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

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

June 29, 2017

MEMORANDUM

SUBJECT: Science and Ethics Review of a Protocol for Field Evaluation of Three Topically-Applied Insect Repellent Products Containing IR3535

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REF: Weeks, Emma, Study Director. (2017) Protocol for "Field Evaluation of Three Topically Applied Insect Repellent Products Containing IR3535 Against Mosquitoes in Florida." Unpublished document. April 23, 2017.

We have reviewed the referenced protocol for field testing for three topically-applied repellent products containing IR3535 against mosquitoes in Florida from both scientific and ethics perspectives. This EPA review evaluates the scientific aspects of the proposed research for an efficacy study to assess the efficacy of three topically applied repellents containing IR3535. 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. All elements of required documentation are provided in the submitted protocol package and supplementary documentation of review by University of Florida Institutional Review Board (IRB).

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, assuming that protocol is amended to address all of EPA's comments. 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 efficacy and protection time of three topically-applied skin repellents. Efficacy at preventing mosquitoes from landing will be expressed as Complete Protection Time (CPT), which is defined as the time between application of the repellent product and the occurrence of the first landing in a 5-minute test, followed by a confirmatory landing within 30 minutes. The research has societal value because people are at risk of contracting mosquito-borne diseases, and such risks can be mitigated by the use of insect repellent products. There are no data showing the necessary efficacy of IR3535 in human studies. The rationale for this testing is to collect data to estimate the CPT for products containing IR3535. As intended, the data resulting from this proposed study will be used to support registration of topically-applied repellents containing IR3535.

2. **Subject Selection:** With regard to the number of subjects who will participate in the field testing of each of the three products, as further explained in EPA's comments in section D.2. and Attachment 5, in order to generate statistically-sound data, the preferred sample size is 13 test subjects for each product at each site (minimum of 5 subjects of each gender). In addition, 2 untreated controls will participate in each field test, so a total of 15 subjects will be involved in each test. On each day, subjects will be randomly assigned to serve as a test subject or untreated control. In addition, 5 subjects will be enrolled as alternates, to take the place of any test subjects who withdraw before or on the day of testing (at least 2 subjects of each gender).

   The efficacy of each product (lotion, wipe, spray) will be determined through field testing at two sites. Each field test will involve a single product. As a result, up to 6 test days with 15 test subjects on each day will be required to conduct the study (up to 90 subjects total). Subjects may choose to participate one, some, or all test days, if they desire and are eligible. When one or more test subjects participate in more than one test day, their 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. Five additional subjects (at least 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. Subjects will be assigned as subjects or alternates randomly during the screening process; on the test day, each person will be randomly assigned as a test subject or untreated control subject. Therefore, a total of 20 subjects (15 subjects and 5 alternates) will be selected for each test day, with up to 120 subjects

(with approximately half of each gender) participating over six test days. The decision as to whether an alternate is needed will occur within the first hour of the test, when the product is applied to test subjects. An alternate who is not needed to replace a test subject will be able to leave.

Subjects will be recruited from the University of Florida and surrounding area, via advertising posted on bulletin boards in the area and through other mediums as necessary in order to ensure that there is equity of access to participate in the study. The advertisement provides information about the study and a phone number and email address for the Primary Investigator (Dr. Emma Weeks). The results of testing IR3535 products should be as generalizable as possible to the target population of skin-applied insect repellent users. Every effort will be made to achieve an appropriate demographic composition of the pool of recruited and enrolled 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 potential hazards associated with these tests including exposure to mosquitoes and disease vectors, physical discomfort of mosquito bites, being outside in a hot, humid climate, adverse reaction to the test substances, unanticipated loss of confidential information, and psychological risks related to pregnancy testing. The protocol notes that risks will be minimized as follows. To mitigate risks from exposure to mosquitoes and disease vectors, the testing sites will be selected from areas that have been monitored weekly for a month prior to the testing, and all mosquitoes captured during the monitoring phase will be tested for pathogens. Testing will not be conducted in areas where mosquito-borne pathogens have been identified. The Primary Investigator will work with the local health departments and mosquito control districts, which have active monitoring programs, to ensure that no vector-borne illnesses have been identified at the field test sites.

   To minimize the discomfort associated with mosquito bites, candidates known to be sensitive to or phobic of mosquito bites will be excluded. In addition, participants will be instructed to wear light, loose-fitting clothing that fully covers their bodies and will be provided with a head net to wear during the testing. Only the area to be treated with the repellent will be exposed to mosquitoes during the test period. In addition, untreated control subjects will only expose their lower leg until the requisite number of mosquito landings have been observed for each period during the testing.

   To protect subjects against the risks associated with being outside for extended periods in a hot, humid climate, subjects will be provided with snacks, water, and other drinks. A shaded area with chairs will be available for subjects' use during the periods between the test periods. To protect against the risk of irritation from exposure to the test substance, people who are sensitive to insect repellents and those with open cuts, scrapes, skin disease and skin problems will be excluded.

EPA's suggested edits to the protocol incorporate additional protections to keep the subjects' identities and results of pregnancy testing private. Practical steps to minimize subject risks have been described in the protocol, and the remaining risks have a low probability of occurrence.

To minimize the risk of contracting any mosquito-borne diseases during the field testing, mosquitoes will be collected by aspirator after they land on subjects but before they bite them. All participants will be trained in aspirating mosquitoes and spotting mosquito landing behavior. To verify that no mosquitoes collected during the study in the study carried any diseases, pooled groups of the mosquitoes will be screened for pathogens. *Ae aegypti* will be screened for RNA of Zika virus. All *Culex* mosquitoes will be screened for RNA of West Nile virus.

To minimize the risk of contracting any mosquito-borne diseases during the lab-based mosquito attractiveness test, the cages will be populated with mosquitoes from a colony reared in the laboratory for over 10 years. Mosquitoes from this colony will also be screened for Zika virus and West Nile virus.

4. **Benefits:** This research offers no benefits to subjects. 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 insect repellent products containing IR3535. These repellent products could lead to fewer mosquito bites and reduced incidents of vector-borne illnesses.

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

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

7. **Informed Consent:** With the agency's comments addressed, 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/identifier. The study records will be maintained in locked cabinets, and electronic files kept on a password-protected computer server

or encrypted electronic storage devices. 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

The University of Florida and the London School of Hygiene and Tropical Medicine/arctec 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). They 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 titled "Revised Protocol with EPA Comments Incorporated." After the HSRB completes its review of the protocol and relays its recommendations to the EPA, the EPA, University of Florida, and London School of Hygiene and Tropical Medicine 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.

The EPA's ethics comments are provided below and organized by section headings used in the protocol. Minor comments on typographical errors have not been included here. Although EPA has proposed adding sections and renumbering, the numbers in EPA's comments refer to the numbering listed in the protocol submitted to EPA. In addition, EPA has provided ethics-related comments on the informed consent materials; these are provided to the HSRB as a separate file.

Study Synopsis

1. Under "Name(s) of Product" and "Type of Product", list the percent active ingredient (e.g., 15% IR3535) for each product formulation. Make this change throughout the protocol and in all related materials, including the informed consent document.
2. Under "Number of Participants", revise the description as follows: "~~The sample size calculations (based on 90% power and a 5% significance level) require 10 participants. Two untreated participants will also~~ Based on power analysis, a sample size of 13 test subjects for each site/product combination in this study design would provide sufficient power (>0.90) to obtain a ratio of the lower limit of 95% CI of the estimated median CPT

/ estimated median CPT is ≥ 0.6, where the ratio of the lower limit of 95% CI of the estimated median CPT/estimated median CPT expresses the precision of estimated median CPT. Two additional subjects will serve as untreated controls for each field test to monitor the landing rate throughout the study. An additional ~~6~~ 5 participants ~~could be~~ will be enrolled as ~~alternatives~~ alternates to replace any test subjects who drop out before testing begins. In total, up to 20 people could be necessary for each test day. Assuming no subjects participate as test, untreated control, or alternate subjects more than once, total of 120 people could be recruited to complete all testing outlined in this protocol." Revise discussion of the number of subjects (test, untreated control, alternates) to be consistent with EPA's recommended sample size throughout the protocol.

3. Under "Study Duration", eliminate discussion of a dose determination study, which would be unnecessary exposure to subjects. In addition, revise the anticipated length of the test period to 16 hours to account for the expected product efficacy length to exceed 12 hours.
4. Under "Inclusion Criteria", revise as follows:
    - Willing to complete mosquito handling training ~~and complete dose determination assays~~
    - Able to withstand exposing the lower leg to mosquitoes for at least 5 minutes at a time
    - Able to operate an aspirator
    - Able to speak and understand English
5. Under "Exclusion Criteria", revise as follows:
    - Students of the primary investigator or any other University of Florida faculty or researchers involved in the study
    - Unable to speak and understand English
6. Under "Investigation Sites", add the primary and any alternate field testing locations.

Objectives – Section 2

7. As discussed and based on product performance data from the study sponsor, amend the maximum duration of the test period to account for the expectation that the repellents being tested will have a protection time of at least 12 hours. Revise test period to 16 hours through the protocol.
8. Add a section titled "3. GLP compliance and Quality Assurance", which should include the following. Please edit the statements related to GLP compliance as appropriate and add the name of the person/entity responsible for QA:
"Good Laboratory Practices, as defined by 40 CFR part 160 will be followed throughout this study.

A representative of [entity to be named] independent Quality Assurance Unit (QAU) will perform all QA duties. The QA representative will conduct critical phase inspections at intervals adequate to ensure study integrity, and maintain written and signed records of each inspection. Records shall identify the study and include the date of the inspection, the phase inspected, the individual conducting the inspection, positive and negative findings, actions recommended and taken to resolve negative findings, the scheduled date for re-inspection (if any), and the date(s) the findings are reported. All inspection findings will be reported to management and the Study Director. Any problems, amendments or

deviations discovered shall be brought to the attention of the sponsor, Study Director and management immediately. The QA representative will review the final reports for accuracy and compliance with GLPs and the protocol. A signed QA statement will be included in the final report that lists the phase inspections that were conducted, their dates, and the dates the findings were reported to management and the Study Director."

9. Add a section titled "4. IRB Review and Ethical Study Conduct", which should include the following:
"4. IRB Review and Ethical Study Conduct

The protocol, informed consent materials, and other supporting information must be submitted to an Institutional Review Board (IRB) for review and must be approved before any portion of the study is initiated, including recruitment of the subjects. To maintain scientific integrity in regards to testing procedure and clarity of the protocol, any revisions made to the protocol as a result of the protocol review process will be reflected directly in the protocol itself.

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 the IRB 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.

All amendments, deviations, and any adverse events will be documented in the final study and reported consistent with IRB reporting procedures. Documentation will include a description of the change, the reason for the change, 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.

This trial will adhere to the principles outlined in the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations. In addition, the study will be conducted in accordance with the ethical principles originating from the Declaration of Helsinki and that comply with current applicable regulatory requirements. They will follow EPA's Product Performance Test Guidelines OPPTS 810.3700: Insect Repellents to be Applied to Human Skin. These studies will be conducted in accordance with all applicable laws, regulations, and IRB requirements.

The IRB responsible for approval and continuing review of this research is:
University of Florida IRB-01
P.O. Box 100173
Gainesville FL 32610-0173"

Study Design – Section 3

10. This is a single-center, two-site field study with all participants testing at least one product at one site. The control for each test ~~is an~~ will be two untreated persons.

Repellent product testing ~~takes~~ will take place in a field setting using ~~10~~ 13 test subjects ~~participants~~ (~~with a 50:50 ratio of males to females~~ with a minimum of ~~three~~ five of either gender) for mosquitoes. Each participant will test a single product at a time (see section 8). All testing of a single product at a single site will occur during one test period (i.e., one day). Two untreated participants will also monitor the landing rate at each test site for each test substance. ~~Throughout the study.~~ An additional 6 participants will be recruited and enrolled as alternates for each test day and will need to be present at the start of each test day in the event they are needed to replace an enrolled subject.

Risks and Benefits – Section 3.2

11. Regarding the testing of mosquitoes for diseases: Confirm that these are the only disease vectors that could be transmitted in the test areas. If other vector-borne illnesses could be present, amend protocol to include testing a subset of collected mosquitoes for all potential vector-borne illnesses.
12. Include a discussion of how subjects will be notified if the screening of the mosquitoes captured on their test day results in detection of a vector-borne illness, and what follow up care will be offered by the study sponsor.
13. Revise the section on the risks of being outside during the test period as follows:
    "Other risks associated with participation in this trial include the risks ~~of~~ associated with being outside in a hot humid climate, such as sunburn, heat stroke. In addition, there is a risk of fatigue due to the length of the test day.
    - Precautions will be taken to prevent sunburn, exposing only minimal skin, wearing a hat etc. Participants will be directed to spend the time between test periods in a covered pavilion/tent with screen doors/walls, etc.
    - Water and other drinks will be provided to prevent dehydration and snacks will be provided to maintain blood sugar levels if necessary.
    - Subjects will be told at the consent meeting and reminded at the training to bring snacks, lunch, and entertainment to occupy their time during breaks between test periods.
    - Subjects will be provided with breaks as needed between the test periods. Chairs and a shaded area will be provided for relief from the sun. Subjects will have an opportunity to eat lunch, dinner, and snacks, and will have opportunities to use the restroom as necessary.
    - In addition, any time remaining immediately following an exposure period and before the start of the following exposure period, subjects will be encouraged to stretch and walk around as needed to try to minimize the discomfort from the length of the testing period, as well as to remain inside the shaded and/or screened area to avoid heat-related illnesses.
    - Where a subject enrolls to participate in more than one test day, at least 72 hours will lapse between each test day to allow subjects to rest and recover.
14. Immediately following the additional text above, add the following additional risks and risk mitigation measures:
    "A potential risk of participation is unintentional release of confidential information.
    - All efforts will be taken to maintain subjects' confidentiality. See the precautions in Section 11.3, "Confidentiality".

There can be psychological stress relating to pregnancy testing.

- In order to minimize the psychological stress, women will be given a private place to take the test, a female member of the study team will verify the test result, and the study director will ensure confidentiality of any test result. The results of the test will not be discussed with or released to anyone besides the subject. The confidentiality of the pregnancy testing will be discussed during the consent process."

15. EPA's regulations prohibit any intentional exposure studies involving pregnant or nursing women. Delete this statement: "~~There is insufficient evidence to fully characterize the risk of IR3535 to pregnant or lactating women. Therefore, pregnant women or women intending to become pregnant will not be included in the study.~~"
16. Revise the discussion of potential benefits of the research as follows: "There will be no direct benefit to participants. Indirect benefits to society will be additional products available to consumers to repel mosquitoes, thereby reducing the potential for mosquito bites and transmission of vector-borne illnesses ~~improved products for prevention of mosquito biting and pathogen transmission~~. The results of this study will inform the product labelling.

Alternatives to Human Study Research – Section 3.3

17. Insert this statement at the beginning of the section: "This study will use human subjects because no reliable models or surrogates have been found to adequately predict the efficacy of topically-applied insect repellents."
18. Please add the following language to the end of the section. "Every effort will be made to protect the subjects in this study from all potential hazards. Products containing IR3535 have been registered by EPA, and the risk assessment has shown that this active ingredient presents little or no hazard when used as directed."

Participant Entry – Section 4

Screening Procedures – Section 4.1

19. Add discussion of how the screening process will be conducted. For example, note that candidates will be screened by phone using a script. Please indicate who will perform the phone screening or send the follow up email (study director, trained study personnel, other entity?).
20. Describe who will conduct the consent/training meeting (study director, specific personnel associated with the study?).
21. Include the following text, with additional information/revisions as necessary to reflect the study procedures: "Individuals who express an interest in participating in response to the recruitment materials 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 University of Florida staff for a training session to learn more about the study and their potential role in it, go over the inclusion/exclusion criteria and the informed consent materials, receive answers to any questions they may have, and to provide informed consent to participate in the study. 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."

Inclusion Criteria – Section 4.2

22. Ensure that the list of inclusion criteria matches the list in the Study Synopsis table.
23. Revise the opening of this section as follows: "~~Volunteers will be healthy individuals and chosen based on their insensitivity to the bites in order to limit any itchiness or discomfort.~~ Volunteers will be ~~included~~ eligible to participate in the study if they meet all of the following criteria:"
24. Revise the paragraph requiring subjects to avoid certain products and activities as follows, to indicate that these activities must be avoided for 24 hours prior to each study day, rather than indefinitely. "Volunteers will be advised not to apply any cosmetics associated with a strong scent, such as perfume, hand cream, body wash, or scented shampoo for the 24 hours immediately preceding the study. Additionally, volunteers will be asked not to drink alcohol or consume spicy foods, i.e., curries, hot peppers and garlic, and to not engage in vigorous exercise for the 24 hours prior to ~~the~~ each test~~s~~. A study staff member will verify each subject's compliance with this request on each test day prior to performing any treatment with a test substance. ~~This will be verified with the participants prior to the commencement of any tests.~~"
25. The protocol indicates that only English-speaking participants will be enrolled. Please add the following statement to this section. "Current repellent 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, so limiting recruitment to English speakers will not result in equity-of-access issues."

Exclusion Criteria – Section 4.3

26. Ensure that the list of exclusion criteria matches the list in the Study Synopsis table.

Withdrawal Criteria – Section 4.4

27. Because study participants will be transported by the study staff to field testing locations, please add specific information about how a withdrawal during the field testing will be handled. Will transportation back to the lab be provided immediately? If so, by whom? Will the subject withdrawing have to find his/her own transportation back? Who will step in to handle aspirating mosquitoes for the partner of the subject withdrawing?
28. Make explicit that withdrawal does not affect benefits for participation before withdrawal by revising as follows: "Participants can withdraw at any time without giving a reason for withdrawing and without forfeiting benefits based on their participation prior to withdrawal."

Randomization and Enrolment – Section 5

29. Break into 2 distinct sections – Enrollment (5.1) and Randomization (5.2).
30. Under 5.1, Enrollment, edit as follows: "Subjects will be enrolled to participate in the study following the process described in Section 8.3. Volunteers will be fully informed before the study and it will be made clear that they can withdraw from the study at any

time and without forfeiting benefits based on their participation prior to withdrawal. Volunteers will be given and asked to read the consent form which must be signed before the test begins."

31. Please add the following statement to the end of section 5.1: "Subjects will be eligible to enroll in more than one test day, but at least 72 hours must elapse between test days involving the same subject."
32. Under 5.2, Randomization, please provide more details about when and how randomization for subjects or alternates will occur.
33. Move the text regarding assignment of subjects to the test or control group under Section 7, Treatments, to Section 5.2 and edit as follows: "~~The participants~~ For each test day, the 15 subjects will be randomly assigned to either a treatment or untreated control group. 15 pieces of folded paper will be placed in a box; two pieces of paper will have the word "control" and thirteen will have the word "treatment". With reference to the participant list, the PI will draw the pieces of paper in order to assign treatments to participants."
34. If a subject participates in more than one test day, he or she should be randomly assigned as a test subject or untreated control subject for each instance of participation.

Adverse Events, Definitions – Section 6.1

35. Regarding this sentence: "Medical judgement should be exercised in deciding whether an AE is serious in other situations", clarify who will be exercising the medical judgment and how they are qualified.

Adverse Events – Serious AEs – Section 6.2.2

36. If risk of anaphylaxis is low/negligible, be explicit about the potential risk and note what precautions will be taken to provide medical assistance on site (e.g., trained first aid person/nurse, epi pens). If there is no or negligible risk of anaphylaxis during field testing, given the screening process and mosquito attractiveness test, then note that in the protocol.
37. EPA suggests that someone trained in first aid be on site during field testing, and/or having a trained nurse on call.
38. Please include how any adverse events during the field testing that require transportation to the hospital will be handled. Will a member of the study team travel to the hospital with the subject? Will the remaining subjects be able to continue the study?

Treatments – Section 7

39. Please specify the products to be tested and the percent IR3535 for each product to be tested.
40. Please move randomization language to section 5.2.
41. Regarding application of the product to a single lower leg, please clarify how the process for selecting the treatment site. Who decides which lower leg? If subjects participate in more than one test day, will the treatment be applied to the same leg, different leg, or randomly assigned on each test day?

Test Methodology – Section 8
Field Sites – Section 8.1

42. Suggest specifying the intended field test locations as well as alternates in the protocol.
43. Please clarify how you will coordinate with local health departments and mosquito control districts to monitor for incidents of vector-borne illness at/near the intended field test locations.
44. EPA and HSRB recommend that field testing occur in areas with active vector-borne illness monitoring programs.

Site monitoring – Section 8.1.1

45. Add dengue and chikungunya to the list of diseases that will be monitored for, and if detected, would necessitate moving the field testing location.
46. Increase the time for detecting vector-borne illnesses from two weeks to four weeks: "To minimize risks to subjects, field testing will not be conducted where WNV, ZIKV, dengue, chikungunya or other mosquito-vectored diseases have been detected within the previous ~~two~~ four weeks. The sites will also not be known ZIKV transmission areas."

Mosquito processing – Section 8.1.2

47. To minimize risks to subjects, ensure that trapped mosquitoes are tested for all possible vector-borne illnesses that could be present in the area based on the species captured, e.g., dengue and chikungunya.

Test Insects – Section 8.2

48. Reference the section on monitoring where site selection is discussed. ("A site will be selected that has an abundance of ZIKV vectors (*Aedes albopictus*), but no previous history of transmission (see section 8.1.1)."
49. Please provide more information about when and how information about mosquitos captured will be shared with subjects. Will you share the results of all tests of mosquitoes captured during a specific test day be shared with all subjects who participated that day, regardless of whether any vector-borne illnesses were detected? Will information be shared only in the event that a vector-borne illness is detected? How will information be shared – phone call, letter, email – and how will you verify that the subjects received the information?
50. If diseases are detected in any of the mosquito testing, what additional monitoring/care will be offered to the subjects who participated in the day(s) where a vector-borne illness was detected?

Volunteer Enrollment – Section 8.3

Recruitment of Volunteers – Section 8.3.1

51. Add a section (4.1) describing in detail the recruitment process. For example, where will advertisements be posted and for how long? What steps will you take to ensure that the recruited population is representative of the general public/users of skin-applied insect repellents? Who will make contact to with people who express an interest in participating?
52. EPA will provide comments on the recruitment script and advertisement separately.
53. EPA recommends revising this section as follows and requests that the researchers provide specific details where necessary: "Recruitment will not begin until EPA and the

HSRB have reviewed the protocol and the associated informed consent document, these documents are revised to address comments from EPA and the HSRB, and the IRB has approved the final versions of these documents.

For each test substance tested, there will be thirteen test subjects (at least five of each gender) and two untreated control subjects selected per testing period. There will be at least 6 alternates per test day who will need to be present on test days until the researchers determine whether they are needed to replace an enrolled test subject. All subjects will be 18 to 55 years of age."

[insert description of advertisement, when/where it will be posted, how people can use the information on the poster to enroll in the study, etc. For example: "*Subjects will be recruited from the Gainesville, Florida area, via advertising through digital and social media. Advertisements will be posted in digital and social media mediums, such as Facebook, Yahoo/Bing, Google and Craigslist. The advertisement will list the phone number and email address for the primary investigator, with instructions for people interested in participating to contact her for more information."*]

54. Explain how recruitment will address the need for the test subjects to be as representative of the general population of skin-applied repellent users as possible. For example, consider adding: "Every effort will be made to achieve the demographic composition, via a stratified random sample of the pool of recruited subjects. The qualifying subjects will be stratified into smaller subgroups according to their race/ethnicity, age, and gender to help ensure that the subjects are as representative as possible of the general population of skin-applied repellent users. The final report will specify the demographics of test subjects who participated in the study, taking into account the availability of test subjects on each test day."
55. Amend protocol to note the total number of subjects that will be recruited for participation to ensure that a representative sample (age, gender, race/ethnicity) can be selected for the field testing on each day. The revised protocol will call for 20 subjects per day (15 test/control subjects, 5 alternates); consider how many additional subjects should be recruited and screened to ensure that you have a sufficiently large pool to choose from. In previously reviewed protocols, researchers screened a pool of participants to determine eligibility that was double the expected number of participants for the study. In this case, that could mean screening up to 240 subjects [(13 test subjects + 2 control subjects + 5 alternates) * 6 test days *2 = 240].
56. Specify that for each test day, the participant pool will include at least 5 subjects of each gender.

Consent and screening – Section 8.3.2

57. The documentation provided to the IRB specifies that only the study director, Dr. Week, will obtain informed consent from subjects. Please indicate in the protocol whether this is the case. If additional staff will be involved, specify their roles and how they are qualified to obtain informed consent.
58. Revise this section to include specific details about who will conduct the consent meeting, what will be covered, how the trainer will ensure comprehension of the informed consent materials prior to obtaining consent from the participants. EPA

suggests revising as follows:
"After the screening is complete, and prior to participating in any study-related procedure, each potential subject will meet in person, either individually or in small groups, with the Study Director for a consent meeting.

During the consent session, the following aspects of the study will be discussed and the following activities completed:

1. Upon arrival, subjects will be asked to provide proof of age with a driver's license, passport, or other valid identification.
2. Subjects will be given the Informed Consent Document (ICD), time to read the ICD, and the opportunity to ask questions about it. The trainer will provide a brief outline of the study including its purpose, the subjects' potential role in the study, the potential length of the study on any given test day, the identity and function of the pesticide to which they will be exposed, the potential hazards associated with the study and steps being taken to mitigate each hazard as addressed in the protocol, and the inclusion/exclusion criteria. The procedures involved with the attractiveness test, training on aspirating mosquitoes, and a 5-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 test substances will be applied to their leg for the future testing as per section 8.5.4 of the protocol, will be informed that they will wear gloves to protect their hands and head nets to protect the head, face and neck, and will be shown how to aspirate mosquitoes.
3. Any questions or concerns about the study will be discussed and answered.
4. The employee conducting the consent session with test subjects will let all training attendees know that if a test subject needs to speak to the study director in private about any aspect of the study, time will be made for this discussion once the general consent session is over.
5. To confirm understanding of the consent form, the following questions will be asked:
    a. What part of your body will be treated and what will be used in this study?
    b. What will you be wearing during the exposure period?
    c. How long will you be exposed to mosquitoes for during the field test for each exposure?
    d. What are the potential discomforts or hazards from this study?
    e. Do you have the freedom to quit or withdraw from the study at any time?
    f. If you quit or withdraw from the study, for how many hours will you be paid?
6. The trainer will recommend that subjects bring their own form of entertainment (book, DVD player, computer, etc.) to minimize participant anxiety and potential boredom during testing procedures. The researcher will have drinks (e.g., bottled water, soft drinks, etc.) and snacks available for subjects during the study day. Researchers will ask subjects if they have any food allergies and make snacks available taking into account the responses. Subjects will be told that they can bring their own lunch and snacks to consume during a break between exposure periods.
7. The trainer will provide test subjects with the study director's contact information (name, email, and phone number) to field any follow up questions. This information will be on the first page of the provided ICD.

8. Female participants will be notified that they will be required to undergo pregnancy testing at the beginning of each testing day.

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. Their eligibility to take part will then be assessed using a participant-completed questionnaire to screen for confounding health conditions that may make them unsuitable for taking part. For females, a negative pregnancy test prior to the training day and every test day is required in order to enrol and maintain enrolment of such a participant. All females will need to confirm that they are not pregnant and do not intend on becoming pregnant throughout the course of the study. See section 8.4.1. for more details. The subject will then be given a photocopy of the signed ICD and testing schedule, and scheduled to attend a mosquito attractiveness test and training session."

Pregnancy testing of females – Section 8.4.1

59. Please clarify whether a pregnancy test is required during the screening/consent process, or only on each day to which the subject could be exposed to mosquitoes. In Section 8.3.2, "a negative pregnancy test prior to the training day and every test day is required in order to enrol and maintain enrolment of such a participant. All females will need to confirm that they are not pregnant and do not intend on becoming pregnant throughout the course of the study." However, this section notes that pregnancy testing will be required "at the beginning of any day when they will be exposed to mosquitoes."
60. Please revise the sentence regarding disposal of pregnancy tests as follows, in order to take precautions to maintain the confidentiality of female subjects and to prevent unintentional disclosure of test results: "Provisions will be made to allow the test subject ~~will~~ to dispose of the test ~~results~~ in a discrete manner (e.g., opaque plastic bags available in the restroom used for testing)."

Attractiveness test – Section 8.4.2

61. If known, clarify whether the attractiveness test and aspirator training will occur in the same visit. In addition, please note whether these two events will occur at the same meeting where informed consent is obtained.
62. Specify whether the mosquitoes used in the attractiveness test will be allowed to probe and bite, or whether they will be blown off or aspirated upon landing. EPA suggests the latter.
63. In order to minimize the potential for exposure to pathogens, EPA requests that if there is a potential for the mosquitoes used in the attractiveness test to bite the subjects, only mosquitoes that have not previously received a blood meal be used.

Insect landing catch training – Section 8.4.5

64. If known, clarify whether the attractiveness test and aspirator training will occur in the same visit. In addition, please note whether these two events will occur at the same meeting where informed consent is obtained.

65. EPA suggests that this section be revised to note what protections will be available to subjects during this training. EPA requests that subjects be instructed to wear long-sleeved shirts and long pants to the training session, and be provided with head nets and gloves to protect skin from mosquito bites.
66. Provide more specific information about the training session: how long is it expected to take? What criteria will be used to determine when a subject is sufficiently qualified to aspirate mosquitoes during the field testing?

Dose determination – Section 8.4.3

67. Although EPA's product performance guidance includes steps for a dose determination phase as part of an insect repellent efficacy study, to minimize the exposure of test subjects, EPA suggests using a standard dose based on previously reviewed and accepted studies.

Test Methodology – Section 8.5

Subject meeting – Section 8.5.1

68. In order to ensure that subjects who are treated complete the study, and to support the scientific validity (and therefore the ethical validity) of the study, EPA recommends that subjects be required to stay on site after treatment until they are transported to the field location. Allowing people to leave after treatment could jeopardize the integrity of the treatment, and would introduce a risk that the subject would not return.
69. Address how transportation from the field back to the location where the repellent was applied will occur. How will you get a subject who wishes to withdraw from the study once in the field back to the lab? Someone who wishes to withdraw during the field portion should not feel compelled to stay in the field, or should be informed in advance that transportation will only be provided at the start and end of the test period.

Subject preparation – Section 8.5.2

70. Include a discussion of how treatment leg will be assigned during the test period.
71. Please revise section to include methods for ensuring the integrity of the application: "Before treatment, study staff will ensure that no subject has participated in another field test for this study in the previous 72 hours. One pant leg will be rolled up securely and the exposed lower leg (ankle to knee), of each participant will be washed with unscented soap and carefully rinsed and dried. With exception of the treated area, the participant's head, hands, trunk, and limbs will be covered with light-colored material through which insects cannot bite. Study staff will verify that participants ~~should~~ avoided alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.) and excessive exercise for at least 24 hours before and throughout the test. In addition, participants ~~should~~ will be reminded to avoid strenuous exercise and sweating during the study, as well as avoiding abrading, rubbing, touching, or wetting the treated area, especially by not rolling down the pant leg or sitting with legs crossed. Subjects will be provided with head nets and gloves to protect exposed skin from mosquito bites during the field testing."

Untreated control participants – Section 8.5.2 (correct to 8.5.3)

72. To minimize potential for mosquito biting and exposure to vector-borne illnesses while

balancing the need to ensure adequate biting pressure during the study, untreated controls only need to expose one lower leg during the monitoring periods until 5 landings occur or for five minutes, whichever occurs sooner. Please revise this section to reflect this limited exposure period.

73. To ensure consistency in subject preparation, please use the same procedure to prepare the negative control subjects and the test subjects. Revise this section as follows: "Two participants per test day will not be treated with a repellent on any limb. Before beginning the test day, study staff will ensure that no subject has participated in another field test for this study in the previous 72 hours. One pant leg will be rolled up securely and the exposed lower leg (ankle to knee) of each participant will be washed with unscented soap and carefully rinsed and dried. For untreated control subjects, the participant's head, hands, trunk, and limbs will be covered with light-colored material through which insects cannot bite, except the prepared lower leg which will be exposed periodically to ensure mosquito biting pressure. Study staff will verify that participants avoided alcohol, tobacco, and scented products (perfume, cologne, hair spray, lotion, soap, etc.) and excessive exercise for at least 24 hours before and throughout the test. In addition, participants will be reminded to avoid strenuous exercise and sweating during the field testing."

Product application – Section 8.5.4 (change to 8.5.5)

74. To minimize the unnecessary exposure of human subjects and because EPA has already established a standard dose based on previously reviewed protocols and completed studies, please edit as follows "The surface area of the lower leg (ankle to knee), will be calculated in order to calculate how much repellent each participant should receive. Study staff will apply a standard dose to the lower leg of each participant. ~~The "typical consumer dose" of the lotion or spray will be applied to the lower leg. With the wipe, the applied amount will be measured by weighing the wipe before and after use.~~"

Continued landing pressure – Section 8.5.5 (change to 8.5.6)

75. To minimize untreated control subjects' exposure to mosquito bites, clarify that as soon as the threshold number of landings has been achieved to demonstrate continued landing pressure, untreated control subjects can lower their pant leg and stop counting the landings on the untreated leg.

Exposure period – Section 8.5.6 (change to 8.5.7)

76. Clarify whether you will collect and identify all mosquitoes landing on test subjects, or only mosquitoes landing on the treated area.

Between exposure periods – Section 8.5.10 (new section)

77. Add a section explaining what subjects can do in the periods between exposures to mosquitoes, and steps the study staff will take to ensure the comfort of subjects. For example, EPA suggests adding "Between periods of exposure to mosquitoes for the test and control subjects, the study director will ensure access to a shaded, screened location with adequate seating to accommodate the subjects. In addition, subjects will have access to cold drinks (water, soft drinks, etc.) and snacks to keep them hydrated and to maintain their blood sugar. As discussed during the consent session, subjects may also

consume food and drinks they brought, read, or engage in other leisure activities. Between exposure periods, test subjects will be reminded not to engage in activities that could abrade the treated leg (e.g., rolling down pants, crossing legs). The study staff will ensure that subjects have access to restroom facilities when necessary."

Follow-up after testing – Section 8.6

78. Please clarify who will make follow-up contact with the subjects (study director, study staff, etc.) and how it will be made (phone, email, etc.).
79. What will happen if the person trying to make follow-up contact is unsuccessful at reaching the subject? Will the data be used? Will the study staff attempt any additional follow up activities?

Safety and Data Monitoring – Section 10

Risk assessment – Section 10.1

80. The first paragraph in this section, beginning with "The Principal Investigator has determined studies of this kind to be 'low risk'" seems related to the suggested additional section on GLP and QA. Please ensure consistency between this section and the new section on GLP/QA.

Adverse events – Section 10.2

81. Provide more detail about how subjects will be monitored and how adverse effects will be handled. For example, who will transport the subject for medical care? EPA recommends that a member of the study team accompany the subject experiencing an adverse reaction to the medical facility. Will testing for all subjects stop, or just the subject who is having an adverse reaction? What will happen with the subjects who may continue testing? How will their transportation be handled at the end of the testing period?
82. Please add the following statement at the beginning of the second paragraph in this section: "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."
83. Explain what steps will be taken to confirm no adverse effects occurred if a subject does not respond to the email following up 72 hours after the test day. Also provide information about how a subject will be contacted if he/she does not use email.
84. Regarding exercising medical judgment about whether adverse effects are serious – explain who will exercise medical judgment, how they are qualified to exercise medical judgment, and under what criteria the judgment will be exercised.
85. Identify someone who is independent of the study (i.e., not a study director, advisor, etc.) who can make the decision about whether or not an adverse effect was serious and/or whether it was related to study participation.
86. Regarding this statement: "In the case of a severe reaction such as anaphylaxis, a trained First Aider will be called immediately and the volunteer taken to the nearest Emergency Room (ER)." See EPA's ethics comment #36 and make similar edits to this section.

Compensation due to adverse events – Section 10.3

87. Revise the following sentence as follows: No additional compensation for adverse events beyond payment for medical expenses related to participation in the study is routinely offered.
88. Add a statement that evaluating adverse effects may require the study personnel to consult with the treating medical personnel, with the subject's consent: "The Sponsor and the Principal Investigator will determine whether the injury is related to the subject's participation in this study. To do this, they may request to consult with the person/facility that provided medical treatment following an adverse effect, which could require your consent."

Regulatory Issues – Section 11

Ethics Approval – Section 11.1

89. Suggest removing this section as the contents should be covered under the proposed addition of section 4.

Consent – Section 11.2

90. Suggest removing this section as consent and withdrawal are covered in many other areas of the protocol.

Confidentiality – Section 11.2

91. Add the following text to the end of the existing language: "The information obtained from subjects taking part in these studies will be used by the researchers, funders, and the sponsor, and will become part of a series of reports (one report for each conducted study). All reports (as well as all study-related records) and will be kept as confidential as possible under local, state, and federal laws. The results of this study are not intended for publication; however, if any of the study-related data are published, subjects' identities will remain confidential.

    All efforts will be taken to maintain the confidentiality of the pregnancy test results. The test results will not be recorded, and will not be disclosed to anyone other than the test subject, the verifying employee, and/or the Study Director. Opaque bags will be available where the pregnancy tests are taken to allow for discrete disposal.

    In addition, 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. No one outside researchers, Sponsor, the IRB, or certain governmental agencies (such as USEPA) will have access to subjects' personal information."

Funding – Section 11.5

92. To provide compensation that is consistent with the principles of justice and respect for persons, and that is not too high as to constitute undue inducement or so low as to be

attractive only to economically-disadvantaged persons, revise the compensation offered to participants as follows: at least $10/hour for up to 8 hours; compensation for any amount of time after 8 hours should be at least $15/hour (time and a half), rounded up to the next hour.

93. Explain how alternates will be compensated for participating in the consent meeting and training session, and how they will be compensated if they are present on test days but not needed to participate.
94. Provide more details about compensation for people withdrawing for various reasons. How will people who begin a test but decide to withdraw without giving a reason be compensated? How will people who do not comply with instructions and are asked to withdraw be treated? How will people who withdraw for health or safety reasons be compensated (i.e., paid for the full day or paid for the time they participated in the study)?
95. Provide detail about how subjects will be paid – direct deposit, check, etc. – and on what schedule. For example, "Subjects will be paid on (e.g., 1st and 15th of the month, at the end of each training or test day) by (mail, wire transfer, check delivered at the office, etc)."

References – Section 12

96. Delete reference one and replace with reference to EPA risk assessment for IR3535.
97. In #3, reference the most recent version of the Declaration of Helsinki (October 19, 2013) - https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/
98. Provide a citation for "Data Protection Act 1998".

D. Summary Assessment of Scientific Aspects of the Proposed Research

The study objective is to assess the duration of three topically applied insect repellent products at preventing landing by mosquitoes on human hosts: "***Primary objective:*** *To determine the efficacy and duration of three topically applied insect repellent products at preventing landing by mosquitoes* ***over*** *12 hours.* (p. 7, Section 2, Objectives). "*The aim of this study is to provide longevity and efficacy data for three topically applied insect repellent products for prevention of mosquito landing*." (p. 7, section 1. Introduction). The testing hypothesis is that the products are expected to prevent mosquito landings on human hosts by a period ≥ 12 hours post application.

On p. 7, section 1. Introduction, it is stated, "*The use of repellent products can provide added* ***personal protection from disease transmission*** *vectors and nuisance bites. New effective repellents would offer an additional option for protection against biting insects. The tests carried out provide important information on the effectiveness of skin repellents, which will be used for label claims to accurately inform consumers and registration purposes."*

The second objective is to determine the typical consumer dose for efficacy testing. However, the Agency recommends that the study use standard doses, as have been used in the most recent mosquito field study reviewed by the HSRB (April 2015). These standard application rates are based on dosimetry tests used in previous studies since 2006 that were reviewed by EPA and

HSRB. The researchers have agreed to amend the protocol to test 3 formulations at the recommended standard dose of 1 g of product/ 600 cm^2 for the wipe and lotion, and 0.5 g/ 600 cm^2 for the pump spray. Therefore, the second objective is no longer relevant.

In this study, landings of wild mosquitoes on human subjects will be used to evaluate the repellency of 3 insect repellent products applied to human skin. The efficacy testing will be conducted against mosquitoes because they are the target insect pest repelled by the product. The study sponsor proposes to conduct the test in two field locations in Alachua County, Florida, most likely a forest or wetland and urban environment, where predominant mosquito species differ. The sites "*will be outside the current hotspot of ZIKV transmission but in an area of high mosquito abundance and diversity. However, efforts will be made to include a site where Aedes albopictus is present"* (p. 12, 8. Test Methodology; 8.1. Field Sites), "…*but* [with] *no previous history of* [pathogen] *transmission*." (p. 13, 8.2 Test insects). The end point of efficacy or unit of measure for determining repellent duration, or complete protection time(CPT), in this experiment will be mosquito landings, and efficacy is measured as CPT. Repellent failure is the exposure period in which two or more mosquito landings occurred in an exposure period, or when one landing occurred in an exposure period and another landing occurred in the subsequent exposure period; the period where the repellent fails is considered the CPT. Sites will be selected based on the absence of mosquito-borne pathogens. *"Field testing will not be conducted where West Nile Virus (WNV), Zika (ZIKV) or other mosquito-vectored diseases have been detected within the previous 2 weeks*." (p. 12, section 8.1.2). Sites will be monitored at least weekly for 24 hours using CDC light traps and BG Sentinel traps during a month prior to study initiation to verify absence of mosquito-borne diseases.

1. **Study Design**

   Experimental design: This will be a field study conducted with human subjects at two ecologically distinct mosquito habitats, most likely a forest or wetland and urban environment, in Alachua County, Florida. At each site, EPA and the study sponsor have agreed that the test groups should consist of 13 treated subjects and two untreated control subjects to monitor landing pressure throughout the duration of the test. Treatments will be applied to lower area on either right or left leg. Subjects at each site will be selected from a pool of informed and consenting volunteers that will be tested for their attractiveness to mosquitos (pg. 14, section 8.4.2. Attractiveness test), and trained to catch/handle landing mosquitoes using aspirators (pg. 15, 8.4.3. Insect landing catch training). At each field site, testing will be conducted for a minimum period of 12 hours. Each subject will undergo five-minute exposures every thirty minutes until repellent failure occurs or end of study period is reached, whatever happens first. To assess CPT, subjects will be paired with a trained member of staff or another participant, and the untreated control subjects will be paired together. Pairs will be separated from other pairs by at least 3m/10 ft apart. Testing will begin 2 hours post application of the repellent product. Five landings on untreated control subjects in each five-minute exposure period is considered the minimum necessary to ensure the mosquito pressure is adequate to determine repellency of the tested product. Adequate mosquito landing during testing will be monitored by 2 control subjects. "*Two negative controls will be completed at each time point to monitor mosquito landing rates (see section 8.5.2). The*

participants will be randomly assigned to either a treatment or untreated control group. ~~Twelve pieces of folded paper will be placed in a box, two pieces of paper will have the word control and ten will have the word treatment. With reference to the participant list, the PI will draw the pieces of paper in order to assign treatments to participants.~~" (pg. 11, Section 7. Treatments). Before efficacy testing, pathogen-free mosquitoes from laboratory colonies (mainly Aedes spp.) will be employed for testing subjects' attractiveness to mosquitoes. Selected participants will be trained in the laboratory for handling and collecting mosquitoes using aspirators. Mosquitoes that land during efficacy testing will be aspirated by the subjects, and labeled with time of collection. Collected mosquitoes will be processed for taxonomic identification and detention of pathogens. Field collected mosquitoes will be identified by genus and species (subspecies or strain if possible), and analyzed for pathogens using reverse transcription and quantitative PCR (RT-qPCR) (pg. 12; 8.1.3 Mosquito pathogen testing). The result of the analyses will be communicated to subjects and reported in the study reported.

Based on new product development data available to the study sponsor, it has been proposed to and accepted by the agency to extend duration of the test from 12 hours minimum to 16 hours. In addition, the Agency has recommended that for the study results to be considered valid, no more than 3 consecutive exposure periods or 15% of non-consecutive exposure periods should be missed due to weather delays or inadequate landing pressure.

2. Statistical Design

The primary objective of the data analysis is to estimate the median protection time with 95% confidence interval. The times to treatment failure will be analyzed using Kaplan-Meier Survival functions for estimation of the median Complete Protection Time with 95% confidence intervals. (pg. 18, section 9.2. Data analysis). The Kaplan-Meir Survival analysis is advantageous since CPTs may not be normally distributed. Kaplan Meier estimator has been accepted by EPA and the HSRB for the Median CPT calculation in past repellent efficacy studies and is also recommended by the World Health Organization for CPT calculation from these non-parametric data sets. The rationale provided for the originally proposed sample size of 10 subjects for efficacy testing is provided on pg. 18, section 9.1.2 Complete protection time, and it is based on data from a field trial in Florida where the 95% confidence interval of the median was calculated from a sample size of 10 subjects with 1 out of 10 individuals censored at 5.5 hours. The confidence width was 3 hours, which corresponded to the lowest CPT of 2.5 hours for a median CPT of 5 hours. Similar calculations yielded the same results with 2 individuals censored out of 10 (pg. 18, section 9.1.2 Complete protection time):

> *"Based on data from a previously completed field trial of mosquito repellents in Florida[8], where the observed CPT ranged from 2.5 to 5.5 hours with a median of 5 hours, and where 1 out of 8 individuals was censored, a calculation of 95% confidence interval of the median was performed for this study based on a sample size of 10 individuals with the same parameters as above with 1 individual censored (i.e. 10%) at 5.5.*

hours. This obtained a confidence width of 3 hours. Regardless of the observed data the lower limit of this 95% confidence interval corresponded to the 3rd lowest observed time. Similar calculations were performed for the same sample size, but with 2 individuals censored (i.e. 20%), which resulted in identical results."

EPA has developed a statistical model (included as Appendix 5 in EPA's comments on the protocol) for determining a statistically valid sample size for this type of field-based insect repellent efficacy study. Based on this model, EPA and the researcher have agreed that the sample size will be adjusted from 10 test subjects to 13 test subjects. EPA suggests retaining 2 subjects to serve as untreated controls. Furthermore, for statistical analysis purposes, the Agency recommends that data collected at the point of subject withdrawal from the test be treated as right-censored when withdrawal occurs prior to confirmed landing, and that treatments be randomly applied to either right or left leg of treated subjects.

On pg. 7, Section 3. Study Design, it is stated, "*This is a single-center, two-site field study with all participants testing at least one product. The control for each test is an untreated person. Repellent product testing takes place in a field setting using 10 participants (preferably with a 50:50 ratio of males to females, with a minimum of three of either gender) for mosquitoes. Each participant will test a single product at a time.* (see Section 8)."

The protocol has been revised to adopt a sample size of 13 subjects for efficacy testing of each product according to EPA recommendations; five additional subjects are proposed to be added as alternate, and 2 untreated subjects are proposed as control subjects for monitoring landing pressure throughout the test. Furthermore, each product will be tested separately at each site on the same day for all subjects.

On pg. 11, Section 7, Treatments, it is stated, "*LivFul Inc. will provide the three products to be tested. The three products have the same active ingredient, IR3535, in a spray, lotion or wipe. Dose determination will be conducted to obtain the typical consumer dose, prior to test commencement (see section 8.4.3.).* ***The product will be applied to the participant's lower leg.*** *The dose will not exceed the maximum daily limit for IR3535 (see Appendix 4 for calculations of the maximum safe dosage of IR3535). Two negative controls will be completed at each time point to monitor mosquito landing rates (see section 8.5.2). The participants will be randomly assigned to either a treatment or untreated control group. Twelve pieces of folded paper will be placed in a box, two pieces of paper will have the word control and ten will have the word treatment. With reference to the participant list, the PI will draw the pieces of paper in order to assign treatments to participants*."

The protocol should explain how the treatment will be randomly applied to either right or left legs of subjects. In addition, the Agency recommends that the product be applied at the standard dose of 1 g of product / 600 cm^2 for all but pump sprays; pump sprays should be applied at a rate of 0.5 g of product / 600 cm^2.

The randomization method should be adjusted according to EPA recommended sample size and total number of subjects (a sample size of 13 subjects for efficacy testing of each product according to EPA recommendations; five additional subjects are proposed to be added as alternates, and 2 untreated subjects are proposed as control subjects for monitoring landing pressure throughout the test).

On pg. 13, Section 8.2. Test Insects, it is stated, "*Field tests for mosquito repellents will be conducted in at least two distinct habitats, most likely a forest or wetland and an urban environment, where the predominant mosquito species differ* (pg.12, Section 8.1. Field Sites)… *Mosquito tests will be conducted where more than one species are present. A site will be selected that has an abundance of ZIKV vectors (Aedes albopictus), but no previous history of transmission. Landing insects will be aspirated or trapped before and during the test, and labeled with the time of collection. After the field study, collected insects will be identified by genus and species, and if possible, by subspecies or strain. The number in each taxon collected in each time period will be reported*." (pg. 13, Section 8.2. Test Insects).

On pg.17, Section 8.5.8. Exposure duration, it is stated, "*In order to ensure an acceptable landing pressure throughout the duration of the study, as well as to preserve wellbeing, maintain morale amongst the volunteers, and work within the limits of daylight available**, the first two hours of testing will be skipped**. Given the typical range of CPT data for IR3535 products[7] it is highly unlikely failure will occur before this time point. Each participant will test all time points. Between time points the repellent will be left on the leg and re-tested every 30 minutes up to 12 hours or until Complete Protection Time (CPT) has been determined. CPT is defined as one landing in the 5-minute test period followed by a second confirmatory landing in the next test period, 30 minutes later, on the treated leg.*"

If subjects' exposure is delayed, the protocol should require a minimum number of complete exposure cycles to ensure valid results. Following a delayed exposure, EPA recommends the subject should complete at least 3 exposure cycles before getting a confirmed landing. In addition, the protocol should describe precautionary methods that will be taken to preserve the integrity of the applied product from time of application to commence of exposure cycles. The revised protocol adopts these recommendations.

The protocol has insufficient information about how the data will be handled in the event of weather that delays test periods and/or of periods with insufficient mosquito landing pressure. Weather delays should be no longer than 3 exposure periods. If the

experiment is delayed for weather, the first exposure period of the weather delay would be used as the duration of CPT for a subject if a confirmed landing occurs immediately following a weather delay**.**

For assessment of landing pressure, landing counts should be collected on exposed skin of untreated control subjects and time of each landing should be recorded to know how long it took to reach 5 landings within 5 minutes. After 5 landings in a 5-minute period, the exposed skin should be covered to protect from mosquitoes. In cases of low mosquito pressure, if a landing occurs followed by no landing on the next exposure period at a time when landings are below minimum on untreated subjects, the first landing should be considered as a confirmed landing in that case

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

Subjects will be exposed to up to three different formulations of repellent products containing IR3535, an EPA-registered pesticide, during the efficacy testing described in the protocol. Subjects will be exposed to mosquito species encountered in the field during efficacy testing, and to mosquitoes from laboratory reared colonies during assessment of subjects' attraction to mosquitoes and mosquito handling training. One limb, lower leg, of each subject will be treated; exposure to the repellent will be continuous throughout the period of the efficacy test. Subjects will be exposed for 5 of every 30 minutes during the efficacy phase to all or some wild mosquito species encountered at field sites. The researchers' calculations of Maximum Safe Dosage of IR3535 appear in Appendix 4 of the protocol. However, the information provided in the protocol does not follow EPA risk assessment procedures. EPA recommends that the study sponsor amend the protocol to reference EPA's risk assessment, rather than data from European risk assessments.

According to the EPA's risk assessment based on data submitted to EPA for registration of IR3535, IR3535 is not a skin sensitizer, is classed as category III for acute dermal toxicity (LD_{50} > 3000 mg/kg in rats), category IV for acute oral and inhalation toxicity (LD_{50} > 5000 mg/kg in rats), and category II for eye irritation. The NOAEL for dermal toxicity is ≥ 3000 mg/kg/day in rats and for oral toxicity is 600 mg/kg/day in rabbits. In its risk assessment, EPA used a 5% dermal absorption factor for IR3535 and based their calculations on an 11.8 kg child and 60 kg adult for a product at 7.5% active ingredient. Since the initial registration, EPA has registered several products containing up to 20.07% IR3535. The products to be tested in the protocol may contain up to 20% IR3535 and Margins of Exposure (MOE) can be calculated as below:

The calculation below is an example for calculating exposure estimates for a product containing 20% IR3535, the worst case scenario of the three products proposed in the protocol, for risk characterizations using 2680 cm^2 as the average area for the lower leg of an adult male (average lower leg area for a female is 2330 cm^2):

(2680 cm^2 x 1000 mg formulation/600 cm^2) ÷ 60 kg = 74.4 mg formulation/kg

(74.4 mg formulation/kg)(0.20 mg a.i./mg formulation) = 14.9 mg a.i./kg

14.9 mg a.i./kg x 0.05 dermal absorption factor = 0.75 mg a.i./kg

Margins of Exposure (MOE) are calculated by dividing the oral NOEL of 600 mg/kg/day as below:

600 mg/kg ÷ 0.75 mg/kg = 800

To reach the EPA's level of concern (MOE ≤ 100), a person would need to make approximately 9 applications in a single day. Although the information in the protocol does not follow EPA's risk assessment procedures, the conclusions are the same; the amount of IR3535 applied in these studies does not exceed a level of toxicological concern to test subjects.

4. Endpoints and Measures

According to the protocol, "*The primary endpoint is the median Complete Protection Time (CPT) of the repellent product. The CPT is defined as the time between application of the repellent product and the occurrence of the first landing in a 5-minute test, followed by a confirmatory landing within 30 minutes, or two landings within the same 5-minute test*." (Section 3.1, Study Endpoints). EPA notes that the endpoint should be mosquito landings, not CPT. The CPT is the measure of repellency signaling product failure.

E. Compliance with Applicable Scientific Standards

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

- Experimental design
- Pre-training of subjects
- Risk minimization

F. EPA Science Comments

The following elements in the protocol must be revised before the research goes forward:

1. In Section 1, protection against disease transmission should not be considered a research objective. Therefore, the first sentence from the paragraph below should be revised to say "**protection from disease vectors.**" "*The use of repellent products can provide added* personal protection from disease ~~transmission~~ vectors and nuisance *bites. New effective repellents would offer an additional option for protection against biting insects."*

2. In Section 1. Study Design, the Agency recommends that no more than 3 consecutive exposure periods or 15% of all exposure periods should be skipped for weather delays or inadequate landing pressure.

3. Clarify the procedure that will be followed in the event of a weather delay. EPA suggests that in the event of a weather delay lasting longer than 3 exposure periods, the study should be terminated for that day. If the experiment is delayed for weather, the first exposure period of the weather delay would be used as the duration of CPT for a subject if a confirmed landing occurs in the test period immediately following a weather delay.

4. The margin of exposure (MOE) should be calculated from EPA's risk assessment based on data submitted to EPA for registration of IR3535. Based on a dermal absorption of IR3535 (5% dermal absorption of the applied dose) subjects in this study will be exposed to a systemic dose rate of 1.67 mg product/600 cm^2 when treated. The value of the oral No Observable Adverse Effects Level (NOAEL) is 600 mg/kg/day and was used in EPA's risk assessment to verify whether subjects will be exposed to an application dose below the level of concern. Replace the MOE calculations in Appendix 4 of the protocol with the data from EPA's risk assessment, provided in section D.3. above.

5. EPA requests that the study sponsor amend the protocol to reference EPA's risk assessment, rather than data from European risk assessments.

6. For statistical analysis, data collected at the point of subject withdrawal from the test should be treated as right-censored when withdrawal occurs prior to confirmed landing.

7. Revise number of subjects and statistical basis for the number of subjects according to the EPA statistical model provided as Appendix 5 to the protocol. The number of subjects for each test day should be 13 test subjects and 2 untreated controls. Five alternates should be recruited for each test day.

8. It is possible that random selection will yield an unequal distribution of males and females. Testing should be conducted with an approximate 50:50 male to female ratio, but in no case should there be fewer than 5 subjects of either gender.

9. In section 9.2, Data Analysis, add a statement indicating that treatments will be randomly applied to either right or left leg of subjects.

10. On p.17, Section 8.5.8. Exposure duration: "*In order to ensure an acceptable landing pressure throughout the duration of the study, as well as to minimize exposure and work within the limits of daylight available**, the first two hours of testing will be skipped**. Given the typical range of CPT data for IR3535 products[7] it is highly unlikely failure will occur before this time point. Each participant will test all time points. Between time*

points the repellent will be left on the leg and re-tested every 30 minutes up to 12 hours or until Complete Protection Time (CPT) has been determined. CPT is defined as one landing in the 5-minute test period followed by a second confirmatory landing in the next test period, 30 minutes later, on the treated leg."

Because subjects' exposure is delayed, the protocol should require a minimum number of complete exposure cycles to ensure valid results. Following a delayed exposure, EPA recommends the subject should complete at least 3 exposure cycles before getting a confirmed landing. In addition, the protocol should describe precautionary methods that will be taken to preserve the integrity of the applied product from time of application to commencement of exposure cycles.

11. The protocol should list potential field sites. EPA recommends that the protocol also include alternate sites. Changing field sites to locations not listed in the protocol must be done through the amendment process and would require approval from the IRB before implementation.

12. For the initial biting pressure evaluation prior to beginning the field study, the minimum landing rate is one landing within one minute. Adequate landing pressure during the field study is 5 mosquito landings per subject within 5 minutes. For assessment of landing pressure, landing counts should be collected on exposed skin of the lower leg of each untreated control subject. The time of each landing should be recorded. After 5 landings, the untreated control subjects' exposed skin should be covered to protect from mosquitoes until the next test period.

13. Revise the protocol to reflect the following: If after the initial mosquito pressure evaluation period, untreated controls do not receive 5 landings in 5 minutes for a given test period, if the first landing occurs on a test subject in the previous test period, followed by no landing during the exposure period at a time when landings are below minimum on untreated subjects, the first landing should be considered as a confirmed landing.

14. Sources of variation such as mosquito species to be likely present at the proposed field sites, habitat characteristics, climate, mosquito pressure at different times of day, and test subjects' attractiveness to mosquitoes, influence CPT. The protocol should discuss how these sources of variation might affect CPT, and to account for or minimize them. For example, if testing during periods low mosquito pressure, explain how those data will be used in the estimation of CPT. EPA recommends that landing pressure should not be below 5 landings within 5 minutes for more than 15% of exposure periods.

15. Power analysis for determination of sample size for efficacy testing has been revised based on EPA's model power analysis (proposed as Appendix 5 for the protocol). EPA recommends a sample size of 13 subjects per product. In addition, testing for a single

product at a single site should be done by all subjects on the same day.

16. The protocol should be amended to include a discussion of whether, and if so, how, the study will be conducted in accordance with good laboratory practices. See 40 CFR 160 for EPA's regulatory requirements related to GLPs and field studies.

Attachments:

1. EPA Protocol Review (Protocol dated April 23, 2017)
2. Completeness checklists
3. EPA's comments on the IRB-approved protocol
4. EPA's comments on the IRB-approved informed consent materials
5. IRB approval of protocol dated April 23, 2017
6. IRB correspondence
7. IRB roster
8. IRB minutes
9. IRB written procedures
10. IRB approved protocol & informed consent materials
11. Original IRB submission – protocol & informed consent materials
12. EPA's comments on the screening and 72-hour follow up scripts
13. EPA's comments on the advertisement

Attachment 1 - EPA Protocol Review

Title: Field evaluation of three topically applied insect repellent products containing IR3535 against mosquitoes in Florida

Date: April 23, 2017

Principal Investigator and any sub-investigators: Dr. Emma Weeks

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1. Societal Value of Proposed Research

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

In this study, human subjects will test the efficacy of three formulations of an insect repellent containing IR3535, an EPA-registered pesticide, against mosquitoes in the field in Florida. The study objective is to assess the duration of three topically applied insect repellent products at preventing landing by mosquitoes on human hosts: "***Primary objective:*** *To determine the efficacy and duration of three topically applied insect repellent products at preventing landing by mosquitoes over 12 hours.* (pg. 7, Section 2, Objectives). *The aim of this study is to provide longevity and efficacy data*

for three topically applied insect repellent products for prevention of mosquito landing." (pg. 7, section 1. Introduction).

The field tests will measure the efficacy of the IR3535 products at repelling mosquitoes in order to establish a median complete protection time for each formulation of the product.

(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 complete protection time for three formulations of skin-applied mosquito repellents containing IR3535 to determine the median length of protection time provided by each formulation.

The rationale for testing is to collect data to establish a median complete protection time. The data supporting currently registered products are not sufficient to establish a median complete protection time for these specific products.

A standardized protocol will enable the EPA to receive consistent and scientifically reliable data about the complete protection time for these products. The field testing data will provide information about: 1) the length of time after treatment before the first confirmed landing by a mosquito; and 2) the bite protection efficacy of new product(s) for EPA registration. Because this is a field test, mosquitoes will not be permitted to bite subjects. The endpoint is landing with intent to bite, and mosquitoes who exhibit this behavior should be aspirated before they can bite the test subjects.

(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 products.

(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 study objective is to assess the duration of three topically applied insect repellent products at preventing landing by mosquitoes on human hosts: "***Primary objective:*** *To determine the efficacy and duration of three topically applied insect repellent products at preventing landing by mosquitoes* ***over*** *12 hours.* (pg. 7, Section 2, Objectives). *The aim of this study is to provide longevity and efficacy data for three topically applied insect repellent products for prevention of mosquito landing*." (pg. 7, 1. Introduction).

The testing hypothesis is that the products are expected to prevent mosquito landings on human hosts by a period ≥ 12 hours post application. However, no explicit hypothesis is stated other than the statement: "*The repellent must repel the mosquito in the presence of the attractive host in order to be truly effective*." (pg. 8, Section 3.3 Alternatives to Human Study Research). EPA recommended that the testing period be extended beyond 12 hours if the hypothesis is that the products may protect for over 12 hours.

A second objective in the proposed study is the determination of "typical consumer dose." EPA recommends elimination of dosimetry testing and that products be tested at the standard dose (1 g/600 cm^2 for aerosols and wipes. For pump sprays the Agency recommends applying 0.5 g product/600 $cm^{2)}$. These application rates are based on dosimetry tests used in previous studies since 2006 that were reviewed by EPA and HSRB.

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

The objective cited may be achieved by the study if the protocol is revised and amended in accordance with EPA's comments on the ethical and scientific aspects of the protocol.

2.1 Statistical Design

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

The original protocol submitted by the University of Florida proposed that 10 individuals serve as test subjects. However, the justification for the proposed sample size is not supported statistically. After consultation with EPA, the University of Florida has agreed to EPA's proposed sample size of 13 test subjects, the methodology for which is described in Appendix 6 of EPA's "Science and Ethics Review of a Protocol for Field Evaluation of Three Topically-Applied Insect Repellent Products Containing IR3535".

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

In efficacy testing, 2 untreated control subjects per field trial are employed to confirm appropriate pest pressure throughout the study. There are no positive controls. Use of two untreated control subjects is appropriate for the study design. No direct

comparisons of treated and untreated subjects are contemplated in the statistical analysis plan**.**

"Two negative controls will be completed at each time point to monitor mosquito landing rates (see section 8.5.2)." (pg. 11, Section 7. Treatments).

"Two participants per test day will not be treated with a repellent on any limb. These participants will be fully clothed to prevent mosquito bites. Untreated control participants will monitor the mosquito activity at regular intervals during the test, by counting and collecting mosquitoes landing on their clothing, to confirm continued acceptable landing pressure. Controls with negative inert substances will not be done to reduce risk to the participants of mosquito biting and pathogen transmission. A positive control will also not be completed, IR3535 is a known efficacious repellent, in this study we are trying to determine the effectiveness (CPT) of the formulations." (pg. 16, 8.5.2. Untreated control participants)

(c) How is the study blinded?

The study is not blinded. Each product will be tested separately at two different locations, and the investigator and subjects will be aware of the identity of the test substance on each day of testing.

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

Subjects will be recruited from a group from the general public. Interested candidates will be contacted by the primary investigator and invited to come to a screening and consent meeting. EPA has suggested that this pool generally represent the demographics of U.S. repellent and/or treated fabric users. At the screening/consent meeting, each person will be selected as a test subject or alternate.

For each test day, 20 subjects will be scheduled to attend, 15 test subjects and 5 alternates that fulfill the inclusion/exclusion criteria and have signed the ICD. The morning of the study, the 15 test subjects will be randomly assigned to either the test or control group by drawing slips of paper with the words "treatment" or "control" and assigning each to the subjects in the order they are listed on the investigator's sheet.

"*The participants will be randomly assigned to either a treatment or untreated control group. Twelve pieces of folded paper will be placed in a box, two pieces of paper will have the word control and ten will have the word treatment. With reference to the participant list, the PI will draw the pieces of paper in order to assign treatments to participants.* (p. 11, Section 8.5.2, Untreated control participants).

(e) Can the data be statistically analyzed?

Yes. See (f) below.

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

According to the protocol, "*The endpoint for Complete Protection Time will be time to treatment failure for each participant test. Treatment failure is the time at which the product no longer provides complete protection, which is determined as the time at which one landing occurs in a 5 minute period, followed by a confirmatory landing within 30 minutes. The times to treatment failure will be analyzed using Kaplan-Meier Survival functions, and from these the median Complete Protection Time and 95% confidence intervals will be calculated."* (p. 18, section 9.2. Data analysis).

EPA has commented that CPT is determined as the time at which one landing occurs in a 5-minute period, followed by a second landing in the same 5-minute period or the subsequent test period 30 minutes later.

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

Yes. However, power analysis for determination of sample size for efficacy testing should be revised according to the latest power analysis simulation conducted by the Agency. The primary objective of the data analysis is to estimate the median protection time with 95% confidence interval. The times to treatment failure will be analyzed using Kaplan-Meier Survival functions for estimation of the median CPT with 95% confidence intervals. (p. 18, section 9.2. Data analysis). The Kaplan-Meir Survival analysis is advantageous since CPTs may not be normally distributed. Kaplan-Meier estimator has been accepted by EPA and the HSRB for the median CPT calculation in past repellent efficacy studies and is also recommended by the World Health Organization for CPT calculation from these non-parametric data sets. The proposed sample size is 13 human subjects for efficacy testing of each product. EPA has recommended amending the protocol's justification for sample size determination to 13 subjects for efficacy testing. Power analysis for determination of EPA's recommended sample size appears in Appendix 5.

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

Yes. Assuming EPA's recommendations about statistical design and sample size are incorporated into the protocol, it will have adequate statistical power if 13 test subjects are used, based on the EPA's latest power analysis (Attachment 5).

2.2 How and to what will human subjects be exposed?

Subjects will be exposed to wild mosquitoes during the field testing. Risks of acquiring a vector-borne illness will be minimized through weekly monitoring the potential test sites for a month prior to testing (p. 12 § 8.1.1), testing mosquitoes captured during the monitoring periods to ensure they do not carry diseases or viruses

(p. 12 § 8.1.3). The protocol should be revised to add EPA's recommendation for coordinating with local public health departments and mosquito control districts with active monitoring programs to ensure no outbreaks have occurred in the test areas. Subjects with known allergic reactions to mosquito bites will be excluded from research participation (p. 13, § 8.3.1).

Subjects will be exposed to repellent product formulations during efficacy testing. Subjects will be exposed to mosquito species encountered in the field during efficacy testing, and to mosquitoes from laboratory reared colonies during mosquito detection and handling training, and assessment of subjects' attraction to mosquitoes. One limb, lower leg, of each subject will be treated; exposure to the repellent will be continuous throughout the period of the efficacy test. Subjects will also be exposed for 5 of every 30 minutes during the efficacy phase to all or some wild mosquito species encountered at field sites.

Calculations of Maximum Safe Dosage of IR3535 appear in Appendix 4 of the protocol. However, the information provided in the protocol does not follow EPA risk assessment procedures. EPA recommends that the study sponsor amend the protocol to reference EPA's risk assessment, rather than data from European risk assessments.

According to the EPA's risk assessment based on data submitted to EPA for registration of IR3535, IR3535 is not a skin sensitizer, is classed as category III for acute dermal toxicity (LD_{50} > 3000 mg/kg in rats), category IV for acute oral and inhalation toxicity (LD_{50} > 5000 mg/kg in rats), and category II for eye irritation. The NOAEL for dermal toxicity is ≥ 3000 mg/kg/day in rats and for oral toxicity is 600 mg/kg/day in rabbits. In its risk assessment, EPA used a 5% dermal absorption factor for IR3535 and based their calculations on an 11.8 kg child and 60 kg adult for a product at 7.5% active ingredient. Since the initial registration, EPA has registered several products containing up to 20.07% IR3535. The products to be tested in the protocol may contain up to 20% IR3535 and Margins of Exposure (MOE) can be calculated as below:

The calculation below is an example for calculating exposure estimates for a product containing 20% IR3535, the worst case scenario of the three products proposed in the protocol, for risk characterizations using 2680 cm^2 as the average area for the lower leg of an adult male (average lower leg area for a female is 2330 cm^2):

(2680 cm^2 x 1000 mg formulation/600 cm^2) ÷ 60 kg = 74.4 mg formulation/kg

(74.4 mg formulation/kg)(0.20 mg a.i./mg formulation) = 14.9 mg a.i./kg

14.9 mg a.i./kg x 0.05 dermal absorption factor = 0.75 mg a.i./kg

Margins of Exposure (MOE) are calculated by dividing the oral NOEL of 600 mg/kg/day as below:

600 mg/kg ÷ 0.75 mg/kg = 800

To reach the EPA's level of concern (MOE ≤ 100), a person would need to make approximately 9 applications in a single day.

"*Participants will undergo a screening evaluation which includes a training (to detect mosquito landings and use an aspirator), a mosquito attraction test, and a dose determination assay. Followed by participation in up to 6 repellency tests for up to 12 hours duration. In total each of the three products is to be tested for 12 hours at each of the two field sites."* (p. 2, Study Synopsis)

"*Field tests for mosquito repellents will be conducted in at least two distinct habitats, most likely a forest or wetland and an urban environment, where the predominant mosquito species differ. The test will most likely be conducted in Alachua county, Florida, USA. This area is outside the current hotspot of ZIKV transmission but in an area of high mosquito abundance and diversity. However, efforts will be made to include a site where Aedes albopictus is present.*" (p. 12, Section 8.1. Field Sites).

(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 establish a median CPT for three products containing IR3535. The data supporting currently registered IR3535 products do not provide this information.

(c) What duration of exposure is proposed?

The exposure period is up to 16 hours; the test substance will be applied, then field testing will begin 2 hours after the application and last for up to 14 hours. Subjects will be exposed to mosquitoes for 5 minutes of every 30-minute period starting at 2 hours after the time of application until 14 hours after the time of application OR until the first confirmed landing. New data from product development indicates the product lasts longer than 12 hours. The protocol is being revised to extend exposure period to 16 hours, and testing to 14 hours after 2 hours of application.

2.3 Endpoints and Measures

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

According to the protocol, *"The primary endpoint is the median Complete Protection Time (CPT) of the repellent product. The CPT is defined as the time between application of the repellent product and the occurrence of the first landing in a 5 minute test, followed by a confirmatory landing within 30 minutes."* (p. 7, Section 3.1. Study Endpoints). EPA notes that the proper endpoint is mosquito landings, not CPT. The CPT is the measure of repellency signaling product failure.

The data collection sheet should be appended to the protocol rather than provided as a separate file.

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

- Alternate subjects will be enrolled to ensure adequate sample size:
  "*The sample size calculations (based on 90% power and a 5% significance level) require 10 participants. Two untreated participants will also monitor the landing rate throughout the study. An additional 5 participants could be enrolled as alternatives*." (Pg. 2, Study Synopsis).
- On pg. 10, 4.4. Withdrawal Criteria, the study protocol explains criteria for subjects withdrawal, removal or termination in the study:
  "*Participants can withdraw at any time without giving a reason for withdrawing. Data collected to the point of withdrawal will be used in the analysis of the study, unless the participant requests that their data is not used, in which case it will be removed from the database. Participants may also be removed at the discretion of the Principal Investigator, where continued participation may affect the safety of the participant or where there is a development of any condition which might interfere with study participation."*
- Withdrawal of subjects will be treated as right-censored data for statistical purposes.
- Subjects will be trained to recognize landings and familiarize themselves with handling of mosquitoes:
  "*Participants will undergo a screening evaluation which includes a training (to detect mosquito landings and use an aspirator…"* (pg. 2, Study Synopsis).
- Subjects will work in pairs, checking each other as well as themselves:
  "*Each treated and untreated control participant will be paired with a trained member of staff or another participant. The two untreated controls will be paired together. Each pair will be located at least 3 m/10 ft apart from other pairs*." (pg. 16, Section 8.5.6. Subject Placement).
- Landings will be verified by a research technician
  "*Under supervision of a trained member of staff or another participant, the number and timing of each landing during each exposure period for each participant will be recorded. All landing insects will be collected for identification by aspiration and labelled with the time of collection (before they have chance to probe or bite)*." (8.5.7. Exposure period).
- For assessment of landing pressure, time of each landing will be recorded to document how long it took to reach 5 landings within 5 minutes.

(c) What QA methods are proposed?

arctec is designated as the Trial Monitoring Center (pg.1):

arctec
Address: Room LG38, LSHTM, Chariot Innovations Ltd., Keppel St, London WC1E 7HT, UK
Tel: +44 (0) 20 7927 2883
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"*This trial will adhere to the principles outlined in the International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines, protocol and all applicable local regulations*." (p. 1, Section Trial Monitoring Center).

The study protocol should be revised to adhere to EPA GLP requirements. The EPA requires that any laboratory or field testing site should adhere to good laboratory practice standards (GLP), 40 CFR Part 160, but the testing site are not required to be GLP accredited. A statement of compliance or non-compliance has to be submitted to inform the EPA if the compliance was met (see 160.12 Statement of compliance or non-compliance). In addition, these rules require that each testing facility include an independent Quality assurance (QA) unit to monitor execution of each protocol and document its conduct in accordance with the GLP regulations. Again, the QA unit does not have to be GLP accredited.

According to 40 CFR §160.17, EPA may refuse to consider reliable for purposes of supporting an application for a research or marketing permit any data from a study which was not conducted in accordance with this part. Therefore, EPA has the discretion to accept or reject any non-GLP study. If it is a credible study and the GLP compliance statement demonstrates that the parts of the study that are non-GLP do not have any influence on the results of the study, the study will be accepted.

"*Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period."* (p. 24, Section 11.6. Record retention).

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

Sources of variation include mosquito species to be likely present at the proposed field sites, habitat characteristics, climate, mosquito pressure at different times of day, and test subjects' attractiveness to mosquitoes.
Assuming EPA's recommendations are incorporated, then uncertainty will be addressed by indicating that no more than 15% of the study period will take place

during low mosquito pressure. Likewise, no more than 15% percent exposure periods should be skipped due to weather related condition.

3. Subject Selection

3.1 Representativeness of Sample

The population of repellent users is presumed to be diverse in age, gender, physical size, general health, attractiveness to biting insects, and other characteristics. EPA recommended that the protocol state explicitly that eligible subjects will be selected to be representative by gender and race/ethnicity of general adult population of repellent users. (p. 13, 8.3.1. Recruitment of volunteers).

(a) What is the population of concern?

The population of concern is people who would purchase and use skin-applied insect repellents.

(b) From what populations will subjects be recruited?

Volunteers will be recruited in and around the University of Florida, and will be representative of the population of concern: "*…eligible subjects the participants will be selected to be representative of age, gender, race/ethnicity of the general population.*" (p. 13, section 8.3.1. Recruitment of volunteers).

EPA recommends that the protocol be amended to describe in detail the recruitment process. For example, where will advertisements be posted and for how long? What steps will you take to ensure that the recruited population is representative of the general public/users of skin-applied insect repellents? Who will make contact to with people who express an interest in participating?

Participants will be recruited from a pool that meet the inclusion and exclusion criteria, as outlined below:

<u>Inclusion criteria</u>

- Able and willing to give fully informed consent
- Able to understand and comply with the study procedures
- Male or female
- Aged 18 to 55 years
- Non-smokers or willing to refrain for 24 hours prior to and during each test
- Willing to undergo a mosquito attraction test (putting an arm into a cage of mosquitoes)
- Willing to complete mosquito handling training
- Able to stand outside for periods of at least 5 minutes at a time
- Able to operate an aspirator
- Able to speak and understand English

Exclusion criteria

- Suspected or known to be sensitive or allergic to, or phobic of, mosquito bites
- Participated in an interventional study (other than a biting insect challenge study) in the previous 3 months
- Participated in a biting insect challenge study in the previous 48 hours
- Diagnosed with any cardiac or respiratory disorder (whether active or inactive)
- Individuals with localized skin disorders affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes
- Allergic to any of the test or reference product ingredients
- Women who are pregnant, nursing or intending to become pregnant
- Previous anaphylaxis
- Aware of having a compromised immune system
- Employees, managers, and spouses of employees of the University of Florida and of the study Sponsor
- Students of the primary investigator or any other University of Florida faculty/researchers involved in the study
- Unable to speak and understand English
- Unable to aspirate mosquitoes

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

The participants should be representative of the population of concern: "…*eligible subjects the participants will be selected to be representative of age, gender, race/ethnicity of the general population.*" (p. 13, section 8.3.1. Recruitment of volunteers).

(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. (p. 9, sections 4.2 and 4.3, Inclusion and exclusion criteria, respectively).

Inclusion criteria

- Able and willing to give fully informed consent
- Able to understand and comply with the study procedures
- Male or female
- Aged 18 to 55 years

- Non-smokers or willing to refrain for 24 hours prior to and during each test
- Willing to undergo a mosquito attraction test (putting an arm into a cage of mosquitoes)
- Willing to complete mosquito handling training
- Able to stand outside for periods of at least 5 minutes at a time
- Able to operate an aspirator
- Able to speak and understand English

Exclusion criteria

- Suspected or known to be sensitive or allergic to, or phobic of, mosquito bites
- Participated in an interventional study (other than a biting insect challenge study) in the previous 3 months
- Participated in a biting insect challenge study in the previous 48 hours
- Diagnosed with any cardiac or respiratory disorder (whether active or inactive)
- Individuals with localized skin disorders affecting the legs (such as eczema, psoriasis, or atopic dermatitis) or open cuts or scrapes
- Allergic to any of the test or reference product ingredients
- Women who are pregnant, nursing or intending to become pregnant
- Previous anaphylaxis
- Aware of having a compromised immune system
- Employees, managers, and spouses of employees of the University of Florida and of the study Sponsor
- Students of the primary investigator or any other University of Florida faculty/researchers involved in the study
- Unable to speak and understand English
- Unable to aspirate mosquitoes

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

None. EPA has suggested that the protocol clarify that students of the primary investigator or any other study staff are not eligible to participate. Subjects may be students of the University of Florida, but not working with anyone involved in the administration of the study.

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

No.

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

Volunteers will be recruited in and around the University of Florida, and will be representative of the population of concern: "*…eligible subjects the participants will be selected to be representative of age, gender, race/ethnicity of the general population.*" (p. 13, section 8.3.1. Recruitment of volunteers).

EPA recommends that the protocol be amended to describe in detail the recruitment process. For example, where will advertisements be posted and for how long? What steps will you take to ensure that the recruited population is representative of the general public/users of skin-applied insect repellents? Who will make contact to with people who express an interest in participating?

Potential subjects will be contacted by someone associated with the study, who will provide more information by phone or email. If the potential subject is interested in participating, he or she will be invited to meet with the study director or other study personnel one on one or in a small group to review the informed consent document. This meeting will cover a brief outline of the study including its purpose, the subjects' potential role in the study, the potential length of the study on any given test day, the identity and function of the pesticide to which they will be exposed, the potential hazards associated with the study and steps being taken to mitigate each hazard as addressed in the protocol, and the inclusion/exclusion criteria. The procedures involved with the attractiveness test, training on aspirating mosquitoes, and a 5-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 test substances will be applied to their leg for the future testing, will be informed that they will wear gloves to protect their hands and head nets to protect the head, face and neck, and will be shown how to aspirate mosquitoes.

(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 through advertisements in local newspapers and potentially through other 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, students or employees of the study director or other faculty and researchers involved in the study are excluded from participation. Finally, any employees, managers, and spouses of employees of the University of Florida and the study sponsor are excluded from participation.

3.3 Remuneration of Subjects

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

The protocol initially proposed paying subjects $10/hour for the field testing, regardless of the total number of hours in a single test day. However, EPA recommends that for any time beyond 8 hours, subjects be compensated at a rate of time and a half (i.e., $15/hour). Subjects will also be compensated for the time they spend in the consent meeting, training course, and mosquito attractiveness test ($20 per meeting/event).

(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?

EPA has requested that the protocol include more specific information about how and when subjects will be paid. According to the materials provided to the IRB, the subjects will be paid by check.

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?

The active ingredient, IR3535, in the 3 proposed products 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 IR3535 is greater than 3,000 mg/kg body weight. IR3535 is not a skin sensitizer.

Results from toxicity testing:

- A primary eye irritation study on rabbits showed that IR3535 is irritant to the eyes. Irritation was observed for 24-48 hours but was all cleared within 7 days.
- A dermal sensitization study in Guinea pigs showed that IR3535 is not a contact sensitizer.
- A primary skin irritation study on rabbits showed that IR3535 is minimally irritating to the skin. All irritation was cleared by 48 hours.
- The single dose acute dermal LD_{50} of IR3535 is >3,000 mg/kg in rabbits.
- The acute oral LD_{50} of permethrin is 5,000 mg/kg in rats,

The NOAEL for dermal toxicity is $\geq$ 3000 mg/kg/day in rats and for oral toxicity is 600 mg/kg/day in rabbits. In its risk assessment, EPA used a 5% dermal absorption factor for IR3535 and based their calculations on an 11.8 kg child and 60 kg adult for a product at 7.5% active ingredient. Since the initial registration, EPA has registered several products containing up to 20.07% IR3535. The products to be tested in the protocol may contain up to 20% IR3535 and Margins of Exposure (MOE) can be calculated as below:

The calculation below is an example for calculating exposure estimates for a product containing 20% IR3535, which is the product with the highest concentration of IR3535 of the three products proposed in the protocol, for risk characterizations using

2680 cm^2 as the average area for the lower leg of an adult male (average lower leg area for a female is 2330 cm^2):

(2680 cm^2 x 1000 mg formulation/600 cm^2) ÷ 60 kg = 74.4 mg formulation/kg

(74.4 mg formulation/kg)(0.20 mg a.i./mg formulation) = 14.9 mg a.i./kg

14.9 mg a.i./kg x 0.05 dermal absorption factor = 0.75 mg a.i./kg

Margins of Exposure (MOE) are calculated by dividing the oral NOEL of 600 mg/kg/day as below:

600 mg/kg ÷ 0.75 mg/kg = 800

To reach the EPA's level of concern (MOE ≤ 100), a person would need to make approximately 9 applications in a single day.

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

Risks to subjects include the risk of exposure to biting mosquitoes, the risk of exposure to disease vectors, the risk of exposure to the test material, risks related to receiving an unexpected result on a pregnancy test, and the risk of a loss of confidentiality.

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

The proposed dose and exposure levels are significantly lower than the established NOAELs for the test material. See the calculations in Section 4.1. To reach the EPA's level of concern (MOE ≤ 100), a person would need to make approximately 9 applications in a single day.