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

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

August 19, 2022

MEMORANDUM

SUBJECT: Ethics Review of Completed Study titled "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes"

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

TO: Linda Hollis, Chief, Biochemical Pesticides Branch
Biopesticides and Pollution Protection Division
Office of Pesticide Programs

REF: Scott P. Carroll, Ph D. (2021) Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray Against Mosquitoes. Report Amendment date May 12, 2022. 380 pages. MRID 51912701.

I have reviewed available information concerning the ethical conduct of the referenced research study, "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes", including Volumes 2 and 3 of the submission that provide the Institutional Review Board Communications Files. The documents submitted to the Environmental Protection Agency (EPA) describe the implementation and results of a field study, the objective of which was to determine the efficacy of skin-applied insect repellents against mosquitoes in field settings.

After reviewing all available documentation, I have determined that the conduct of this study met applicable ethical standards for the protection of human subjects of research, and that the requirements for documentation of ethical conduct of the research were satisfied. If the research is determined to be scientifically acceptable, I find no barrier in regulation to the EPA's reliance on this study in actions under the Federal Insecticide, Fungicide, or Rodenticide Act (FIFRA) or the Federal Food, Drug, and Cosmetic Act (FFDCA). If the research is not scientifically valid, it would not be ethical to rely on it.

In addition, under 40 CFR 26.1604, EPA is required to seek input from the Human Studies Review Board (HSRB) for intentional exposure human studies covered by EPA's Human

Studies rule that are initiated after April 7, 2006. EPA will share this study and all associated support documents, as well as EPA's science and ethics reviews of the study with the HSRB for their review. This memorandum and its attachments constitute EPA's ethics review.

Completeness of Submission

The materials provided by the study sponsor satisfied the requirements of 40 CFR 26.1303. A checklist indicating how each requirement has been satisfied is provided in Attachment 1.

Summary Characteristics of the Research

MIMIKAI sponsored this study in order to determine the complete protection time (CPT) or duration of efficacy of a skin-applied repellent (MIMIKAI Lilly Pilly) containing 11% Oil of Lemon Eucalyptus (OLE, also known as Citriodiol) and 7.75% 2-undecanone (methyl nonyl ketone or MNK) against wild populations of mosquito species. Testing was conducted by Carroll-Loye Biological Research (CLBR). The field tests of each product formulation were held at two different sites in Northern California. The study initiation date was February 17, 2020. Field testing occurred on September 26, 2021, and October 3, 2021. The study completion date was October 28, 2021, and the study was closed out by the IRB on November 16, 2021.

Human subjects were used because no reliable models or surrogates have been found to adequately predict the duration of efficacy of topically-applied insect repellents. The repellent test product (IR3535) has been registered by EPA and has already been found to present little or no risk when used as directed. The precautions taken to mitigate hazards associated with the study were consistent with the approved protocol.

Required Reviews of Protocol & Ethics-Related Chronology

On December 24, 2020, Advarra IRB approved the protocol dated December 23, 2020, informed consent form, and recruitment materials. Advarra's IRB is registered with FDA and OHRP, and has a Federal-wide Assurance approved by OHRP (00023875). Advarra is fully accredited by the Association for the Accreditation of Human Research Protection Programs (AAHRPP). Satisfactory documentation of the IRB procedures and membership is on file with the Agency. Documentation regarding IRB approval of the protocol, consent and recruitment materials has been provided to the HSRB members with the background materials for this review.

An IRB-approved draft protocol was submitted to EPA for review. The protocol and EPA's review[1], dated March 25, 2021, were discussed at a public meeting by the HSRB on April 20, 2021. The HSRB concluded that "[t]he research proposed … is likely to meet the applicable

[1] Fuentes, Hull-Sanders, Arling. Science and Ethics Review of a Protocol for Field Evaluation of Skin-Applied Mosquito Repellent Product Containing Oil of Lemon Eucalyptus. March 25, 2021. https://www.epa.gov/sites/default/files/2021-04/documents/1c._epa_science_ethics_review_memo_w_att_mimikai_mosquito_mim-006_3-25-21.pdf

requirements of 40 CFR part 26, subparts K and L, if the recommendations made by the EPA and HSRB are adequately addressed".[2]

In follow-up to the HSRB meeting, the researchers revised the protocol and related materials to address comments, including the EPA and HSRB comments described in Attachment 2, and submitted the revised documents to the California Department of Pesticide Regulation (CDPR) and Advarra IRB for final review and approval of the protocol and materials prior to initiating the study. CDPR approved the study on July 14, 2021 (pp. 358-9) and Advarra approved the Amendment 3 of the protocol, used in the study, on September 11, 2021 (p. 360). Advarra terminated oversight of the study on November 16, 2021.

The registrant submitted a study report to EPA on November 17, 2021. In response to correspondence from EPA (see the EPA Science Review Memo), the registrant amended and resubmitted the study report. This review pertains to the amended study report, dated 12 May 2022, MRID 51912701.

Recruiting

A total of 57 persons were recruited for the study and 46 completed the consent process (20 male, 26 female). Recruitment was conducted in the Davis and Sacramento regions of California, using Craigslist, the UC Davis ECOSOCIAL list serve, the UC Davis Entomology club email newsletter and word-of-mouth (p. 13). Subjects for both field test days were recruited from a single pool, given the geographic proximity of the test locations, which was a possibility identified in the protocol (p. 53) The protocol called for keeping recruitment open until a pool of 60 candidates was identified, but recruitment was closed when 57 candidates responded.

The list of respondents was randomized, and the study director or a member of the research team contacted those who expressed an interest in participating by phone using an IRB-approved script (p. 15). This communication provided potential subjects with more information about the study, including the process for consenting, the screening process, eligibility criteria, and requirements for participation. Those who were qualified based on these criteria were invited for a meeting with the study team and to review the informed consent document. Of these, six candidates did not return voicemails left by study staff, one candidate could not schedule a time for a consent interview, and two candidates were deemed ineligible after the exclusion criteria screening by phone was completed.

Consent and Enrollment

According to the study report, 46 individuals completed the informed consent process and signed the IRB-approved consent form (p. 15). Consent meetings were held in person or via video call. Subjects were provided with copies of documents as outlined in protocol section 3.4. During the consent meeting, a trained member of the study staff member read the consent form. This included an outline of the study, including its purpose, the subjects' potential role, the length of the study on a test day and overall, the pesticide to which subjects would be exposed,

[2] Cavallari, Jennifer. April 20-21, 2021 EPA Human Studies Review Board Meeting Report. https://www.epa.gov/system/files/documents/2021-07/042021-hsrb-meeting-report-final.pdf p. 8.

risks of participation and how they would be mitigated, and the eligibility criteria. Female subjects were informed about the prohibition on enrolling pregnant and nursing women, and the study requirement to take a pregnancy test on each study day on which they would be exposed to the test substance or mosquitoes. In addition, the consent process included a demonstration of the repellent application, attractiveness testing, and aspirator use. The process also highlighted that participation was completely voluntary. Subjects were permitted to ask questions, and then were asked questions to ensure their comprehension of the consent form and study procedures. In addition, during the consent process, candidates were screened according to the eligibility criteria. If the person was determined to be eligible, the study staff verified the subject's age with a government-issued identification.

A total of 14 individuals completed the consent process in person at an outdoor location, signing the consent form following the process described above. The remaining 32 subjects, who attended the meeting and consented to participate virtually, were asked again at their first lab visit whether they still wanted to participate, were reminded they were free to withdraw at any time, and were offered the opportunity to ask questions about the research. After confirming a continued desire to participate, they were asked to initial all pages and sign the consent form. All subjects received a copy of their signed consent forms.

Subjects met the eligibility criteria outlined in the protocol (pp. 53-54) and were screened during the consent process (p. 15). Eligibility was confirmed through the subject screening, mosquito attractiveness test, aspirator training, and pregnancy testing for female subjects. Subjects were eligible to participate if they were willing to consent, between 18 and 60 years old, and able to speak and understand English. People were not eligible to participate if doing so would pose a risk to their health (allergic or sensitive to mosquito or arthropod bites, allergic to the test substance, skin disorders and/or open cuts/scrapes on the legs, previous anaphylaxis, compromised immune system, prone to heat-related illness), if they were unwilling to refrain from using certain products before the testing (repellents, perfumed products, alcoholic beverages, tobacco), or if they did not spend time outdoors on a regular basis. Individuals who were deemed unattractive to mosquitoes or who were unable to successfully demonstrate the ability to aspirate mosquitoes during the training phase were also excluded. Additionally, pregnant or nursing women, and employees of the Study Director or study sponsor, as well as their spouses and immediate family members, were not eligible.

After completing the consent process, subjects were scheduled for a training visit. As all test sites were located in northern California, this entailed the subject visiting the CLBR laboratory facility to be tested for attractiveness to mosquitoes, to be trained to use an aspirator, and to have their limbs measured so the study staff could calculate the appropriate dose of the test substance. Mosquito attractiveness testing and aspirator training were conducted according to the protocol. All subjects were deemed attractive and demonstrated proficiency in using the aspirator as outlined in the protocol.

Following successful completion of the screening visit, subjects were eligible to participate in one or more field tests.

Demographics

A total of 57 individuals were recruited to participate in the study, and 46 of those consented to participate in the study. Of the 46 persons who consented, there were 20 males and 26 females. Subjects' ages ranged from 19 years old to 65 years old.

Randomization and Test Day Procedures

Prior to the test day, the research director called or emailed the subjects to remind them about the study requirements (pp. 22-23). On each test day, subjects were invited to assemble at the CLBR lab. In a deviation from the protocol, more than the specified 20 consented subjects were invited to assemble at the test location. On the first test day, 25 subjects arrived at the test site. On the second day, 22 subjects arrived at the test site. Subjects were reminded again about the study requirements to avoid perfume, alcohol, smoking, and repellents prior to and during the test period, as well as about the freedom to withdraw at any time without penalty (p. 23). Subjects' skin was inspected according to the protocol and females who were required to take a pregnancy test and share the results with a female member of the study staff did so (p. 192). All subjects were qualified to continue participation in the study. Subjects were randomly assigned roles as test (13), control (2), or alternate (5) subjects. Once all 20 slots were filled any remaining subjects were compensated and dismissed. Using a mix of personal and rental vehicles, subjects went from the lab to the field test location, approximately 80 minutes away. On each test day, 4-5 subjects transported themselves to the field site and the remaining subjects were transported by the researchers. Upon arrival at the field site, subjects washed the forearm to be used for treatment, sprayed it with diluted ethanol, then dried it. The test substance was applied to each test subject's forearm by a researcher wearing gloves and using a finger cot. All subjects going into the field (test and control) were prepared with gloves and tape at the wrist and elbow to protect against mosquitoes from biting outside of the treatment/control assessment area.

No subjects withdrew, were excluded during the test day screening, or removed by the study director. No alternates were needed to replace test subjects; they were dismissed from participation within the first two exposure periods.

To start the testing, two untreated control subjects' forearms were exposed to assess landing pressure p. 24. Once landing pressure was established, the test subjects began 5-minute exposures to mosquitoes every 30 minutes. Repellency was measured as the time between application of a test substance and the first confirmed landing. A "landing" occurred when a mosquito landed on the treated test skin of a subject. A First Confirmed Landing (FCL) was defined in the protocol as when a second landing occurs within 30 minutes of the first landing. There was a discrepancy between the definition of FCL in the protocol and the consent form (a second landing within 30 to 60 minutes of the first landing). The staff followed the more conservative definition from the consent form. After the initial landing pressure assessment, the two control subjects exposed one forearm every 30 minutes to assess landing pressure, stopping at the sooner of five minutes or after five mosquitoes landed.

When a test subject received a confirmed landing or at the end of the test day, subjects removed the tape and gloves, washed their skin, and were released from study participation.

Study staff were available to provide transportation to the subjects who completed participation at the time they finished if necessary.

Safety Precautions

The protocol discussed potential risks associated with these tests including exposure to the test material, biting mosquitoes and vector-borne pathogens, physical risks from being outside during the day, unanticipated loss of confidential information, and psychological risks related to pregnancy testing. Risks were appropriately minimized as follows: Individuals with skin conditions that could be exacerbated by exposure to the test substance or who had a known allergy to the test substance or repellents were excluded from participation. Subjects were provided with soap and water to wash their treated limbs immediately following their participation in the study. A medical professional examined subjects' skin on the test day prior to and following exposure, and there were no instances of adverse reactions to exposure to the test substance.

Mosquitoes used in the attractiveness test and aspirator training were lab-raised and never received a human blood meal (p. 307). The protocol requirements for trapping and testing mosquitoes prior to conducting field testing and coordination with the local health departments and mosquito control districts to ensure no vector-borne illnesses were detected at the test sites were followed. Testing was conducted in areas where no mosquito-borne vectors were identified through the researchers' trapping and testing program, and there were no reports of incidents from the local public health services within a week of testing. Subjects were trained in aspirating mosquitoes for the field portion of the test to avoid mosquitoes biting after landing and to collect the mosquitoes for pathogen testing.

Subjects who might have adverse reactions to mosquito bites or the test substance, or who might have difficulty standing outside for extended periods were excluded per the study's eligibility criteria. Subjects were provided with a head net and gloves, and instructed to wear clothing that fully covered their bodies during the testing. Only the area to be treated with the repellent was exposed to mosquitoes during the test period. In addition, untreated control subjects only exposed their forearm until the requisite number of mosquito landings were observed for each period during the testing or until five minutes elapsed, whichever occurred first. At each test site, a shaded, screened area with chairs, with snacks, water, and other drinks was available for subjects' use during the periods between the test periods.

No adverse effects were reported by study participants.

Confidentiality

The study followed the measures outlined in the protocol regarding confidentiality. Subjects were identified by numbers on study documentation, rather than by name. Pregnancy tests were conducted in private, and the results were only communicated with a female member of the study team to confirm eligibility of female subjects to participate.

Compensation

Each subject received compensation consistent with the protocol and informed consent document. Compensation was $25 per hour for each hour of participation in each phase (consent, training, test day) rounded up to the next hour (p. 49). Subjects received their payment in person at the end of each visit (p. 49). No subjects withdrew or were removed from participation in this study.

Protocol Amendments and Reported Deviations

The protocol was amended a total of three times following review by the EPA and the HSRB and prior to the initiation of testing (pp. 115-163). The first amendment, approved by Advarra IRB on December 24, 2020, updated the original submission to the Advarra IRB and changed the amount of MNK in anticipation of submission of the protocol to the EPA for review (pp. 115-135). Amendment 2 was initiated following review by EPA and the HSRB, and addressed the recommendations following the public meeting held in April 2021 (pp. 136-162). Amendment 3 corrected a typographical error (p. 163).

The EPA noted an issue related to amendment approval dates. The effective dates of some amendments predate the IRB's approval of the amendments. For example, for Amendment 1 the amendment date is listed as December 23, 2020 (p. 135). However, the IRB did not approve the amendment until December 24, 2020 (Volume 2, p. 189). The discrepancies in the effective dates did not affect subject safety or welfare; however, amendments are not effective until the IRB has reviewed and approved them. The EPA recommends that in future studies, the effective dates of amendments and protocol revisions be listed as "IRB approval date" or left blank at the time of submission to the IRB and added after IRB approval.

Seven deviations to the study were included in the report. Several deviations related to subject recruitment and enrollment. First, the protocol called for recruiting a pool of 40 subjects, 20 per site. The Study Director increased enrollment to 46 subjects, and asked 25 subjects to show up for the first test day and 24 subjects to show up for the second test day (deviation 5). Individuals were assigned as control (2), test (13), or alternate (5) subjects on the test day. Those who were not assigned any of these roles were dismissed. Additionally, subject randomization and balanced enrollment between genders deviated from the protocol requirements (deviations 6 and 7). However, none of the deviations negatively impacted subjects' safety or welfare.

On the test day, a discrepancy between the protocol and consent form about how a confirmed landing is defined was identified. The protocol followed the EPA guidelines, scoring a confirmed landing as a landing followed by another landing within 30 minutes (the same or next exposure period). The consent form scored a confirmed landing as one that occurred within the same exposure or in two of three consecutive exposure periods (i.e., 60 minutes). The definition of confirmed landing from the consent form was used (deviation 3). This definition is more conservative and aligns with the consent given by subjects. It did not impact the health or safety of subjects.

Two deviations related to the measurement and application of the test substance. Deviation 1 substituted pre-weighed finger cots for the protocol-specified pre-weighed gloves.

Deviation 2 noted that only subjects' forearms were measured to calculate the appropriate test dose, rather than the protocol-specified measurement of both forearms and lower legs, based on the expected behavior of mosquitoes present at the test sites. Deviation 4 noted that at certain points, subject pairs were closer than the 10' minimum specified in the protocol.

None of the protocol amendments or deviations negatively impacted the subjects' health or welfare.

Recommendations

For future studies, EPA recommends the following:

- Include details about subject recruitment, consent, enrollment, test day participation, and compensation in the study report. Additionally, include the procedures followed for pre-testing mosquito trapping and consultation with local public health agencies.
- Make clear in the protocol and the consent form whether subjects will be recruited to participate in more than one test day.
- Closely review the protocol and consent form to ensure consistency prior seeking final approval of the documents.
- Ensure that the protocol clearly states that all amendments to the protocol, regardless of whether they are related to subject safety or informed consent, must be reviewed and approved by the overseeing institutional review board prior to implementation. *See* 40 CFR 26.1108(a)(3)(iii) compared with the language on pages 70-71 of the study report.

Applicable Ethical Standards

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

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

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

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

Findings

Pregnancy testing of female subjects was conducted on each day of testing. No pregnant or lactating women were enrolled in the study. All subjects who participated in study were at least 18 years old. Therefore, 40 CFR §26.1703 does not prohibit reliance on this research.

40 CFR §26.1705 requires that EPA have "adequate information to determine that the research was conducted in substantial compliance with subparts A through L of this part." Within this range, only subparts K and L are directly applicable to the conduct of third-party research such as this. After reviewing all available information, I conclude that this study was conducted in substantial compliance with subparts K and L.

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

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

Conclusion

This study reports research conducted in substantial compliance with the requirements of 40 CFR 26 subparts A through L, and with the protocol for research that was reviewed by EPA and the HSRB according to the standards at 40 CFR 26, Subpart P. In its conduct, this study met applicable ethical standards for the protection of human subjects of research, and requirements for documentation of ethical conduct of the research were satisfied. From EPA's perspective, if this study is determined to be scientifically valid and relevant, there is no regulatory barrier to EPA's reliance on it in actions under FIFRA or §408 of FFDCA. This research and EPA's reviews will also undergo review by the HSRB.

Cc: Shannon Borges
Clara Fuentes
Angela Myer
Menyon Adams

Attachment 1: §26.1303 Completeness Checklist
Attachment 2: Responsiveness to EPA and HSRB Ethics Comments on Draft Protocol

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

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

Requirement		Y/N	Comments
(a) Copies of all of the records relevant to the research specified by § 26.1115(a) to be prepared and maintained by an IRB	§1115(a)(1): Copies of • all research proposals reviewed, • scientific evaluations, if any, that accompany the proposals, • approved sample consent documents, • progress reports submitted by investigators, and reports of injuries to subjects.	Y	Appendices 16.1, 16.2, 16.6
	§1115(a)(2): Minutes of IRB meetings which shall be in sufficient detail to show • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of • members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; • a written summary of the discussion of controverted issues and their resolution.		Requested from the IRB
	§1115(a)(3): Records of continuing review activities.	Y	IRB Volume 1
	§1115(a)(4): Copies of all correspondence between the IRB and the investigators.	Y	IRB Volume 1
	§1115(a)(5): • A list of IRB members identified by name; earned degrees; representative capacity; indications of experience such as board certifications, licenses, etc., sufficient to describe each member's chief anticipated contributions to IRB deliberations; • any employment or other relationship between each member and the institution, for example, full-time employee, a member of governing panel or board, stockholder, paid or unpaid consultant.	Y	IRB Volume 2
	§1115(a)(6): Written procedures for the IRB in the same detail as described in § 26.1108(a) and § 26.1108(b).	Y	IRB Volume 2
	§1115(a)(7): Statements of significant new findings provided to subjects, as required by § 26.1116(b)(5).	N/A	
(b) Copies of all of the records relevant to the information identified in § 26.1125(a)-(f)	§1125(a)(1): The potential risks to human subjects	Y	Study Report
	§1125(a)(2): The measures proposed to minimize risks to the human subjects;	Y	Study Report
	§1125(a)(3): The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	Study Report
	§1125(a)(4): Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	Study Report
	§1125(a)(5): The balance of risks and benefits of the proposed research.	Y	Study Report
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.	Y	Study Report
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.	Y	Study Report
	§1125(d): A description of the circumstances and methods proposed for presenting information to potential human subjects for the purpose of obtaining their informed consent.	Y	Study Report
	§1125(e): All correspondence between the IRB and the investigators or sponsors.	Y	IRB Volume 1
	§1125(f): Official notification to the sponsor or investigator, in accordance with the requirements of this subpart, that research involving human subjects has been reviewed and approved by an IRB.	Y	IRB Volume 1
(c) Copies of sample records used to document informed consent as specified by §26.1117, but not identifying any subjects of the research		Y	Study Report
(d) If any of the information listed in paragraphs (a) through (c) of this section is not provided, the person shall describe the efforts made to obtain the information.		N/A	

Attachment 2

Responsiveness to EPA and HSRB Ethics Comments on Draft Protocol

EPA Recommendation	Action taken by Study Sponsor
Remove requirement for subjects to wear Tyvek suits during testing	Removed from the protocol
Randomize assignment of subjects as control or test subject, rather than recruiting individuals to consent and participate as control or test subjects	Protocol and consent materials were revised to address this comment
Revise consent materials to include a concise description of key information, as well as step-by-step demonstrations of all procedures that will occur during the attractiveness testing, aspirator training, limb measurement, and field testing. Include in the protocol discussion of consent the steps that will be taken to ensure comprehension of the consent materials prior to inviting individuals to consent to participate	Protocol and consent materials were revised in accordance with these recommendations
Update compensation section to include information about when and how compensation will be paid, the rate for alternates, compensation for subjects who withdraw or complete testing early	Protocol was amended to include this information
Revise protocol to acknowledge risks associated with COVID-19 and the precautions that will be taken	Protocol was amended to include this information
Replace “bites”, “landing with intent to bite” and “LIBes” with landings	Protocol was amended except for some typographical errors carried over from previous versions of the protocol
Clarify that pregnancy testing must be conducted anytime female subjects will be exposed to mosquitoes or the test substance	Protocol was amended to include this information

EPA Recommendation	Action taken by Study Sponsor
Explain how adverse events will be evaluated and reported to the IRB. Additionally, include in the protocol a requirement for a trained professional to evaluate subjects' skin to ensure they are eligible to participate on the test day, and again at the end of the test period	Protocol was amended to address these comments
Clarify how subjects will be transported to the test site and at whether subjects who reach CPT before the end of the test day will be free to leave or will need to wait for study staff to provide transportation	Protocol was amended to address these comments

HSRB Recommendation	Action taken by Study Sponsor
Do not use Tyvek suits in testing; if Tyvek suits are used, add a question and exclusion criteria for potential subjects about history of heat-related illness or injury.	Protocol was revised to remove Tyvek suits for subjects
Ensure that any COVID-19 related steps are included in the protocol	Protocol was revised to include COVID-19 related precautions
Consider removing the option to use latex gloves as they pose a greater allergenic risk than other glove options.	Protocol was revised to refer to latex-free gloves throughout
Clarify in the protocol the criteria to be used to judge whether subjects are in poor physical health	"Poor physical health" was removed as an exclusion criterion for subjects
Revise the protocol to specify that standard first aid materials are commonly-acquired over the counter materials	Protocol was revised to specify that the first aid materials are over the counter and to include examples (bandages, antiseptics, and mild topical and oral antihistamines)