HSRB Human Studies Review Board

May 9, 2025

Monique E. Tadeo, MS, CIP
EPA Human Subjects Research Review Official and Director,
Program in Human Research Ethics and Oversight
United States Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: April 3, 2025, EPA Human Studies Review Board Meeting Report

Dear Ms. Tadeo:

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of a study protocol involving human subjects.

On April 3, 2025, the HSRB considered a study protocol for an evaluation of topically applied insect repellent products containing Citriodiol, Oil of Lemon Eucalyptus against mosquitoes in the field. Briefly, the goal of the proposed study is to determine the efficacy and duration of protection of two topically applied insect repellent products at preventing landing by mosquitoes. The study will be conducted at two field sites in the United States. The median complete protection time (CPT) for the product is noted as the efficacy endpoint.

The HSRB's responses to the charge questions, along with detailed comments and recommendations for the EPA to consider, are provided in the enclosed final meeting report.

Sincerely,

Philip Day

Philip Day, Ph.D.
Co-Chair, HSRB

Julia A. Sharp

Julia Sharp, Ph.D.
Co-Chair, HSRB

Report of the U.S. Environmental Protection Agency Human Studies Review Board

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HSRB Science Reviewer
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EPA Contact

Emily Sokol, Designated Federal Officer

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Contents

List of Acronyms

CFR	Code of Federal Regulations
CI	Confidence Interval
CPT	Complete Protection Time
EPA	Environmental Protection Agency
HSRB	Human Studies Review Board
mCPT	median Complete Protection Time
OLE	Oil of Lemon Eucalyptus

HSRB Meeting Report

SAST-001 (2024): Submission of "Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field" March 22, 2024. Sponsored by Citrefine International Ltd. Moorfield Rd. Yeadon, Leeds. LS19 7BN U.K. MRID 523504. Protocol version 0.1 as amended March 11, 2024. IRB approved March 7, 2024. 117 p.

Introduction

On April 3, 2025, the Human Studies Review Board (HSRB) considered the scientific and ethical charge questions related to a study protocol titled "Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field." The Environmental Protection Agency (EPA) sought the HSRB review of the study protocol in accordance with the applicable requirements of 40 Code of Federal Regulations (CFR) part 26.

Review Process

The Board conducted a public meeting on April 3, 2025. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-10408-01-ORD). This Final Report of the meeting describes the HSRB's discussion, recommendations, rationale, and consensus in response to the charge questions on ethical and scientific aspects of the research.

For each agenda item, the Agency staff presented their review of the scientific and ethical aspects of the research. Each presentation was followed by clarifying questions from the HSRB. The Board solicited public comments and then proceeded to address the charge questions under consideration. The HSRB discussed the science and ethics charge questions and developed a consensus response to each question. For each of the charge questions, the Chair called for the Board to vote to confirm concurrence on a summary statement reflecting the Board's response.

For their evaluation and discussion, the Board considered materials presented at the meeting, research articles, and related materials, the Agency's science and ethics reviews of the research studies, the Agency's statistical analysis of the research data, comments from the Public, and oral comments from Agency staff during the HSRB meeting discussions. A comprehensive list of background documents is available at https://www.epa.gov/scientific-leadership/hsrb-april-3-4-2025.

Charge Questions and Context

Charge to the Board – Science

Is the protocol "Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field" likely to generate scientifically reliable data, useful for estimating the amount of time each of the products tested repels mosquitoes?

HSRB Response

The research proposed in the protocol "Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field" is likely to generate scientifically reliable data, useful for estimating the amount of time the product tested repels mosquitoes, given the comments and recommendations provided by the EPA and HSRB are adequately addressed.

Science Review

The objective of the proposed study is to "*determine the efficacy and duration of protection of two topically applied insect repellent products at preventing landing by mosquitoes*." Both products contain the same active ingredient, i.e., oil of lemon eucalyptus (OLE), which is already registered with the U.S. EPA, sold under the registered trade name Citriodiol® and identified by the CAS No. 1245629-80-4. The OLE contents of these two products are 10-15% and 30% respectively. These two products will be tested against natural populations of mosquito species of public health importance within the genera *Aedes*, *Anopheles*, and *Culex*, across two field sites in the United States.

The study will comprise a minimum of four completed test days (two products and two field sites). A power analysis determined a sample size of 13 participants provides sufficient power for each formulation for each of the two sites. 20 individuals (i.e., healthy adult volunteers) will be needed for each field test day. 13 participants will be enrolled as the treated group and each of them will receive product treatments on one lower leg. Two participants will be enrolled as the untreated group to monitor mosquito landing pressure at each field site and during the test days. An additional five participants will be also enrolled as the alternate group to replace potentially withdrawn volunteer(s) from the treated or untreated groups. For each group of 13 participants receiving repellent at least six will be female and six will be male. The two controls will be evenly split between female and male. Computer randomization will be used to assign individuals to treatment (repellent) or control and also to assign spray to either the left or right leg. This will be done separately for females and males.

The two study sites will be selected based on preliminary trapping and monitoring and must contain the mosquito genera of interest and no vector-borne diseases detected within a 25-mile radius. Participation will span a minimum of 6 days, beginning with 1 day for screening and consent, 1 day for attractiveness testing and aspirator training, 1 day of field testing, and 3 days of post-field adverse effects monitoring. Participation would be longer if individuals participate in more than 1 test day. Repellent dosage application is based on limb surface area. Each site will test the 10-15% OLE aerosol on one day and the 30% OLE on a different day. Participants will be paired together in the field except for one participant who will be paired with a study staff. Likewise, each control will be paired with study staff. There will be nine stations at each field site, separated by at least 3 meters, and paired participants will rotate through stations at 30-minute intervals.

1 hour after the treatment, mosquito landings on the treated or control exposed lower legs of each volunteer will be monitored to assess mosquito repellent efficacy. The exposure period to wild mosquitos will be 5 minutes. The 5-minute exposure will be repeated every half hour for 6 (for the 10-15% OLE product) or 10 hours (for the 30% OLE product), or until Complete Protection Time (CPT) can be established by more than half of the volunteers in the treated group.

For each volunteer in the treated group, "the primary endpoint is the first confirmed landing, which is used to estimate the median Complete Protection Time (mCPT) of the repellent product. The CPT is defined as the time between application of the product and the occurrence of the first confirmed landing. First confirmed landing is a landing followed by another landing within a 5-minute exposure period, or a landing in an exposure period immediately followed by an exposure period in which a landing occurred." CPT data from all the volunteers in the treated groups will be used to estimate the mCPTs of the two repellent products. The mCPT will be estimated and occurs when over half the participants experience a confirmed landing. Adverse events during field testing and follow-up will be recorded as a safety endpoint.

Statistical Analysis

The sample size calculation was based on previous work by the EPA statisticians to be used for field-based skin-applied mosquito repellent efficacy studies. Simulation was used based on a Weibull distribution to obtain a certain level of power to precisely estimate mCPT. The simulation varied four parameters: sample size, mCPT, the level of precision of estimated mCPT (K), specifically the lower limit of the 95% Confidence Interval (CI) of estimated mCPT/estimated mCPT), and the variation of the CPT distribution (P5MR), specifically CPT5th %/mCPT). Setting P5MR ≥ 0.5 for the variation of CPT data and K at 0.6, power exceeds 90% for a sample size = 13 and mCPTs ranging from 2-8 hours.

The statistical model that will be used to model time to treatment failure (i.e., first confirmed landing) is the Kaplan-Meier curve and from this the mCPT will be estimated along with the 95% CI based on the log-log transformation. The model will be run separately for each formulation (10-15% and 30%) and each site (4 models). Participants who withdraw during the field testing will be included in the analysis as right censored.

Comments

- Untreated group: The untreated group is only used to confirm mosquito landing pressure at the field site and during the test period; they are not used to compare with results from the treated group. Therefore, it is not necessary for the exposure period of the untreated group to be the same as that of the treated group. The Guideline suggests "*at least one mosquito landing within 1 minute,*" while this protocol requires "*five landings per 5 minutes per control person.*" Although the landing pressure is the same, the HSRB is concerned that the increased exposure duration may be unnecessary and potentially increase health risks for untreated participants (such as from mosquito bites).

- Mosquito attractiveness assessment: In Section 6.2, it states mosquito attractiveness of all volunteers will be assessed using only one mosquito species (*Aedes aegypti*). However, the study objective includes three target genera (*Aedes, Anopheles, and Culex*, Section 2). The Guideline suggests, "*Before the test, subjects should expose their untreated forearms to the **target** insects in a test cage to establish their attractiveness.*"

- Treatment rates:

    - Information on application rates is provided in Section 6.4, which appears to be based on the amount of products, not the amount of OLE. More OLE will be applied to volunteers for the product containing 30% OLE.

    - The protocol states that one formulation will be 10-15% OLE. It would be preferable to have the same dosage level (either 10 or 15%) to increase internal validity by reducing extraneous variability.

- Treatment methods: The treatment methods are described in Section 6.7.5, which appear to be different from how both products will be used when they are marketed. This is a deviation from the Guideline, which specifies "the formulated product should be used as it is or will be marketed, in the same type of container and bearing the same directions for use."

- Right-censoring data: As mentioned in Section 5.4, there have been products in similar formulations or containing similar percentages of OLE that are already registered in United States and other countries. Data from these already registered products may support study design and minimize the likelihood of having right-censoring data, which may hamper the development of mCPTs.

- Descriptive statistics: Descriptive statistics and/or other data summaries that will be included in the analysis to understand the characteristics of participants are not well documented in the methods.
- Landing measurement protocol: The protocol is unclear in terms of how the time to treatment failure for each participant is recorded and if this could result in inadvertent left censoring.
- Pairing of participants: The protocol states that participants will be paired during the field study, but it is unclear if pairing influences the endpoint leading to observations that may not be independent, which would affect the confidence bounds for mCPT, resulting in lower precision than assumed.
- Spatial independence: It seems questionable that stations 3 meters apart is sufficient distance to prevent a diffusion effect, such that participants in one pair attract mosquitoes away from those in a nearby pair. The World Health Organization guidelines, for example, suggest a minimum of 20 meters separation between stations.
- Sample size and design balance: If mosquitoes are attracted to females and males differently, then the pairing could affect the endpoint. To minimize the effect, the six pairs should be balanced (two female-female pairs, two male-male pairs, two female-male pairs). It is also unclear if male/female strata will be analyzed as a factor in the model, in which case sample size may be inadequate, n=13 would be the minimum in each stratum according to the power analysis.
- Power analysis: The precision parameter of K=0.6 for determining sample size does not result in a narrow CI. For example, a mCPT of 5 hours would have a 95% CI of 3-7 hours, which leaves a reasonable amount of uncertainty.
- Site-specific models: It appears Kaplan-Meier analyses will be performed separately for each site. Precision can be increased by including both sites (for the same formulation concentration) if it is determined survival curves are similar across sites.
- Treatment efficacy: An objective listed in the protocol is to determine efficacy, but the statistical analysis only includes estimating mCPT among the treated. There needs to be a comparison group to establish efficacy, and this would require a comparison of the treated versus the controls.
- Participant alternates: The plan is to have five alternates each day in case participants drop out. It seems including them in the study, if study logistics permit, would add information and increase precision. If the additional information (knowledge) gained exceeds the risk of more people being exposed, it would be worthwhile to allow alternates to participate. Additionally, on page 28 of the EPA review, it is stated that there are two alternates.
- Participant withdrawal: As recommended by the EPA reviewers, if an individual withdraws early during the test day it would be worth considering adding an alternate participant to increase usable data. Another possibility is to include all alternate participants who are present on test day. Also, it is unclear what happens to a pair when one individual of the pair withdraws from the study.
- Mosquito landing: Who must observe a landing is unclear. From protocol Section 6.7.8, it could be read as either the participant who experiences the landing, the partner who witnesses the landing, or a trained member of staff. The method that is most accurate should be used. This could be the participant must witness the landing, anyone with the participant who witnesses the landing even if the participant does not, or both the participant and the witness.

- Ticks: On page 28 of the EPA review, there is mention of ticks that are out of context for this mosquito study.

Recommendations

- Untreated group: The HSRB recommends decreasing the exposure period of the untreated group, as long as the mosquito landing pressure has been confirmed. Please refer to Section (k)(6) in the Guideline.
- Mosquito attractiveness assessment: The HSRB recommends assessing mosquito attractiveness for all the target genera considered in this study. Otherwise, the Board recommends discussing why assessment using only one mosquito species (*Aedes aegypti*) is considered sufficient to conclude the attractiveness will be the same/similar for all the other target mosquito species.
- Treatment methods: The HSRB recommends applying the products in the same way when they are marketed. The Board understands that both products are in aerosol formulations, and it may be challenging to quantify the actual amounts of products applied to the target areas. To determine treatment rates for aerosol products, the HSRB recommends considering the method mentioned in Section (i)(4) of the Guideline (page 24).
- Treatment rates:
    - Clarify in Section 6.4 whether the additional application will be based on the product containing OLE, or additional amounts of OLE.
    - Clarify dosages to be used in the study, particularly why a range of "10-15%" is used instead of choosing 10% or 15%. Also, clarify if using a range may potentially impact internal validity.
- Right-censoring data: Since this study is still in protocol phase, the HRSB recommends considering using data from these already-registered products to improve study design and minimize the likelihood of having right-censoring data, which may hamper the development of mCPTs. This may be of greater importance for the product containing 30% OLE. Please refer to Section (k)(7) of the Guideline (page 28) for recommendations that may help minimize the likelihood of right-censoring data.
- Descriptive statistics: The HSRB recommends providing details of descriptive statistics and/or other data summaries that will be included in the analyses to understand the characteristics of participants, those excluded, and drop-outs to help with assessing the validity and generalizability of the study.
- Landing measurement protocol: The HSRB recommends ensuring that the estimate of time to landing is not biased. That is, clarify in the protocol how the time to treatment failure for each participant is recorded and if this could result in inadvertent left censoring.
- Pairing of participants: The HSRB recommends addressing participant pairing as described in the study design if this impacts confidence bounds. Consider Kaplan-Meier survival models that explicitly account for clustered data.
- Spatial independence: The HSRB recommends justifying the distance between sampling locations of 3-meters apart with a reference to the Product Performance Guideline.
- Sample size and design balance: The HSRB recommends balancing pairs in the study design. (e.g., two female-female pairs, two male-male pairs, two female-male pairs) to minimize the pairing effect in the data. Additionally, clarify if there are planned analyses of sub-groups. For

example, if men/women are to be analyzed comparatively, then n=13 is the minimum number needed per stratum.

- Power analysis: The HSRB recommends providing additional information about precision in the sample-size determination and why K=0.6 was used, as this value does not result in a narrow CI.
- Site-specific models: The HSRB recommends clarifying the impacts and efficiency of performing site-specific models instead of including both sites with the same OLE concentration if survival curves are not statistically different across sites.
- Treatment efficacy: The HSRB recommends addressing your definition of "efficacy" in the protocol and how you will assess efficacy in absence of comparing treatment and control groups.
- Participant alternates: The HSRB recommends considering alternate-individual participation to increase data if the additional information gained exceeds the risk of more people being exposed. On page 28 of the EPA review, the text should be updated to reflect that there will be five alternates.
- Participant withdrawal: The HSRB recommends considering if alternates should be used during test days if a participant withdraws to increase usable data. Also, clarify the disposition of the pair when a single participant of that pair withdraws from the study.
- Mosquito landing: The HSRB recommends clarifying potential inaccuracy in determining landing and who must observe a landing, as this is unclear in the protocol (Section 6.7.8).
- Ticks: On page 28 of the EPA review, the HSRB recommends providing an appropriate update to the text relevant to this mosquito study.

Charge to the Board – Ethics

If amended to address the EPA's and the HSRB's recommendations, is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

HSRB Response

The research proposed in the protocol "Evaluation of Topically Applied Insect Repellent Products Containing Oil of Lemon Eucalyptus (Citriodiol) Against Mosquitoes in the Field" is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L, if the recommendations made by the EPA and the HSRB are adequately addressed.

Ethics Review

Participant Selection and Recruitment

The proposed study will recruit up to 20 individuals per day per product (13 participants, two controls, and five alternates) for a total of up to 80 enrolled participants. Individuals will be recruited via advertising through traditional, digital and social media. While some text is available in Appendix 1 of the submitted materials, detailed recruitment materials (advertisement, emails, posters etc.) were not included for review. Only English speakers will be recruited. Participants determine their own eligibility and self-exclusion. The study staff will screen and consent remotely. Pregnant, nursing, and lactating women are excluded from the study.

Under Section 6. Test Methodology, 6.1. Pregnancy Test the protocol states: "Women may be exempt from the pregnancy test if they are considered to not be of childbearing potential. This means either permanently sterile (through hysterectomy, bilateral salpingectomy or bilateral oophorectomy) or post-

menopausal (no menses for 12 months without an alternative medical cause). Women will need to give signed confirmation of their eligibility for exemption but will not be required to disclose the reason. "The Study Director can choose to exclude women if they believe the statement of non-childbearing potential to be incorrect." This last sentence is overly broad and problematic. This situation refers to an individual potential participant and is not a justification to exclude "women" from the study. All research protocols rely on participants to be honest in their answers (i.e. asking the participant who smokes if they refrained for the past 24 hours).

Participants will need to be able to operate the aspirator.

Informed Consent Process

The informed consent document states that participation is voluntary and the participant may end participation without penalty. The goal of the research, general procedures, and potential risks are included. The consent form states there will be no direct benefit to participants. Rules regarding the 24 hours before the experiment are noticeable in the consent form. The consent form is organized according to the components of the study: (1) screening and consent, (2) training and (3) one to four field tests lasting 6-10 hours is clear (spaced at least 72 hours apart). The process includes sample questions posted to confirm participants' understanding of the study protocol. Study staff will contact subjects by phone 2-3 days before the test day to confirm exposure to COVID-19 and remind individuals of instructions prior to arrival for the study.

Risks and Benefits

There are no direct benefits to research participants in this study. The risks are stated in the protocol and consent. Mitigating activities such as light, loosely fitted clothing that covers their body is included in the study. Participants will be given breaks, snacks, and hydration during testing. Sunburn and mosquito bites will be treated.

Review Summary

Informed consent meets the requirements for the protection of human participants. In general, the consent form is clear, concise, and includes sufficient details. Exculpatory language is not included. The consent form includes an explanation of the purposes of the research and the expected duration of the individual's participation, a description of the procedures to be followed, and identification of OLE as the active ingredient of the test products. The consent also includes descriptions of reasonably foreseeable risks or discomforts to the participant; potential benefits to others, and a statement regarding the intent to maintain research participants' confidentiality. The research involves no more than minimal risk. Compensation for participation based on participation is clear. There is space to include whom to contact with questions. Statements regarding voluntary participation, withdrawal, and minimum period regarding the retention of data are also included in the consent.

Comments

- The protocol states "this research does not offer benefits to the participants, so limiting recruitment to English speakers will not result in equity-of-access issues." This analysis and application of the principle of justice is lacking. The opportunity to participate in research is a benefit in and of itself.
- The principle of justice should not be limited to only those studies that are expected to provide a direct benefit to participants. Individuals should have the opportunities to participate, to then

exercise their autonomy by deciding whether to participate. There does not appear to be sufficient justification regarding the exclusion of individuals over 55 years of age. The protocol only references excluding individuals over 55 "as they are more vulnerable to the effects of SARS-CoV-2 infection."

- The protocol allows for research participants to "drive in their own vehicles if they wish." It is unclear how an adverse event occurring to or from the site would fall under the sponsor's obligations for any medical injury.
- Under Section 6.9. Follow-up, the protocol states "in the event that a mosquito captured during testing is found to carry an arbovirus, the Study Director will contact the participants testing that day. Contact will be made via telephone." It is unclear what actions will be taken by the staff and what possible health interventions could be recommended to the participant.
- The consent provides a minimum number of years for record retention but does not provide a maximum.
- The protocol and consent form use the terms sex and gender interchangeably.
- Consent states "Your gender may be reported with the data as the coded identifier above, where M is for male and F is for female." This is a field in the data collection sheets and it is unclear why gender would not be recorded.

Recommendations

- The HSRB recommends amending the protocol to remove the phrase in **bold** and *italics*: "This research does not offer benefits to the subjects, ***so limiting recruitment to English speakers will not result in equity-of-access issues.***"
- The HSRB recommends amending the protocol to include a more robust justification for excluding participants over 55 years of age.
- The HSRB recommends amending the protocol to remove the statement "the Study Director can choose to exclude women if they believe the statement of non-childbearing potential to be incorrect" and amend the protocol to verbally confirm their eligibility for exemption after signing the confirmation, reiterating that the participant will not be required to disclose the reason.
- The HSRB recommends using terms "sex" or "gender" consistently in one document.
- The HSRB recommends amending the protocol and consent to state explicitly that a participant is part of the study and will be paid for the time it takes to drive to the study site if in their own vehicle and clearly state when the study participation ends if driving their own vehicle from the site.
- The HSRB recommends amending the protocol and consent to state what medical actions need to be taken if a captured mosquito is found to carry an arbovirus to clarify if this is a discussion regarding watching for symptoms, recommendation to see a health care provider, or the possibility of treatment. The CDC website does not provide detailed or actionable information.
- The HSRB recommends amending the consent to state the maximum of years to keep data or state that data will be stored indefinitely.
- The HSRB recommends amending the consent to state "your gender will be reported with the data as the coded identifier above, where M is for male and F is for female."

Recommendations for Future Studies

There are no additional recommendations for future studies.