "bite protection provided by treated uniforms." (p. 6 of 54, §2.1.1)
7. Add "disease-free" immediately before "mosquitoes." (p. 6 of 54, §2.1.2)

8. Since this is a lab study, all subjects who withdraw should be replaced. It is insufficient to continue with the remaining subjects. Please revise accordingly. (p. 6 of 54, §2.1.4)
9. Does the applicant wish to pursue claims of 75 washings (or more)? If so, additional FR-ACU/ACU permethrin-treated uniform fabric samples could be added here. (p. 7 of 54, §2.2.1)
10. It should be noted here that these fabric samples will be treated with permethrin according to the Invexus process. (p. 7 of 54, §2.2.1.2-§2.2.1.4 and §2.2.1.6-§2.2.1.8)
11. Change "For logistical reasons in consideration of the fact that mosquitoes will bite test subjects and to limit the discomfort of bites" to: "To determine the bite protection against each species individually, and limit the discomfort associated with mosquito bite, one species…" (p. 7 of 54, §2.2.2)
12. Replace "test substances" with "permethrin-treated and untreated uniform fabrics." (p. 7 of 54, §2.2.2)
13. Testing is to be done on different species on different days, so testing for control attractiveness needs to occur against each species individually regardless of whether the test is conducted on the same or different days. Specify that this is a way to minimize the potential effects of residual permethrin that might be absorbed into the skin (e.g., carryover contamination). (p. 7 of 54, §2.2.2)
14. Add "the mosquitoes" immediately after "place." (p. 7 of 54, §2.2.3)
15. Insert "arm" between "your" and "during." (p. 9 of 54, §2.2.6(5)(b))
16. Instead of "pesticide treated," specify "permethrin-treated." (p. 10 of 54, §2.2.9)
17. Add "characteristics" immediately after "physical." (p. 10 of 54, §2.3.3)
18. Replace "attractiveness for mosquitoes" with "attractiveness to mosquitoes." (p. 10 of 54, §2.3.3)
19. Replace "i.e." with "e.g.," add "CO_2 output" immediately after "e.g.," and add commas as necessary. (p. 10 of 54, §2.3.3)
20. Please clarify what "followed all their directions" means. (p. 14 of 54, §2.5.7)
21. Revise §2.6.13 (pp. 15-16 of 54). "If a subject is found to be unattractive to mosquitoes at the first exposure (i.e. number of confirmed control bites is less than 10% when testing ACU fabric; less than 20% when testing FRACU fabric), they will be replaced with an alternate subject. In the event that a subject is determined not to be attractive to the mosquitoes, when the study is underway, they will not be allowed to continue to participate in the study. The study will continue with the remaining subjects." How will you confirm this is attributable to a subject's low attractiveness and not due to mosquito behavior? Please clarify.
22. Revise §2.7.1 (p. 16 of 54). It should be noted that subjects that have a noticeable smell of fragrance products will not be allowed to participate since this may confound results. Also, revise "12 hours" to "24 hours."
23. Not all of these diseases are locally transmitted in the US. Please revise. (p. 18 of 54, §2.10.2.3)
24. Revise §2.10.2.3 (p. 18 of 54). Malaria is a disease – not a disease-causing organism. *Plasmodium* spp. cause malaria.
25. Insert "spp." immediately after "*Plasmodium*." (p. 18 of 54, §2.3.4 – section added in a subsequent draft.)
26. If the protocol notes that no vaccine is available for chikungunya, it should be noted that one does exist for yellow fever. Alternatively, do not mention vaccines at all in this protocol. (p. 19 of 54, §2.10.2.8)

27. This section needs to be reworded. It would be impossible for lab-reared mosquitoes to transmit disease-causing pathogens. One option is to edit the beginning of this section to read, "To eliminate the risk of….." (pp. 19-20 of 54, §2.10.2.9)
28. Revise §2.10.2.9 (pp. 19-20 of 54). Mosquitoes are the vectors. Diseases cannot be vectors. All scientific names should be italicized. If the abbreviated genus convention is used, a period should go after the abbreviation. Further clarify why the mosquitoes can be considered disease-free."
29. The supplier lab needs to be identified. (p. 20 of 54, §2.10.2.9)
30. Bandages, antiseptics, and hydrocortisone cream may affect a test subject's attractiveness to mosquitoes. Therefore, it should be noted that subjects who use these items may need to withdraw after doing so. (p. 20 of 54, §2.10.2.11)
31. Describe the fabrics a bit more, e.g., composition of fabric types and openness vs. tightness of the weave. (p. 21 of 54, §3.0)
32. "FR-ACU and ACU treated fabrics ('test substances')" need to be defined earlier in the protocol. (p. 21 of 54, §3.0)
33. "The treated fabrics will contain no more than 0.5% permethrin." How will this be confirmed? (p. 21 of 54, §3.0)
34. Revise §3.0 (p. 21 of 54). It is insufficient to say "between 0 – 0.5%." This should be either 0% or 0.5% +/- and the variation should be provided. The variation should be within the certified limits for the product.
35. Revise §3.1.1.1-3.1.1.8 (p. 21 of 54). Test substances should align with Section 2.2.1 as well as the title page. It should be clear that treated fabrics contain permethrin.
36. Since no positive control will be used, this section (§4.2) should be deleted altogether. (p. 22 of 54, §4.2)
37. Add "(e.g., left)" immediately after "only one forearm." (p. 22 of 54, §5.2)
38. Revise §6.3 (p. 22 of 54). Specify the type of tape and gloves to be used. "Double" should be added immediately before "gloved" so that this section matches the description earlier in the protocol.
39. Add "(e.g., left)" immediately after "just one forearm" if only one fabric type is to be tested. Also specify that control fabrics will be tested first to confirm attractiveness, and that washing will occur with unscented soap and water. (pp. 22-23 of 54, §6.4)
40. Testing needs to be conducted on one day for each subject if this part of the protocol is to be followed as written. Controls cannot be used beyond the day they are conducted so each subject's testing would need to be completed in one day if section 6.4 is to be followed as written. We recognize that that is your current intent. We want to highlight that if a test subject withdraws at any point during the study, per EPA's earlier comment, an alternate would need to be tested in order to ensure that a full set of subjects is tested for the study; this may necessitate another day of testing for that alternate depending on if/when a test subject withdraws. (p. 23 of 54, §6.4.1)
41. Revise §6.4.2-6.4.4 (p. 21 of 54). These all need to indicate permethrin-treated.
42. Revise §6.4 (p. 23 of 54). Use "testing on a different day" instead of "another day of testing," as this could be construed as testing over two days.
43. Specify length of the drying cycle. (p. 24 of 54, §6.5.7)
44. Revise §7.1.1 (p. 24 of 54). Add the two species to be tested after "following two species." Change "The following species of mosquitoes" to "These mosquito species were…" Change "disease-causing" to "diseases" as the current wording doesn't make sense.
45. Add location: Carlisle, PA. (p. 24 of 54, §7.1.2)

46. Specify whether the aspirator used will be a vacuum-type aspirator or a mouth-operated one. (p. 25 of 54, §7.1.5)
47. Revise §7.2.2 (p. 26 of 54). This needs to align with the subject selection procedures described earlier.
48. Humidity likely will need to be higher than 30%. (p. 26 of 54, §7.3.1)
49. Replace "repellency" with "attractiveness." (p. 26 of 54, §7.4.3)
50. Revise §7.4.4 (p. 26 of 54). Any mention of washing subjects' forearms needs to specify that unscented soap and water will be used. Also, it should be determined if subjects are attractive to mosquitoes – not to the control fabrics.
51. Replace "Repellency" with "Attractiveness." (p. 27 of 54, §7.6)
52. Revise §7.6.1 (p. 27 of 54). Add "in the cages" following "after aspirating the mosquitoes to ensure all mosquitoes released…" Also add the approximate number of minutes mosquitoes will be placed in a freezer.
53. In addition to the total number of mosquitoes with confirmed bites, it would be important to record the exact total number of mosquitoes in each cage (The number of mosquitoes with confirmed bites + no bites). (p. 28 of 54, §7.6.4)
54. Revise §7.6.6 (p. 28 of 54). Test subjects should not be conducting their own control exposures. This should be done by the study investigators to the test subjects.
55. Revise §8.2.1 (p. 28 of 54 of 54). This is not a protocol designed to test a repellent. Replace "repellent" with "insecticide-impregnated fabric."
56. Revise §8.2 (pp. 28-31 of 54) entirely. See input from EPA statisticians below:
    The original protocol submitted by i2LResearch USA, Inc. proposed that 8 individuals serve as test subjects. However, the justification for the proposed sample size provided in the initial protocol appears to pertain to studies where Complete Protection Time (time from application to a confirmed mosquito bite) is evaluated, which is not applicable for this study design where Percent Bite Protection will be evaluated. After consultation with EPA, i2LResearch USA, Inc. has agreed to EPA's proposed sample size described below.

    EPA has done a power analysis for a similar study previously reviewed by HSRB. In this past HSRB study submission, the bite-through rate of the control group (non-treated FRACU fabric) was assumed to be set as 20% and 50%. In the proposed study design, i2LResearch USA, Inc. indicated that the bite-through rate of the control ACU fabric is expected to be about 10%. The aforementioned study previously reviewed by HSRB examined bite-through rates of FRACU fabric only – not ACU fabric. FRACU fabric is constructed with a lower fiber density in comparison to ACU fabric (pp. 26-27 of 54, §3.0), and therefore presents less of a physical barrier to mosquitoes attempting to bite through it. Consequently, bite-through rates with control ACU fabrics are expected to be lower than with control FRACU fabric. This affects the number of test subjects required to achieve an acceptable level of statistical power to answer the charge question presented to the HSRB.

    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 ACU fabric is 10% and the true percent bite protection of the ACU fabric is at least 80%. The Agency's 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 6%, the study requires a sample size of 15 subjects, given that a true bite-through rate in the control is 10% and the true percent bite

protection is 80% (Table 4). To reach 80% power of achieving the width of the 95% confidence interval of the estimated percent bite protection of less than 3%, our simulations indicate that the study requires a sample size of 10 subjects, given that a true bite-through rate in the control is 75% and the true percent bite protection is 80% (Table 5). A detailed report of the Agency's power analysis is presented in Attachment 2. 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).

Note that the desired precision (expressed as the 95% confidence interval half-width) differs between the FRACU and the ACU, with the ACU half-width criterion being set at 6% and the FRACU at 3%. 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 assumed parameters. That is, increases in the number of subjects beyond these numbers do not substantially narrow half-width the 95% confidence interval, 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 confidence interval. 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% confidence interval 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.

57. Section 8.3.2 (p.30 of 54) – The investigator proposed a formula to calculate the percent protection time

    i. % Bite Protection = $[(B_{NC}/F_C) – (B_T/F_C)]/ (B_{NC}/F_C)$

    ii. Where:

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

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

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

    b. and proposed to use RM-ANOVA to analyze the repeated measure data.

    c. The use of RM-ANOVA is not justified if the investigators propose to use the percent bite protection of each fabric × treatment on each subject as the response variable in the analysis. One of important assumptions in ANOVA (or RM-ANOVA) is the response variable is continuous and is not bounded. However, the percent bite protection is bounded between 0 to 100% and using ANOVA can result in an upper bound 95% CI for bite protection that exceeds 100%. EPA statisticians suggest the investigators need to utilize a generalized linear

model for binomial distribution using a log link function, using subject as random effect (SAS PROC GLIMMIX) to analyze the data of this study design.

d. For example, since the endpoint in this study is the total count of mosquitoes with confirmed bites out of total number mosquitoes in each testing cage, the investigators may wish to consider using generalized linear mixed-effect models for count data (with link = log and offset = log(total number of mosquitoes in each cage)) to analyze the repeated measures data and estimate the % Bite Protection.

58. Alternatively, the investigators may consider to evaluate the efficacy of the product by using the Odds Ratio (odd of bite of a treatment/odd of bite of the control) of bites instead of % Bite Protection. In this case, the generalized linear mixed-effects models can be used with a link = logit. (p. 30 of 54, §8.3.2)
59. Identify this formula as Abbott's formula. (p. 30 of 54, §8.3.2)
60. "F_C = female insects in the cage that are capable of biting at the start of the 15 minute exposure period." Earlier it is stated that 200 ± 25 mosquitoes will be used per test cage. How will this number be recorded if an approximation is used? It should be noted in section 7.6 that all mosquitoes will be counted after the test is completed and mosquitoes are frozen. (p. 30 of 54, §8.3.2)
61. The investigators mentioned and referenced a website for Kaplan-Meier estimator. EPA statisticians don't believe that the Kaplan-Meier Estimator is relevant or can be used in this study. More specifically, it appears that the investigators copied information from other studies and pasted into the protocol of this study. However, Kaplan-Meier Estimator might be used in those other studies, but it will not be used in this study because this study is designed to measure bite protection, not the "time to event" measure of the Kaplan-Meier statistic. (p. 31 of 54, §8.3.5)
62. Raw numbers for mosquitoes with visible blood in the abdomen (obviously fed) as well as those mosquitoes which need to be crushed to see that blood feeding occurred should be provided. (p. 31 of 54, §9.1.1.5)
63. The storage and disposal process should be described in more detail than what was provided. (p. 35 of 54, §12.2)
64. Add lines for time started and time stopped for each exposure. (p. 36 of 54, Appendix I – raw data sheet)

Attachments:

1. EPA Protocol Review
2. Sample Size Estimation for Design of Mosquito Laboratory Studies
3. EPA Completeness Checklists
4. i2LResearch Chart Depicting 5 Test Days

Attachment 1 - EPA Protocol Review

Title: Laboratory bioassay to assess the efficacy of permethrin applied to military uniform fabric for protection from mosquito bites (*Aedes aegypti* and *Anopheles quadrimaculatus*), after 0, 20 and/or 50 washings

Date: September 29, 2016

Principal Investigator and any sub-investigators: Timothy Foard, Study Director

Participating Laboratory:
i2LResearch USA, Inc.
1330 Dillon Heights Avenue
Baltimore, MD 21228-1199

Sponsor:
Arjan Giaya PhD, MBA
LaunchBay LLC
27 Ireta Rd, Shrewsbury, MA 01545

IRB:
Schulman Institutional Review Board
4445 Lake Forest Drive, Suite 300
Cincinnati, OH 45242.

1. Societal Value of Proposed Research

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

In this study, military uniform fabric will be treated with permethrin. Treated fabric will be compared to untreated fabric to determine if the treatment can decrease mosquito bites to human skin that is covered by the sleeve. Specifically, this study will determine the bite protection level of permethrin-treated U.S. Military Flame Resistant Army Combat Uniforms (FRACUs) and Army Combat Uniforms (ACUs) treated at an application rate of 0.52%, and to assess the bite protection performance after 0x, 20x, and 50x washes against two species mosquitoes (*Aedes aegypti* and *Anopheles quadrimaculatus*). (p. 4 of 54, §1.1.1)

(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 that can be used to evaluate the bite protection of fabrics that are treated or impregnated with substances that repel or reduce arthropod bites to determine if the treated fabrics provide sufficient protection against mosquitoes.

The rationale for testing is to collect data to show that military uniforms impregnated with 0.52% permethrin through the Invexus^{TM} process will provide ≥90% mean bite protection against mosquitoes for up to 50 washings. The data supporting currently

registered military uniforms impregnated with 0.52% permethrin do not show ≥90% efficacy through 50 washes using human subjects.

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 the permethrin treatment compared to bites received through the untreated control fabric; 2) the relative bite protection capability of one fabric type (FRACU) *vs.* another fabric type (ACU) if both are tested on each test subject; and 3) the bite protection efficacy of a new product(s) for EPA registration. Because these data are acquired in a laboratory setting, there are fewer associated risks than determining where the optima lie using wild-type mosquitoes in a field setting. Bites are measured in these studies by the presence of a blood meal in the abdomen of the female mosquito (pp. 27-28 of 54, §7.6.2-7.6.4).

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

EPA will review the study to satisfy product specific efficacy data requirements and acceptable label claims for repellent 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. No previous testing of this product against mosquitoes under the proposed use pattern has been conducted.

(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.

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 assess the efficacy of up to two permethrin treated Army Combat Uniforms: (ACU) and Flame Resistant Army Combat Uniforms (FRACU). The materials will be tested unwashed, 20 times washed, and 50 times washed, for protection against bites by mosquitoes. The fabric is treated with permethrin via the Invexus™ process. The data collected in the study will be used to support product registration." (p. 4 of 54, §1.1.1).*

(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 and amended to explain, in more detail, the following items noted on pages 23-28 of this review.

2.1 Statistical Design

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

The original protocol submitted by i2LResearch USA, Inc. proposed that 8 individuals serve as test subjects. However, the justification for the proposed sample size provided in the initial protocol appears to pertain to studies where Complete Protection Time (time from application to a confirmed mosquito bite) is evaluated, which is not applicable for this study design where Percent Bite Protection will be evaluated. After consultation with EPA, i2LResearch USA, Inc. has agreed to EPA's proposed sample size described below.

EPA has done a power analysis for a similar study previously reviewed by HSRB. In this past HSRB study submission, the bite-through rate of the control group (non-treated FRACU fabric) was assumed to be set as 20% and 50%. In the proposed study design, i2LResearch USA, Inc. indicated that the bite-through rate of the control ACU fabric is expected to be about 10%. The aforementioned study previously reviewed by HSRB examined bite-through rates of FRACU fabric only – not ACU fabric. FRACU fabric is constructed with a lower fiber density in comparison to ACU fabric (pp. 26-27 of 54, §3.0), and therefore presents less of a physical barrier to mosquitoes attempting to bite through it. Consequently, bite-through rates with control ACU fabrics are expected to be lower than with control FRACU fabric. This affects the number of test subjects required to achieve an acceptable level of statistical power to answer the charge question presented to the HSRB.

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 ACU fabric is 10% and the true percent bite protection of the ACU fabric is at least 80%. The Agency's 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 6%, the study requires a sample size of 15 subjects, given that a true bite-through rate in the control is 10% and the true percent bite protection is 80% (Table 4). To reach 80% power of achieving the width of the 95% confidence interval of the estimated percent bite protection of less than 3%, our simulations indicate that the study requires a sample size of 10 subjects, given that a true bite-through rate in the control is 75% and the true percent bite protection is 80% (Table 5). A detailed report of the Agency's power analysis is presented in Attachment 2. 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).

Note that the desired precision (expressed as the 95% confidence interval half-width) differs between the FRACU and the ACU, with the ACU half-width criterion being set at 6% and the FRACU at 3%. 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 assumed parameters. That is, increases in the number of subjects beyond these numbers do not substantially narrow half-width the 95% confidence interval, 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 confidence interval. 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% confidence interval 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.

(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 set as described on p. 26 of 54 in §7.2.1. 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. Both arms will serve as a control treatment replicate, one for FRACU fabric and the other for ACU fabric.

(c) How is the study blinded?

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

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

Subjects will be recruited from a group from the general public at least three times greater than the number required for the study. A recruitment firm will initially advertise to potential subjects and compile a pool of potentially interested subjects who respond to a secure study website and fill out a form with their contact information. This pool will generally represent the demographics of the members of the military who are the intended users of the treated clothing.

For each test day, 15 test subjects and 15 alternates that fulfill the inclusion/ exclusion criteria will be selected from the pool of subjects by a subject allocation table via appropriate software (such as Excel or Minitab). The selection will be achieved by randomly selecting the test subjects' assigned code numbers. EPA recommends 15 alternates, however only 10 of the alternates (5 males and 5 females) would need to be on site on the morning of the test day; it's unlikely that more than 10 subjects will withdraw on the test day. The decision as to whether an alternate is needed will occur within the first 2 hours of the test, during the preparation time and control exposure, but before all the treatment exposures.

As described in Section 2 of this review, the preferred sample size is 15 test subjects for testing the ACU fabric. The preferred sample size is 10 test subjects for testing the FRACU fabric. Ten subjects will test both fabrics simultaneously (one fabric type per arm) and, in this instance, one species of mosquitoes will be tested per test day.

The 5 additional test subjects needed for the additional ACU replicates will test both mosquito species simultaneously by wearing ACU fabric on both arms and exposing each arm to one of the two species being tested. Each test subject serves as his/her own control. All test subjects will be exposed to both untreated and treated ACU and FRACU fabric. The additional 5 subjects will be exposed to only untreated and treated ACU fabric.

(e) Can the data be statistically analyzed?

Yes. See (f) below.

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

Based on the Board's past recommendation, 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, SPSS, R, S-Plus, and Stata (p. 31 of 54, §8.3.4-8.3.5).

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

The analysis will provide the overall bite protection values for each treatment group and the controls. The analysis employed by EPA statisticians addresses mean bite protection values and associated uncertainties.

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

EPA has done a power analysis for a similar study previously reviewed by HSRB. In this past HSRB study submission, the bite-through rate of the control group (non-treated FRACU fabric) was assumed to be set as 20% and 50%. In the proposed study design, i2LResearch USA, Inc. indicated that the bite-through rate of the control ACU fabric is expected to be about 10%. The aforementioned study previously reviewed by HSRB examined bite-through rates of FRACU fabric only – not ACU fabric. FRACU fabric is constructed with a lower fiber density in comparison to ACU fabric (pp. 26-27 of 54, §3.0), and therefore presents less of a physical barrier to mosquitoes attempting to bite through it. Consequently, bite-through rates with control ACU fabrics are expected to be lower than with control FRACU fabric. This affects the number of test subjects required to achieve an acceptable level of statistical power to answer the charge question presented to the HSRB.

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 ACU fabric is 10% and the true percent bite protection of the ACU fabric is at least 80%. The Agency's 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 6%, the study requires a sample size of 15 subjects, given that a true bite-through rate in the control is 10% and the true percent bite protection is 80% (Table 4). To reach 80% power of achieving the width of the 95% confidence interval of the estimated percent bite protection of less than 3%, our simulations indicate that the study requires a sample size of 10 subjects, given that a true bite-through rate in the control is 75% and the true percent bite protection is 80% (Table 5). A detailed report of the Agency's power analysis is presented in Attachment 2.

Note that the desired precision (expressed as the 95% confidence interval half-width) differs between the FRACU and the ACU, with the ACU half-width criterion being set at 6% and the FRACU at 3%. 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 assumed parameters. That is, increases in the number of subjects beyond these numbers do not substantially narrow half-width the 95% confidence interval, 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 confidence interval. 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% confidence interval 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.

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 FRACU and ACU fabric and formed into "sleeves" by using clips to secure the two leading edges (connecting the parallel edges) (p. 22 of 54, §6.1). The test material's active ingredient, permethrin, has a low acute and chronic risk profile (see section 4 below) Subjects with known allergic reactions (§8.1.2) are excluded from participation in the test.

Subjects will be exposed to laboratory reared populations of mosquitoes free of mosquito-borne pathogens in the laboratory (p. 6 of 54, §2.1.2). Subjects with known allergic reactions to mosquito bites will be excluded from research participation (p. 18 of 54, §2.10.2.2).

(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 military uniforms impregnated with 0.52% permethrin through the Invexus™ process will provide ≥90% mean bite protection against mosquitoes for up to 50 washings. The data supporting currently registered military uniforms impregnated with 0.52% permethrin do not show ≥90% efficacy through 50 washes using human subjects.

(c) What duration of exposure is proposed?

The exposure period is eight 15-minute periods (2 hours total) for both arms of each subject.

However, for the 5 subjects who will only test the ACUs, they will be exposed to 4 different ACUs (the control and fabric with 50x, 20x, and 0x washings). Because they can test both mosquito species at the same time (one per arm), the duration of exposure for those 5 subjects will be 1 hour instead of 2.

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 females mosquitoes in each test. The proportion of blood-fed/total will be calculated and expressed as a percentage value. This calculation will be performed for untreated control sleeves and treated sleeves (0x, 20x, and 50x washes).
- For each test set, the treatment % bite values will be corrected to account for the bite-through values in the untreated control using Abbott's Formula.
- The overall % bite protection will be calculated and expressed as a mean value for each treatment: 0x, 20x, 50x washes for coats and trousers.

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

The data form for each 15 minutes sleeve test is presented in Appendix I on page 36 of 54.

(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 assist subjects with placing the test sleeves on their arms and excluding all exposed skin from mosquito exposure.
- Laboratory technicians will assist subjects with insertion and removal of their arms in/from the cages.
- Laboratory technicians and the study director will track test sleeve samples and closely monitor the testing.
- Alternate subjects will be enrolled to ensure adequate sample size.
- Counts of blood-fed mosquitoes and the total number of mosquitoes in the cage will be determined by a research technician.
- The test sleeve samples will be assayed by the Analytical Unit (p. 24 of 54) and the amount of permethrin reported as a surface concentration of permethrin in units of mg/cm^2, which is commonly done for treated fabrics.

(c) What QA methods are proposed?

As explained in §1.2.2 on p. 4 of 54 a separate, professional Quality Assurance Unit (QAU) will inspect the study: "Quality assurance of this study will be carried out in accordance with Good Laboratory Practice (GLP) Standards 40 CFR 160. Written reports of all findings from the Quality Assurance Officer will be provided to the study director and management. Any part of the study found by the Quality Assurance Officer to be likely to affect the integrity of the study will be brought the attention of the study director. A statement signed by the Quality Assurance Officer listing the phases inspected, inspection dates, and dates reported to the study director and management will be included in the final report. All deviations and amendments will be recorded and reported as per GLP guidelines.

The quality assurance unit of the analytical laboratory will provide the study director and the study director's management with relevant data, process, and report audits to meet Environmental Protection Agency GLP requirements."

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

Uncertainty is addressed in the experimental design and selection of the number of subjects as described in §2.3. The objective of the data analysis is to estimate the mean level of bite protection and associated 95% confidence intervals for different 'treatments' [i.e. different combinations of fabric types (FRACU and ACU), number of washes, and mosquito species]. The numbers of blood-fed and total female mosquitoes found with treated and control fabric for each subject will be analyzed using a generalized linear model for binomial distribution using a log link function, using subject as random effect (SAS PROC GLIMMIX) (p. 31 of 54, §8.3.4).

2. Subject Selection

3.1 Representativeness of Sample

(a) What is the population of concern?

The population of concern is U.S. military personnel who would wear ACUs and FRACUs treated with permethrin.

(b) From what populations will subjects be recruited?

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. A Spanish language advertisement will also be posted online using the same media, plus an online Spanish language newspaper that advertises within the recruitment area. The advertisement 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. The results of testing the permethrin-treated fabric should be as generalizable as possible to the target population of permethrin-treated fabric users. Researchers will try to ensure that the ethnic groups represented in the demographics of the members of the military who are the intended users of the treated clothing have the opportunity to volunteer for the study. Every effort will be made to achieve the appropriate demographic composition, via a stratified random sample of the pool of recruited subjects. The final study will specify the demographics of subjects who participated in the study, based on gender, age, and ethnic background, due to availability of test subjects on each test day.

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

The researchers will research and identify the demographics of the members of the military who are the intended users of the treated clothing and will try to ensure that recruitment pool is representative of the associated demographics.

(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 assuming EPA's comments, identified in red below, are incorporated.

<u>Individual Inclusion/Exclusion Criteria</u>

The subject must be between 18-55 years old and provide proof of age with a driver's license, passport or other valid identification.
The subject must be able to read and speak English fluently.
The subject must not be an immediate employee of LaunchBay or of i2L, or be immediately related to employees or owners of either company. "Immediately related" includes spouses or the parent of spouses, their adult children, siblings, cousins, nephews, and aunts.
The subjects must have a reliable form of transportation to get to and from the i2L laboratory.
Subjects must feel they are healthy enough to participate in the study and do not have any health conditions that may affect the study or be worsened by the wearing of insecticide-treated fabrics.
The subjects must be willing to be exposed to and bitten by mosquitoes and cannot be phobic of mosquito bites.
The subjects must have no known allergies or sensitivities to mosquito bites, insect repellents or insecticide-treated fabrics.
Subject must be a user of treated fabrics, insect repellent products and/or other products used to repel biting mosquitoes.
The subjects must not be hypersensitive or allergic to latex or skin care products. The subjects must be free from open cuts, scrapes, skin disease, and skin problems such as eczema, psoriasis or atopic dermatitis.
The subjects must be willing to wear short sleeves on their scheduled test day(s) (other clothing choices will be optional).
The subjects must agree to inform the Study Director or other staff if they have violated any study-related restrictions in the previous 12 hours (see 'Restrictions', below) as soon as possible, so a decision can be made whether to continue inclusion of the subject in that day's testing.
The subjects must be able to sit in a chair for at least 15-minute mosquito exposure durations (with breaks for limb stretching and movement given at reasonable intervals).
Confirmation will be needed that the mosquitoes in the study are attracted to the subjects' untreated skin (this confirmation will occur in two parts: first an attractiveness test will be conducted on each subject and second during the test, when each subject acts as their own negative control.)
The subjects must be willing to follow the study procedures as explained and be willing to sign an ICD.
The subjects must not be pregnant or be breast-feeding. To confirm that participating test subjects are not pregnant, at the beginning of each test day, female subjects will be required to perform an over–the-counter pregnancy test that will be supplied by i2L.

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

None. People with a relationship to the study sponsor or testing facility are excluded from becoming subjects. The subject must not be an immediate employee of

LaunchBay or of i2LResearch, or be immediately related to employees or owners of either company. “Immediately related” includes spouses or the parent of spouses, their adult children, siblings, cousins, nephews, and aunts.

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

No.

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

Recruiting Subjects:

Subjects will be recruited from the 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. A Spanish language advertisement will also be posted online using the same media, plus an online Spanish language newspaper that advertises within the recruitment area. The advertisement 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. The results of testing the permethrin-treated fabric should be as generalizable as possible to the target population of treated-fabric users. Researchers will try to ensure that the ethnic groups represented in the demographics of the members of the military who are the intended users of the treated clothing, have the opportunity to volunteer for the study. Every effort will be made to achieve the appropriate demographic composition, via a stratified random sample of the pool of recruited subjects. The final study will specify the demographics of subjects who participated in the study, based on gender, age, and ethnic background, due to availability of test subjects on each test day.

Informing Subjects:

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, listen to the other information to be provided by researchers during training as described the protocol, and receive answers to any questions the subjects may have. Contact information is included on the consent form for any individual who has additional questions or if further clarification is desired, after they have attended the training session.

Individuals will continue to be contacted until a pool of potential subjects exists that is at least two times that required for the study, 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. If desired, interested individuals may provide an email or mailing address to which the informed consent form can be sent for advance review.

Prior to participating in any study-related procedure, each potential subject will meet in person with the Study Director, or another designated i2L staff member who is fully familiar with the protocol and the consenting procedure, for a training session. Logistics for the training session and details to be shared with subjects during the training are listed in the protocol. Test subjects will be asked to provide proof of age with a driver's license, passport, or other valid identification upon arrival to the training session. The potential subjects will be provided with copies of the Informed Consent Document (ICD) and will then be asked to read the entire document. After the potential subjects have completed reading this document, the staff member leading the discussion will ask the subjects if they have any questions regarding the information in the consent form, the study and their role in the study. Any questions will be answered. The trainer will let subjects know that if a private matter needs to be discussed regarding their role in the study, then time will be made for this discussion once the general training session is over. Each subject will be asked comprehension questions listed in the protocol to help ensure their understanding of the consent form. The potential subject will be given ample time to ask and have all questions answered.

If an individual still wishes to enroll in the study, he or she will be asked to sign the ICD, which will be witnessed by the staff member who led the consent discussion. The subject will then be given a photocopy of the signed ICD and testing schedule. All test subjects will attend a training session prior to participation in each test day. The only exception is if a test subject is participating in a second day of testing and their last training session occurred within two weeks prior to their second test day.

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

Subjects will be recruited from the Baltimore, Maryland area, via advertising through digital and social media. There will be no connection or communication between the researchers and the potential subjects' employers, which minimizes the potential for coercion or undue influence. In addition, employees of the study director or sponsor are excluded from participation; more specifically, subjects cannot be an immediate employee of study sponsor LaunchBay or of i2LResearch, and cannot be immediately related to employees or owners of either company. "Immediately related" includes spouses or the parent of spouses, their adult children, siblings, cousins, nephews, and aunts.

3.3 Remuneration of Subjects

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

Each subject will be paid $30 for taking part in each training session**.** 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.

For each test day, test subjects will be paid $104.00 ($13 per hour) for any length of participation up to 8 hours (with exceptions noted below). 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.

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 and after the control exposure, but before all the treatment exposures. If an alternate is asked to replace a subject, he or she will be paid at the same rate as other test subjects, as described above.

If the Study Director or other i2L USA staff ask a subject to withdraw from the test and they have 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.

The Study Director or other designated i2L USA 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 nearest hour) at a rate of $13.00 per hour. This will not affect payment for any previous test days that had been completed.

(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 checks sent in the mail, or hand delivered while they are on site at the i2L facility. i2L issues checks on the 15th and on the last day of each 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 MOEs are 6,700 and 26,000 for military personnel and garment workers, respectively. 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:

- 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 in rabbits study 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 risks to subjects of the proposed research?

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

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

To eliminate the risk of contracting any mosquito-borne diseases, the study will be conducted only with laboratory-reared mosquitoes, which are not known 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 (*Aedes aegypti*) and malaria pathogens (*An. quadrimaculatus*). In addition, the supplier will document that these laboratory-reared mosquitoes are disease free, and that they have never received a blood meal.

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

A 2006 Occupational and Residential Exposure Risk Assessment for permethrin identified a dermal NOAEL of 500 mg/kg/day, based on a 21 day dermal toxicity study in rats. Dermal absorption of permethrin is estimated to be 15% or less. Given the size of the fabric samples proposed in this study design (144 in^2) and the amount of permethrin applied during the impregnation process (0.125 mg/cm^2), the amount of permethrin per fabric sleeve is calculated as 116 mg/sleeve. Assuming an average subject weight of 70 kg, the maximum dose (assuming no loss of permethrin) is 1.69 mg/kg/subject. This maximum dose is more than 300x less than the dermal NOAEL observed in rats.

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

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?

<u>Physical discomfort of enduring multiple mosquito bites.</u>

- Candidates who are allergic, hypersensitive to or phobic of mosquito bites are excluded.
- Subjects are alerted in the consent form to the possibility of experiencing a skin reaction to mosquito bites, and are advised to inform the study director or other staff member, if they believe they are having a reaction.
- Over-the-counter topical anti-itch gel or cream to relieve itching will be available for use by subjects after completion of the study.
- A nurse familiar with the protocol will be on-call to provide advice or assistance in case medical advice is needed during the test day.

<u>Exposure to mosquitoes and mosquito-borne diseases</u>.

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. 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 (*Aedes aegypti*) and malaria pathogens (*An. quadrimaculatus*). In addition, the supplier will document that these laboratory-reared mosquitoes are disease free, and that they have never received a blood meal.

Adverse reaction to test substances.

- Candidates who are known to be sensitive to insecticide-treated fabrics or insect repellents are excluded.
- It is recommended that the protocol exclude subjects with cuts, scrapes, skin diseases or skin conditions such as psoriasis, atopic dermatitis or eczema. These conditions could increase the possibility of a reaction to test material.
- 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.

Psychological risks related to pregnancy testing.

- The protocol provides for discrete handling of the pregnancy testing that is required of female subjects on each test day.
- Female subjects self-administer the pregnancy test in a private bathroom.
- After completing the test, each female subject is asked if she would like to continue in the study. If her answer is no, then no further questions are asked; she will not be asked to share the result with anyone. If her answer is yes, the result of the pregnancy test will be verified by only one member of the research team who will be female.
- For females who proceed with the testing, the result of the pregnancy test is not recorded and kept confidential.

Unanticipated Loss of Confidential Information

- As noted above, all efforts will be taken to maintain the confidentiality of the pregnancy test results. The test results will not be recorded, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director.
- In addition, the subjects' identities and participation in the study 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.

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

The study may be terminated early if adverse events occur among the subjects, by LaunchBay's decision, or for other reasons. The decision to terminate will be made by LaunchBay in conjunction with the Study Director.

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 protocol section 2.10.10.

If a subject is unattractive to target mosquito species during the control exposure, they will not continue in the study.

In addition, the consent form states that:

"*The Study Director or the Study Sponsor can stop your participation at any time without your consent for the following reasons:*

- *If any condition or circumstance may jeopardize your welfare, such as your experiencing increased risks or adverse reactions;*
- *If you fail to follow directions for participating in the study;*
- *If it is discovered that you do not meet the study requirements;*
- *If the test day or study is cancelled."*

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

The protocol discusses the following:

- 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.
- 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.
- The on-call nurse 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.
- Any problems or adverse effects will be promptly reported to LaunchBay and the IRB consistent with IRB reporting procedures.
- Subjects will be informed in a timely manner both orally and in writing of any significant new findings discovered during the course of this study which may influence their continued participation. The Study Director and recruitment firm

will keep on file the phone numbers, email addresses, and street addresses for each study participant as a means to contact them if needed.

- New findings will also be reported, in writing, to LaunchBay and the IRB in a timely manner consistent with IRB reporting procedures.
- The nearest local hospital to i2L's laboratory will be located and directions identified prior to any study-related procedures taking place.
- Should a Type 1 allergic reaction (i.e. anaphylaxis) occur on the test day or if any other serious injury or medical issue occurs, 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 or Principle Investigator will abort the test day.
- 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, they should inform i2L staff and seek medical advice. All adverse effects will be reported to LaunchBay and the IRB within five business days of their being noted, or within the same day in the case of serious adverse effects. LaunchBay will also comply with FIFRA section 6(a)(2) adverse effects reporting requirements as applicable.
- If i2L or LaunchBay learns of new findings or new information relating to the safety or hazard of any of the test substances, i2L will contact the subjects and advise them accordingly both orally and in writing in a timely manner. The IRB will also be advised consistent with IRB reporting procedures.

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

Subjects are clearly and repeatedly informed that they may remove themselves for any reason from the study at any time. All subjects are asked to immediately tell the study director or study staff if they believe they are experiencing a reaction or feel ill during the study. The consent form also states that if, after participating in the study, a subject believes he or she has become ill as a result of their participation in the study, they should contact i2LResearch, with two different numbers provided (for during and after office hours) or contact the on-call nurse anytime, 24-hours a day. The nurse's telephone numbers is provided.

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

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

The protocol does not provide an end date for post-exposure monitoring or follow-up. So, the duration is long enough to discover adverse events which might occur. The consent form states:

"In Case of Injury

If you are injured as a result of wearing the permethrin-treated fabric or from procedures done for the purpose of this study, the Study Sponsor 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. There are no plans to provide other compensation beyond that which is listed in this informed consent document. You will not lose any of your legal rights or release the Sponsor, the study staff, or study site from liability for mistakes or intentional misconduct by signing this consent document.

Questions

If you have any questions about this study or suffer a research-related reaction, call i2L USA at 410-747-4500, or call 202-905-1401 after office hours. There will also be an on-call nurse for non-emergency related queries related to your participation in the study (410-598-7436).

If you have any questions about your rights as a research subject, and/or concerns or complaints regarding this research study, you should write to Schulman IRB 4445 Lake Forest Drive – Suite 300, Cincinnati, Ohio 45242, or call toll-free 1-888-557-2472 during business hours Monday – Friday 8:00 a.m. to 6:00 p.m. EST.

If you have a serious reaction to the test material, the study director will tell you what ingredient(s) it contains.

You do not waive (give up) any of your legal rights by signing this consent form."
-End of excerpt from consent form -

Regarding the protocol, section 9.2.9 states:

"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, they should inform i2L staff and seek medical advice. All adverse effects will be reported to LaunchBay and the IRB within five business days of their being noted, or within the same day in the case of serious adverse effects. LaunchBay will also comply with FIFRA section 6(a)(2) adverse effects reporting requirements as applicable." – *End of excerpt* -

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

The consent form states:

"In Case of Injury
If you are injured as a result of wearing the permethrin-treated fabric or from procedures done for the purpose of this study, the Study Sponsor 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. There are no plans to provide other compensation beyond that which is listed in this informed consent document. You will not lose any of your legal rights or release the Sponsor, the study staff, or study site from liability for mistakes or intentional misconduct by

signing this consent document."

The protocol states:

"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."

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 up to two permethrin-treated military uniforms, specifically U.S. Army Combat Uniforms (ACU) and U.S. Army Flame Resistant Army Combat Uniforms (FRACUs). The permethrin-treated materials will be tested unwashed, 20 times washed, and 50 times washed, for protection against bites by mosquitoes. The fabric is treated with permethrin via the Invexus™ process. The data collected in the study will be used to support product registration. Invexus™ Insecticide Treatment is a process by which the fabrics are treated with permethrin in a continuous, roll to roll treatment process in factory. The permethrin formulation is applied and adhered to fabric via a proprietary process that minimizes energy usage and process waste. The target levels of mean bite protection are ≥90% for the unwashed, 20 times washed, and 50 times washed permethrin-treated fabrics. The research has societal value because U.S. military personnel serving domestically and abroad are at risk of contracting mosquito-borne diseases, but the data supporting currently registered military uniforms impregnated with permethrin do not show ≥90% efficacy through 50 washes in human studies. The rationale for this testing is to collect data to show that military uniforms impregnated with permethrin through the Invexus™ process will provide ≥90% mean bite protection against mosquitoes for up to 50 washings. As intended, the data resulting from this proposed study will be used to support registration of either or both of LaunchBay's Invexus™ treated ACU and FRACU.

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

One beneficiary will likely be the sponsor who is seeking EPA-registration for

permethrin-treated clothing. Indirect beneficiaries would include the U.S. military soldiers who would wear permethrin-treated uniforms and civilians who may benefit from wearing permethrin-treated clothing.

(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 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 has full AAHRPP accreditation.

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

Yes.

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

Yes.

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

This is a protocol for third-party research involving what EPA has interpreted to be intentional exposure of human subjects to a pesticide. The study is being conducted with the intention of submitting the resulting data to EPA under the Federal

Insecticide Fungicide and Rodenticide Act (FIFRA). Thus, the primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply.

8. Informed Consent

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

Yes.

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

Yes.

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

Yes.

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

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

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

N/A

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

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

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

<u>Informing Subjects</u>:

Individuals from the recruitment 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, listen to the other information to be provided by researchers during training as described the protocol, and receive answers to any questions the subjects may have. Contact information is included on the consent form for any individual who has additional questions or if further clarification is desired, after they have attended the training session.

Individuals will continue to be contacted until a pool of potential subjects exists that is at least three times that required for a test day, 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. If desired, interested individuals may provide an email or mailing address to which the informed consent form can be sent for advance review.

Consent Meeting:

Prior to participating in any study-related procedure, each potential subject will meet in person with the Study Director, or another designated i2L staff member who is fully familiar with the protocol and the consenting procedure, for a training session. Logistics for the training session and details to be shared with subjects during the training are listed in the protocol. Test subjects will be asked to provide proof of age with a driver's license, passport, or other valid identification upon arrival to the training session. The potential subjects will be provided with copies of the Informed Consent Document (ICD) and will then be asked to read the entire document. After the potential subjects have completed reading this document, the staff member leading the discussion will ask the subjects if they have any questions regarding the information in the consent form, the study and their role in the study. Any questions will be answered. The trainer will let subjects know that if a private matter needs to be discussed regarding their role in the study, then time will be made for this discussion once the general training session is over. Each subject will be asked comprehension questions listed in the protocol to help ensure their understanding of the consent form. The potential subject will be given ample time to ask and have all questions answered.

If an individual still wishes to enroll in the study, he or she will be asked to sign the ICD, which will be witnessed by the staff member who led the consent discussion. The subject will then be given a photocopy of the signed ICD and testing schedule.

Training:

All test subjects will attend a training session prior to participation in each test day. The only exception is if a test subject is participating in a second day of testing and their last training session occurred within two weeks prior to their second test day.

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

Candidates are offered repeated opportunities to decide not to participate; participants are offered repeated opportunities to withdraw. Subjects will be

recruited from the Baltimore, Maryland area, via advertising through digital and social media. There will be no connection or communication between the researchers and the potential subjects' employers, which minimizes the potential for coercion or undue influence. In addition, employees of the study director or sponsor are excluded from participation; more specifically, subjects cannot be an immediate employee of study sponsor LaunchBay or of i2LResearch, and cannot be immediately related to employees or owners of either company. "Immediately related" includes spouses or the parent of spouses, their adult children, siblings, cousins, nephews, and aunts. Recruitment of alternate subjects reduces the likelihood that subjects might be reluctant to withdraw.

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. 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 the day of testing. The test results will not be recorded, and will not be disclosed to anyone other than the test subject, the verifying female employee, and/or the Study Director.

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

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

"Your Rights
You have been given an opportunity to discuss with i2L USA personnel any aspects of this document which are not clear to you. You have been informed that your consent must be freely given after you are certain that you understand the nature of the test, its purpose, and the procedures to be used, together with the discomforts, risks or other adverse effects you may experience during or after the test. **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.** You must contact the Study Director or any member of the study team if you wish to stop participating in this study. After you read, and sign to indicate your agreement to participate, you will receive a copy of the signed consent form for your files." – End of excerpt -

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

Each subject will be paid $30 for taking part in each training session**.** 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.

For each test day, test subjects will be paid $104.00 ($13 per hour) for any length of participation up to 8 hours (with exceptions noted below). 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.

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 and after the control exposure, but before all the treatment exposures. If an alternate is asked to replace a subject, he or she will be paid at the same rate as other test subjects, as described above.

If the Study Director or other i2L USA staff ask a subject to withdraw from the test and they have 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.

The Study Director or other designated i2L USA 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 nearest hour) at a rate of $13.00 per hour. This will not affect payment for any previous test days that had been completed.

Attachment 2

Note to HSRB: Please see separate file entitled, “Attachment 2 to EPA Review Memo on Protocol - Sample Size Estimation for Design of Mosquito Laboratory Studies.”

Attachment 3
§ 26.1111 Criteria for IRB approval of research
Protocol for Mosquito Bite Protection from Permethrin-treated 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 Bite Protection of Permethrin-treated 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 Bite Protection from Permethrin-treated 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	

40 CFR 26.1125 Submission of proposed human research for EPA review
Protocol for Bite Protection of Permethrin-treated 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	Please see note below.*
	§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.		See note	I2LResearch has provided the correspondence between i2L and Schulman Associates. EPA expects to receive the corresponding minutes on Sept 30, 2016 and will forward that to the 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

*i2LResearch discusses the advertisement to be used but needs to submit it to EPA and the overseeing IRB for review and approval prior to implementation.

Attachment 4 – i2LResearch Chart Depicting 5 Test Days

			Test Day 1	**Test Day 2**
Subject	Left arm	Right arm	*Aedes aegypti* required per exposure to both fabrics	*Anopheles quadrimaculatus* required per exposure to both fabrics (200 are required; however at least 400 mixed sex adults need to be reared to accomplish this)
1	control ACU	control FRACU	400	800
1	50x ACU	50x FRACU	400	800
1	20x ACU	20x FRACU	400	800
1	0x ACU	0x FRACU	400	800
2	control ACU	control FRACU	400	800
2	50x ACU	50x FRACU	400	800
2	20x ACU	20x FRACU	400	800
2	0x ACU	0x FRACU	400	800
3	control ACU	control FRACU	400	800
3	50x ACU	50x FRACU	400	800
3	20x ACU	20x FRACU	400	800
3	0x ACU	0x FRACU	400	800
4	control ACU	control FRACU	400	800
4	50x ACU	50x FRACU	400	800
4	20x ACU	20x FRACU	400	800
4	0x ACU	0x FRACU	400	800
5	control ACU	control FRACU	400	800
5	50x ACU	50x FRACU	400	800
5	20x ACU	20x FRACU	400	800
5	0x ACU	0x FRACU	400	800
			8000	**16000**

There will be at least a 1 month break in between test days 1/2 and test days 3/4. This will be required in order to ensure the supply of required numbers of mosquitoes, especially *Anopheles quadrimaculatus*, as they will need a sufficient period of focused and uninterrupted rearing in order to maintain the fitness of the colony and to supply the necessary numbers for test day 4. Furthermore, we feel that keeping test days 1/2 and 3/4 closer together (e.g. *Aedes aegypti* tested one week, *Anopheles quadrimaculatus* tested the following week) will better suit test subjects' schedules in regards to their participation in the study assuming some subjects may choose to participate in up to two test days.

Subject	Left arm	Right arm	**Test Day 3** *Aedes aegypti* required per exposure to both fabrics	**Test Day 4** *Anopheles quadrimaculatus* required per exposure to both fabrics (200 are required; however at least 400 mixed sex adults need to be reared to accomplish this)
6	control ACU	control FRACU	400	800
6	50x ACU	50x FRACU	400	800
6	20x ACU	20x FRACU	400	800
6	0x ACU	0x FRACU	400	800
7	control ACU	control FRACU	400	800
7	50x ACU	50x FRACU	400	800
7	20x ACU	20x FRACU	400	800
7	0x ACU	0x FRACU	400	800
8	control ACU	control FRACU	400	800
8	50x ACU	50x FRACU	400	800
8	20x ACU	20x FRACU	400	800
8	0x ACU	0x FRACU	400	800
9	control ACU	control FRACU	400	800
9	50x ACU	50x FRACU	400	800
9	20x ACU	20x FRACU	400	800
9	0x ACU	0x FRACU	400	800
10	control ACU	control FRACU	400	800
10	50x ACU	50x FRACU	400	800
10	20x ACU	20x FRACU	400	800
10	0x ACU	0x FRACU	400	800
			8000	**16000**

There will be another break of at least 1 month in between test days 3/4 and test day 5, for the same reasons as stated above.

Test Day 5

Note: This test day would only include the 5 additional subjects needed for testing the ACU fabric. (As a reminder, the testing of the FRACU fabric only requires 10, not 15, subjects.)

Subject	Left arm	Right arm	*Aedes aegypti* required per exposure to both fabrics	*Anopheles quadrimaculatus* required per exposure to both fabrics (200 are required; however at least 400 mixed sex adults need to be reared to accomplish this)
11	control ACU	control ACU	200	400
11	50x ACU	50x ACU	200	400
11	20x ACU	20x ACU	200	400
11	0x ACU	0x ACU	200	400
12	control ACU	control ACU	200	400
12	50x ACU	50x ACU	200	400
12	20x ACU	20x ACU	200	400
12	0x ACU	0x ACU	200	400
13	control ACU	control ACU	200	400
13	50x ACU	50x ACU	200	400
13	20x ACU	20x ACU	200	400
13	0x ACU	0x ACU	200	400
14	control ACU	control ACU	200	400
14	50x ACU	50x ACU	200	400
14	20x ACU	20x ACU	200	400
14	0x ACU	0x ACU	200	400
15	control ACU	control ACU	200	400
15	50x ACU	50x ACU	200	400
15	20x ACU	20x ACU	200	400
15	0x ACU	0x ACU	200	400
			4000	**8000**