EPA-HSRB-21-2

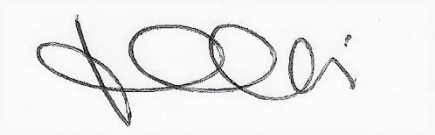
Dr. Jennifer Orme-Zavaleta
EPA Science Advisor
Office of the Science Advisor
1200 Pennsylvania Avenue, NW
Washington, DC 20460
Subject: April 20-21, 2021 EPA Human Studies Review Board Meeting Report

Dear Dr. Orme-Zavaleta,

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of two study protocols involving human participants. On April 20, 2021, the HSRB considered a study protocol for field evaluation of skin-applied mosquito repellent product containing Oil of Lemon Eucalyptus and Methyl Nonyl Ketone. Briefly, the goal of the proposed study is to determine the duration of efficacy of protection of the skin-applied product in repelling mosquitoes. On April 21, 2021, the HSRB considered a study protocol for evaluation of a skin-applied tick repellent product containing Oil of Lemon Eucalyptus and Methyl Nonyl Ketone. Briefly, the goal of the proposed study is to determine the duration of efficacy of protection of the skin-applied products in repelling ticks.

The HSRB's responses to the charge questions presented at the meetings on April 20 and 21, 2021 along with detailed rationale and recommendations for their conclusions are provided in the enclosed final meeting report.

Signed,



Jennifer Cavallari, ScD, CIH

Chair, EPA Human Studies Review Board

INTRODUCTION

On April 20 and 21, 2021, the United States Environmental Protection Agency (EPA or Agency) Human Studies Review Board (HSRB or Board) met to address the scientific and ethical charge questions related to a study protocol "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray Against Mosquitoes" (Protocol No. MIM-006) and a study protocol "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray with Ticks under Laboratory Conditions" (Protocol No. MIM-007). In accordance with 40 CFR 26.1603, EPA sought HSRB review of the study protocol.

REVIEW PROCESS

The Board conducted a public meeting on April 20 and 21, 2021. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL- 10017-40-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 completed and proposed research.

For each agenda item, the Agency staff presented their review of the scientific and ethical aspects of the proposed research, with each presentation followed by clarifying questions from the Board. The HSRB solicited public comments and next proceeded to address the charge questions under consideration. The Board 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, study protocol, related materials and documents provided by the study sponsors, the Agency's science and ethics reviews of the study protocol, as well as oral comments from Agency staff and the investigators during the HSRB meeting discussions. A comprehensive list of background documents is available https://www.epa.gov/osa/april-20-21-2021-meeting-human-studies-review-board.

"Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray Against Mosquitoes." Protocol No. MIM-006

Charge to the Board- Science:

Is the protocol "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray Against Mosquitoes" likely to generate scientifically reliable data, useful for estimating the amount of time each of the product tested repels mosquitoes?

HSRB Response:

The research proposed in the protocol "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray Against Mosquitoes" is likely to generate scientifically reliable data, useful for estimating the amount of time the product tested repels mosquitoes provided that the comments and recommendations provided by the EPA and HSRB are adequately addressed.

The HSRB also has specific comments, recommendations and additional minor points which are described in the discussion below.

HSRB detailed response and rationale:

The HSRB reviewed the protocol and related documentation for the evaluation of a skin-applied insect repellent product containing 11% Oil of Lemon Eucalyptus (OLE, also known as Citriodiol; CAS 1245629-80-4) and 7.75% 2-undecanone (methyl nonyl ketone or MNK; CAS 112-12-9) for use against three genera of mosquitoes in a field- based setting. Lab-based study procedures will occur before the field testing to evaluate subjects' attractiveness to mosquitoes and to train them on how to use an aspirator. The study was designed to establish the median complete protection time, or mCPT, to support the registration and labeling of the product. The protocol was submitted by Carroll-Loye Biological Research and is sponsored by Mimikai. The review assessed the scientific aspects of the proposed research according to the EPA guideline OPPTS 810.3700 – Insect Repellents to be Applied to Human Skin, as well as the recommendations from the EPA and HSRB.

The first protocol dated February 17, 2020, submitted to the EPA was reviewed and deficiencies were identified. Subsequently, a revised protocol was submitted on December 23, 2020, to the institutional review board (IRB). After IRB approval, the revised protocol was submitted to the EPA. For the HSRB's review, the EPA provided both the February 17 and December 23, 2020, protocols for review; however, the EPA's and the HSRB's reviews only cover the December 23, 2020, protocol.

Study Overview

Briefly, the protocol describes the recruitment of 40 study participants[1] to take part in a field-based study. The study plan includes one laboratory visit prior to field-testing to take measurements for skin surface area, evaluate attractiveness to mosquitoes, and train participants on using aspirators. On the study day, participants will be transported to one of two field-study test sites. There will be two ecologically-distinct test sites with a diversity and abundance of target mosquitoes. Potential locations for test sites include northern and southern California, Minnesota, Louisiana, Arizona, and Florida.

Two untreated control subjects will be used to monitor adequate landing pressure five minutes before the study begins, and every 30 minutes thereafter for the duration of testing. Adequate landing pressure is five landings within five minutes or less on each control subject. After the 5 minute period to measure landing pressure is completed, each treated subject will undergo five-minute exposures at 30 minute intervals until CPT occurs for that subject, signaling repellent failure, or the end of study period is reached, whatever occurs first. The First Confirmed Landing (FCL) is a landing followed by a second landing within 30 minutes of the first. CPT is the measurement for residual repellency or time to product failure from time of product application. CPT is measured as a single value for each subject. The testing may take up to 12 hours, with up to 16 hours including transport to the field-study test site.

The product will be applied at the standard dose of 0.5g/600 cm^2 for testing repellency to either the non-dominant arm or a leg that was previously washed with unscented soap and 70% alcohol. The dose will be applied to the skin using a tuberculin syringe and spread over the surface by a gloved researcher.

[1] At each of 2 sites, there will be 13 treated subjects, 2 control subjects, and 5 alternates.

The risk assessment for OLE is based on the EPA risk assessment for p-menthane-3,8-diol (PMD), which is the active component in OLE. OLE contains 65% PMD according to EPA's risk assessment [EPA Memorandum Feb. 4, 1999; Biopesticide Registration of Citriodiol (a mixture of chemicals, containing 65 % PMD as the key ingredient)]. Mimikai Lilly Pilly is categorized as Toxicity Category IV for all route of exposures (MRIDs 510641-03 through 510641-08 in Protocol Appendix 6).

We agree with and support the EPA recommendations as presented in their scientific review and report and would like to suggest some additional recommendations for consideration.

Recommendations:

- The HSRB recommends monitoring field sites prior to testing to confirm the presence and sufficient numbers of a diversity of mosquito species.
- The HSRB recommends adding additional information to the study protocol clarifying how the application of the material "…dispensed from tuberculin (1 ml) syringes by researchers wearing surgical gloves who apply it to treated subjects by spreading evenly over the area to be treated using one finger in a light rubbing motion" represents (or is consistent with) the end-user application of pump spray. EPA noted this method of application is consistent with the dosage and distribution on skin with the bag-in-valve applicator of the product. This justification for using the study application should be explicitly stated in the protocol.
- The protocol states:

  > Given that we consider end user safety essential for the fundamental ethics of conducting this study, the Sponsor proposes generating and providing dermal absorption data for MNK as applied in the form of end product we will be testing, if feasible, or of the active ingredient. No subject recruitment will begin until the dermal absorption study is completed and reviewed by EPA, and the study shows that the dermal absorption rate of MNK is such that the estimated MOE will be at an acceptable level.

  Based on information provided by the EPA, it appears that a dermal absorption study will not be occurring. The information about this proposed study should be removed from the protocol.

- Field testing is proposed in several states (i.e., California, Arizona, Florida, Minnesota, and Louisiana). The protocol states

  > There will be 40 total subjects (20 male and 20 female). We will assign subjects to the first trial by randomly choosing one of the two genders, randomly choosing 10 subjects from that gender, and then repeating the process for the other gender. This will give us a list of 20 subjects (10 males and 10 females). We will assign the remaining 20 subjects to the second trial.

  The HSRB recommends the protocol discuss how the recruitment and randomization strategy will work if the test sites are geographically remote (Page 14, *Sampling Frame* in Section 3.2 of the protocol). This information could also be included or referenced earlier in the protocol to further avoid confusion regarding recruitment strategy and study conduct across disparate geographical locations.
- Section 1.2 of the protocol makes a number of factual assertions (e.g., CDC notes substantial consumer interest in new and effective insect repellent products) but provides no citations for these statements. This should be corrected to be consistent with other parts of the protocol where analogous statements are backed up with citations.
- Page 17, line 30. Please indicate if there are any dietary restrictions, e.g., avoid spicy foods or garlic.
- Page 20, line 889. It is stated that "Alternate subjects may return later to replace subjects that initiate testing but withdraw before useful data are generated." It is unclear if this is feasible for testing done in remote locations. If this procedure is used, please indicate how alternate subjects be notified that they are serving as a replacement.

Statistical Review

The sample size for this study was appropriately computed using EPA guidance and calculations. There will be 13 individuals enrolled in the study with 2 untreated controls for each field trial. Five alternates will be included for a total of 20 participants per field trial or 40 total participants. The participants will be randomized to treatment or control maintaining a 50:50 male to female ratio in the sample; the 2 untreated control individuals will be one from each gender. Treatment will be applied to the non-dominant arm; no randomization description is provided for treatment application to lower legs.

"If subject withdraws during the field test and before the withdrawing subject has received a confirming landing, the subject's data will be retained if his or her total exposure duration is > 90% of the mean of subjects who did not withdraw, provided that not more than 3 of 13 subjects have so withdrawn" (§4.7.7; p. 29). "If more than 3 of 13 subjects withdraw prematurely, those with the briefest participation will be replaced first" (4.7.7; p. 30).

The design of the two experiments is that of a completely randomized design with randomization of treatment conditions to subjects within males and within females. Kaplan-Meier survival functions are appropriate to estimate the mCPT and 95% confidence intervals.

Recommendations:

In addition to the EPA Science Comments, please consider the following recommendations.

- On page 32, please clarify that the "number of mosquitoes that attempted to bite their own treated skin during that five-minute period" is the actual number of landings on treated skin in that five-minute period. Additionally, the note "in a typical test of a reasonably effective repellent, dozens of '0' landing values will be recorded for each '1' or '2'" is unclear (line 1587). The recording of a '1' or '2' is unclear; please clarify which will be recorded and under what circumstance.
- On page 32, please clarify that if at least 50% of subjects have data that are right-censored, the mCPT will not be computed. This is appropriate on line 1613. 'm' could be clarified to be 'median'.
- Clarification should be added that the analyses will be conducted for each of the field studies separately. This clarification can be added to line 1612 in the study protocol on page 32.
- On page 33 (line 1641), please clarify how long of a pause will be considered for a second landing to be confirming for the first landing. EPA has indicated that there should not be a reason for pause based on their recommendations to the study protocol.

Charge to the Board - Ethics:

Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Response:

The research proposed in the protocol "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray Against Mosquitoes" is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L, if the recommendations made by the EPA and HSRB are adequately addressed.

Ethics review:

40 CFR Part 26, Subparts K and L

Subpart K—Basic Ethical Requirements for Third-Party Human Research for Pesticides Involving Intentional Exposure of Non-pregnant, Non-nursing Adults

Basic ethical requirements for third-party human research for pesticides involving intentional exposure of non-pregnant, non-nursing adults include *inter alia*:

IRB Approval. As per EPA Science Ethics Review of MIM-006, approval from a registered and accredited IRB was solicited and granted:

> 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.

Informed Consent, with Documentation. The process for informed consent to participation in the study is as follows:

- Interested candidates contact study staff via phone or email to learn more about the study and to determine whether they meet the eligibility criteria.
- Those who meet study criteria and remain interested in participating will then meet with the study staff.
- Study staff will review eligibility criteria with potential participants and inform female subjects about the requirement for pregnancy testing.
- Those whose eligibility is again confirmed will then be orally provided with information about the study, including a step-by-step description of what the study requires of participants.
- Participants will be told that they can ask questions and meet privately with the Study Director at any time and that they are free to withdraw from the study at any time without forfeiting any benefits to which they are entitled.
- Prospective participants will then be provided with the consent form, the Experimental Subjects' Bill of Rights, a copy of the protocol, and any supporting documents.
- Staff will read aloud the consent form and Bill of Rights, and answer any questions from the prospective participant.
- Prospective participants will be again reminded that they are not obligated to consent to enroll and that they are free to withdraw from participation at any time without penalty.
- Should prospective participants then decide to enroll, they will sign the consent form and the Experimental Subjects' Bill of Rights.
- Study participants will then be given copies of their signed consent form and Experimental Subjects' Bill of Rights.

<u>Subpart L—Prohibition of Third-Party Research for Pesticides Involving Intentional Exposure of Human Subjects who are Children or Pregnant or Nursing Women</u>

§ 26.1203 Prohibition of research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.

All subjects who will participate in this study will be at least 18 years old. An equal number of adult male and female participants will be enrolled (20 and 20) and female subjects will be given pregnancy tests so that those with positive results can be excluded. Testing will be done in private and a single female member of the research team will discuss results with prospective participants.

Recommendations:

In summary, we agree and support the EPA Ethics comments and suggestions. We emphasize and suggest consideration of the following recommendations:

Prevention of heat stress

- Further clarification on what participants will wear (light clothes vs. Tyvek) during testing periods is required; otherwise, the study forms need to be harmonized and reflect the final proposed design. The HRSB recommends that no Tyvek suits be used; it is our understanding that Tyvek suits have not been utilized in other studies and present a heat stress risk. Due to heat related concerns, add a question and exclusion criteria for potential volunteers concerning previous history of heat related injury (e.g. heat exhaustion, heat stroke, etc). Individuals that have experienced this in the past are more prone to future events. Excluding them from the volunteer pool could reduce overall risk to subjects. Furthermore, the HRSB recommends that fans be used in the enclosure for the comfort of the participants.

COVID-19 precautions

- Ensure that any special additional steps that need to be put into place for COVID19 precautions, e.g., masks, social distancing at the lab during testing, etc. are in the protocol.

Minor clarifications

- The protocol indicates that subjects are to be offered latex, nitrile or vinyl gloves. Use of latex gloves should be reconsidered because they pose a greater allergenic risk than the other options.

- The protocol indicates that subjects in ‘poor physical health’ will be excluded from participating. We recommend indicating the criteria and justification for how participants will be ‘judged to be in poor physical health’.
- Page 12, line 511. Recommend specifying that standard first aid materials are commonly acquired OTC (over the counter) materials.

"Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray with Ticks under Laboratory Conditions." Protocol No. MIM-007

Charge to the Board- Science:

Is the protocol "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray with Ticks under Laboratory Conditions" likely to generate scientifically reliable data, useful for estimating the amount of time each of the product tested repels ticks?

HSRB Response:

The research proposed in the protocol "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray with Ticks under Laboratory Conditions" is likely to generate scientifically reliable data, useful for estimating the amount of time the product tested repels ticks provided that the comments and recommendations provided by the EPA and HSRB are adequately addressed.

The HSRB also has specific comments, recommendations and additional minor points which are described in the discussion below.

HSRB detailed response and rationale:

The HSRB reviewed the protocol and related documentation for the evaluation of a skin-applied insect repellent product containing 11% Oil of Lemon Eucalyptus (OLE, also known as Citriodiol; CAS 1245629-80-4) and 7.75% 2-undecanone (methyl nonyl ketone or MNK; CAS 112-12-9) for use against three species of ticks in a lab-based setting. The study was designed to establish the median complete protection time, or CPT, to support the registration of the product. The protocol was submitted by Carroll-Loye Biological Research and is sponsored by Mimikai. The review assessed the scientific aspects of the proposed research according to the EPA guideline OPPTS 810.3700 – *Insect Repellents to be Applied to Human Skin*, as well as the recommendations from the EPA and HSRB.

The first protocol dated February 17, 2020, submitted to the EPA was reviewed and deficiencies were identified. Subsequently, a revised protocol was submitted on December 23, 2020, to the institutional review board (IRB). After IRB approval, the revised protocol was

submitted to the EPA. For the HSRB's review, the EPA provided both the February 17 and December 23, 2020, protocols for review; however, the EPA's and the HSRB's review only cover the December 23, 2020, protocol.

Study Overview

Briefly, the protocol describes the recruitment of at least 33 study participants[2] to take part in a lab-based study. The study plan includes up to four study days. The first laboratory visit includes orientation, consent, measurements for skin surface area, evaluation of attractiveness to ticks, and training and practice on tick manipulation. Repellency testing occurs on the second day and may extend to three or more days.

Briefly, a new tick is exposed to the repellent treated forearm for three minutes at 15 minute intervals. The First Confirmed Crossing (FCC) is a crossing followed by a second crossing within 30 minutes of the first. Complete Protection Time (CPT) is the measurement for residual repellency or time to product failure from time of product application. Each subject serves as his or her own control through the screening of actively questing ticks on the untreated forearm prior to exposure to the repellant on the treated forearm. CPT is measured as a single value for each subject. The testing may take up to 14 hours.

The product will be applied at the standard dose of 0.5g/600 cm^2 for testing repellency to the non-dominant arm that was previously washed with soap and 70% alcohol. Both control and treated forearms will be arranged identically. The dose will be applied to the skin using a tuberculin syringe and spread over the surface by a gloved researcher.

The researchers are using an external quality assurance organization.

We agree with and support the EPA recommendations as presented in their scientific review and report and would like to suggest some additional recommendations for consideration.

Recommendations:

- The HSRB agree with the recommendation of the EPA that additional clarification is needed to indicate how many tick species will be tested on each day and how the tick species will be allocated per subject. The HSRB would like to emphasize that only one

[2] There will be 25 treated subjects per tick species and 8 alternates.

species of tick should be used on a subject on any given test day. Furthermore, tick species should be randomly allocated to subjects as appropriate.

- Occasionally language refers to the fact that ticks will only be used once on any subject. While a tick is only used on one subject, the tick is placed or used within that subject "up to twice", given that ticks are first tested for "questing" and then they are used in the study if "questing" is successful. The HSRB recommends clarifying this language.
- The HSRB recommends clarifying and minimizing the number of researchers who will be applying the test material. The HSRB also suggests indicating the amount of test material that is anticipated to be left on the glove and any differences in application versus when the material is sprayed on.

Statistical Review

The purpose of this study is to examine the CPT of an insect repellent, Mimikai Lilly Pilly, containing 11.0% OLE and 7.75% MNK as its active ingredients, against three tick species, *Ixodes scapularis*, *Dermacentor variabilis,* and *Amblyomma americanum.* The product will be tested in the laboratory on 25 subjects per species at the standard dose of 0.5g/600cm^2 for up to 14 hours.

The sample size is 25 subjects per species based on the EPA's recommended sample size of 25 test subjects for testing repellency a single tick species (p.16). Therefore, a sample size of 25 per species is appropriate for producing reliable and valid results. Male and female subjects will be randomly selected from a pool of 44 candidates. Randomization will be limited to treated and alternate subjects. Subjects proved unattractive to ticks during pre-test training will not be chosen.

One arm from each subject will not be treated with the test product and serve as the control for screening ticks so actively questing ticks can be used in the testing. The other arm will be treated with the test product and serve as the treatment group (§4.2, p.16). Each subject will be his or her control. Treatment application to right or left arm will not be randomized. Treatment will be applied to non-dominant arm of subject. Participants will be randomly assigned as test or alternate subjects (p.17).

Kaplan-Meier survival analysis will be used to calculate the median CPT from the CPT for each participant per tick species (p. 17). The Kaplan-Meier procedure is a nonparametric

method used to estimate the probability of survival past given time points. Kaplan-Meier Survival Analysis is advantageous since CPTs data may not be normally distributed. The Kaplan-Meier procedure does not assume that the data should follow a specific parametric distribution. The Kaplan-Meier procedure accounts for censored observations. Therefore, Kaplan-Meier estimator is most appropriate for calculating the median CPT. Thus, Kaplan-Meier estimator is appropriate to answer the research question.

In the study, proposed statistical methods are appropriate to answer the research question. In addition, the proposed design has adequate statistical power to answer the research question. Therefore, the protocol "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone based Repellent Spray with Ticks under Laboratory Conditions" is likely to generate scientifically reliable data, useful for estimating the amount of time each of the product tested repels ticks.

Recommendations

We agree and support the EPA comments and suggestions. Our recommendation is as follows:

- The HSRB recommends that the time to an event or censorship (known as the "survival time") should be clearly defined and precisely measured. The Kaplan-Meier method requires the survival time to be recorded precisely.

Charge to the Board - Ethics:

Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Response:

The research proposed in the protocol "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-based Repellent Spray with Ticks under Laboratory Conditions" is likely to meet the applicable requirements of 40 CFR part 26, subparts K and L, if the recommendations made by the EPA and HSRB are adequately addressed.

Ethics review:

The research objective of the study is to determine the duration and efficacy of the test material [Mimikai Lilly Pilly Repellent containing 11% Oil of Lemon Eucalyptus (OLE or Citriodiol) and 7.75% 2-undecanone (methyl nonyl ketone or MNK)], when applied at a typical consumer dose to skin, in repelling the following tick species: Deer tick (blacklegged tick) – *Ixodes scapularis*; American dog tick – *Dermacentor variabilis*; and Lone star tick – *Amblyomma Americanum*. The measure of efficacy is the CPT.

Study procedures for recruiting, screening, selecting, training, and (if needed) withdrawing and replacing study participants are described in the protocol and in the EPA scientific review. The conditions under which participants will participate in testing, including actions taken to minimize risks to participants where possible, and procedures for measuring CPT for ticks (including analysis) are also adequately described in the protocol. Both OLE and MNK are registered with the EPA for use in skin-applied repellents at or above the concentrations used in Mimikai Lilly Pilly Repellent and the EPA scientific review found the risks due to exposure to be low. These are lowed further via several exclusion criteria described in the EPA review.

Five risks to participants are discussed in the protocol: exposure to test material, exposure to ticks and tick-borne illness, physical stress of test conditions, and psychological risks associated with disclosure of pregnancy testing results. These risks and actions taken to minimize them are outlined in the EPA scientific and ethics review.

The protocol excludes pregnant and lactating women and individuals under 18 years of age. The consent has been reviewed and approved by Advarra IRB and appears generally appropriate, with the exceptions noted in the EPA ethics review.

Recommendations:

In summary, we agree and support the EPA ethics comments and suggestions. We emphasize and suggest consideration of the following recommendations:

1. *COVID-19 Precautions*. The HSRB would like to emphasize the need to revise the protocol and consent to acknowledge the risks associated with COVID-19 and to describe precautions that will be followed. HSRB recommends that researchers should consider all available prevention and assessment methods for COVID-19 including but not limited to rapid testing, temperature checks, and other suggested guidelines (e.g., symptom

questionnaires) recommended by national, state, county, and city guidelines at the time of the study. Ensure that any special additional steps that need to be put into place for COVID19 precautions, e.g., masks, social distancing at the lab during testing, etc. are in the protocol.

2. *Exclusion criteria*. The HSRB recommends that spending a significant amount of time outdoors is not an appropriate inclusion criteria.
3. *Diversity and Representation:* The protocol indicates that biological ethnicity may relate to the attractiveness to biting arthropods and therefore will recruit subjects with a breadth of ethnicities. The HSRB recommends that additional information be provided on how subjects from a variety of ethnicities will be recruited either be recruiting within targeted communities or other methods.