December 14, 2022

H. Christopher Frey, Ph.D.
Assistant Administrator
Office of Research and Development
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: December 14, 2022 EPA Human Studies Review Board Meeting Report

Dear Dr. Frey:

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of a study involving human participants. On December 14, 2022, the HSRB considered the Study Report *Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions*. Briefly, the study reports the results of the complete protection time of MIMIKAI Lilly Pilly tick repellent based on laboratory studies using human subjects. The results of the study will be used to inform the registration and labeling requirements.

The HSRB's responses to the charge questions presented at the meeting on December 14, 2022 along with detailed rationale and recommendations for their conclusions are provided in the enclosed final meeting report.

Sincerely,

Lisa Corey, Ph.D., DABT
Co-Chair, HSRB

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

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

HSRB Board Members

EPA Contact

Tom Tracy, 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
FCC	First Confirmed Crossing
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLP	Good Laboratory Practices
HSRB	Human Studies Review Board
IRB	Institutional Review Board
mCPT	median complete protection time
MNK	methyl nonyl ketone
OLE	oil of lemon eucalyptus
ORD	Office of Research and Development
POD	point of departure
SACHRP	Secretary's Advisory Committee for Human Research Protections
UC	University of California

HSRB Meeting Report – MIM-007

Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions. Sponsored by MIMIKAI and conducted by Carroll-Loye Biological Research. Study Completed January 5, 2022.

Introduction

On December 14, 2022, the Human Studies Review Board (HSRB) considered the Study Report MIM-007, "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions". Briefly, the study reports the results of the complete protection time (CPT) of MIMIKAI Lilly Pilly mosquito repellent based on laboratory studies using human subjects. The results of the study will be used to inform the registration and labeling requirements.

Documents Provided by the EPA and Reviewed by the HSRB

Note: The study report (1a below) is also Attachment 1 to EPA's Science Review. It has not been included in the file for EPA's Science Review Memo.

1a. MIM-007 Study Report 1-5-2022 [N.B. – this is also referenced as Attachment 1 in EPA's Science Review]

1b. MIM-007 Volume 2 – IRB Communications File

1c. EPA Science Review MIM-007

1d. EPA Ethics Review MIM-007

1e. EPA Ethics Review Attachment 3

1f. EPA Ethics Review Attachment 4

Review Process

The Board conducted a public meeting on December 14, 2022. 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.

The Agency staff presented their review of the scientific and ethical aspects of the research. Each presentation was followed by clarifying questions from the Board. The HSRB considered public comments presented by the sponsor and then proceeded to address the charge questions. 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, 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 and oral comments from Agency staff during the HSRB meeting discussions. A comprehensive list of background documents is available at https://www.epa.gov/osa/december-14-2022-hsrb-meeting-0.

Charge Questions and Context

Charge to the Board – Science

Did the research summarized in "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions" generate scientifically reliable data, useful for estimating the amount of time the product tested repels ticks?

HSRB Response

The research summarized in "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions" generated scientifically reliable data that is useful for estimating the amount of time the product tested repels ticks provided the recommendations and limitations provided by the HSRB and EPA are considered.

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

Science Review

The objective of the study "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions", is to investigate the repellent CPT of a ready-to-use product (MIMIKAI Lilly Pilly repellent) against three "laboratory-reared" tick species in a lab-based setting, and this information is used to support product registration. The protocol of this study has been previously reviewed and approved by both U.S. Environmental Protection Agency (US EPA) and HSRB. The sponsor has indicated any non-adherence to the approved protocol (Study Report, page 6). The HSRB reviewed the study report submitted by MIMIKAI, Inc and the science review document prepared by US EPA during this round of review.

Three tick species (Lone star (*Amblyomma americanum*), Blacklegged (*Ixodes scapularis*), and Brown dog (*Rhipicephalus sanguineus*)) were targeted. A total of 31 human subjects were recruited (17 men and 14 women, 25 treated subjects and 6 alternates), and all subjects were screened for their attractiveness to ticks. The subject selection and randomization were done according to the EPA approved protocol and a final sample size of 25 subjects (selected from a pool of 44 candidates of which 31 were consented), was used per treatment, according to the power calculation previously reviewed and approved by the Agency.

The test material is MIMIKAI Lilly Pilly repellent, which is in a liquid formulation containing 11% (w/w) of oil of lemon eucalyptus (OLE) and 7.75% (w/w) of methyl nonyl ketone (MNK) as active ingredients. The test material was applied to the non-dominant forearm of each subject at a nominal rate of 0.5g/600cm^2 and was dispensed onto the skin using a syringe and applied by spreading the product on the skin of the forearm (from wrist to elbow) using one-gloved finger.

Data collection for determination of complete protection time (CPT) involved measurement of directional movements (palm line to forearm line) of an actively questing tick over a designated section of a subject's treated forearm (See Figure 1, p. 4, EPA Report). Every 15 minutes, a new, "actively-

questing" tick was placed on the treated arm and observed for its movement toward the treated forearm for three minutes. The following experimental measurements were made for each of the tick species over each of the subjects (p. 27, the Study Report):

1. CPT: It is "the time from application of a repellent until efficacy failure", which is the First Confirmed Crossing (FCC) in this study.
2. FCC: It is "the first crossing confirmed within 30 minutes by another similar event." For a sample calculation, see data for Subject 4 (Study Report, p. 301)

Kaplan-Meier Survival Analyses were used by the study to calculate the median complete protection time (mCPT) and 95% confidence intervals (95% CI) for each tick species. The mCPT (95% confidence interval (CI)) in minutes for the ticks *Amblyomma americanum, Ixodes scapularis*, and *Rhipicephalus sanguineous* are 287 (263 - 452), 538 (438 - 675) and 293 (168 - 374) minutes, respectively. These figures showed high variability by inspection, also indicated by the wide CIs.

The Sponsor was silent on the issue relating to the lower limit of the 95% CI for *R. sanguineus* being less than 4 hours. This is a concern relating to underperformance of the Lilly Pilly repellent (see EPA Report, p. 18). The EPA review (p. 3), concluded that CPT data obtained by the Sponsor "provides scientific data that support a CPT of 4 hours on the product label."

The HSRB have a few specific comments and recommendations to the study report and the US EPA science review document.

Science comments to the MIM-007 study report

- No discussion of inert ingredients was included.
- The sponsor was silent on the issue relating to the lower limit of the 95% CI for *R. sanguineous* being less than 4 hours although suggests it may be species differences. The concern of product underperformance of the Lilly Pilly repellent is discussed in the EPA Review on p. 18.

Comments to Study Report 2

- p. 97 section 1.3.3 states: "certified disease free." However, there is no "certification" and for BerTek there is no data at all. This is followed by informing the subjects that there is "no risk". There is no data to support this. Later (p.99, section 1.3.7) they use the language "extremely small" and on p. 201 "no risk of concern" which could more appropriately be replaced with "*de minimus*."
- p. 202: In the second paragraph, the phrase "or another source of laboratory-reared ticks with documented pathogen-free status' is inserted after the phrase 'Oklahoma State University Department of Entomology and Plant Pathology Tick Rearing Facility'. There is no documentation of pathogen-free status from the other source (BerTek) of ticks. This is also an issue for statements on Risks/Discomforts on pages 217, 238, 257, 273, 290 and 326.

Comments to the US EPA science review

- Editorial comment: Page 5, fifth paragraph, line 11-13. It is suggested to rephrase this sentence. The current sentence makes it difficult to understand the rationale of not using tweezers because "forceps were better than paintbrushes/tweezers."

- Page 11, Reported deviation #10. It is unclear how it was concluded the current 3cm-in-3 minute scoring criteria "was more conservative" than the original 1-min stipulation. Discussions are recommended to clarify.

Re: Response to 90-day Preliminary Technical Screening Results (EPA)

- p. 4 Graphs of species overall survival. The x-axis for each species is different making a comparison difficult.

Recommendations

The HSRB offers the following recommendations:

1. Editorial recommendations: 1) Page 10, fifth paragraph, second line: "YY" and "ZZ" here serve as place holders and should be replaced with exact numbers; 2) Page 17, third paragraph, line 5: change "do" to "due."

2. This study used a nominal application rate at 0.5g/600cm^2 (Page 10). It was unclear how this rate was selected and how this value relates to the application rate of the future end-use product. **The HSRB recommends including this information in the final report.**

3. On p. 33 and 156, there is mention of mosquitos. **Throughout the report, 'mosquitos' should be changed to 'ticks'.**

4. The study report lacks demographic information on the study participants. On Page 40, it states common demographic factors including age, race and gender were collected, but only information on gender was presented in the report. **The HSRB recommends that all demographic information be included in the report.**

5. The MOE calculations for OLE shown on Page 49 are confusing, and the purpose of these calculations are unclear. The first two tables calculated MOEs based on forearm treatments, which seems to be for the purpose of assessing risks for study participants. However, for each participating subject, only one arm (non-dominant) was treated, and the treated skin area was measured individually (388-671 cm^2). The treatment rate was around 0.5g/600cm^2. Therefore, it is unclear how the applied grams in these two tables (1.0 and 1.20 g) were determined and why generic surface area was used instead of specifically measured values. **The purpose and choice of exposure information should be clearly explained in the study report.**

6. The third and fourth tables on Page 49, are used to provide "conservatively estimated exposures for the general context of product end users." Since child exposures cannot ethically be assessed, considering the end-use product will also be available to children who have greater surface area to body weight ratio, **the report should include a discussion of use in children related to the data provided on healthy adults. EPA should verify the MOE calculation as there may be an error.**

Recommendations (continued)

7. US EPA indicated it was in the process of updating its insect repellent test guideline (OPPTS 810.37001). **This should be noted in EPA's report and what aspects, if any, will be impacted by changes.**

8. Section 3.1 states: "Final composition is not determined until enrollment is completed and will likely vary among regions. The relevant demographics of the participants will be reported. Based on review of the scientific literature regarding individual differences in repellent performance and attractiveness to blood-feeding arthropods, we conclude that any departure of this study from a sampling frame more reflective of race, age and ethnicity will be unlikely to influence the representativeness of the results, or their generalizability to the greater population of skin-applied repellent users." **This paragraph should be dropped. The only demographic reported was sex and they have no evidence regarding age or ethnicity.**

9. The high experimental variability observed in determining CPT as observed from the Kaplan Meier plots is another concern. For example, **there should be discussion of why the CPT for *I. scapularis* much higher than those for the other two ticks tested**.

Study Report 2 Recommendations

10. On pages 79, 191, 206, and 441, there is reference to mosquitos. **Throughout the report, 'mosquitos' should be changed to 'ticks'.**

11. The HSRB agrees with EPA's comments to the sponsor: The protocol should explain clearly what "documented pathogen-free status" means and how it will be achieved, as well as naming all sources from which ticks might be procured. **If possible, the protocol should include information from all potential sources of ticks about their process for screening tick colonies and how they will document the pathogen-free status.**

12. **Any change to the protocol related to subjects' safety and/or consent must be submitted to and approved by the overseeing IRB prior to implementation.** Deviation from requiring documentation of a tick colony's pathogen-free status impacts subjects' safety and consent and requires a protocol amendment to implement.

13. The response citing little impact from mosquito data is of questionable relevance and **should be dropped from the study report.**

Statistics comments to the study report

When the Agency used the same statistical methods recommended in the protocol in an attempt to replicate the study results, the mCPT points estimates matched but they obtained 95% CIs that differed from the 95% CI reported by the study.

The difference was found related to the default transformation methods of the different statistical software (SAS used by the agency versus R used by the study). The study results were similar, however, to adhere to the study protocol, the standard log-log transformation of the data is recommended to be further used by the registrant instead of only the log transformation. According to the Agency, the previous method (data log-log transformation), used simulations of sample size versus power analysis.

Recommendations

The HSRB offers the following recommendations:

While the reported and unreported study protocol deviations don't appear to obviously interfere with the statistical analyses, to avoid interpretation errors, it is perhaps recommended at minimum that some sensitivity statistical analyses should be further done, as additional evidence/reassurance of the overall study results validity. **After reviewing the study deviations in relation to the collected data, and overall study results, the following further sensitivity analyses recommendations emerged according to the potential confounders, and/or potential source of errors:**

1. The study statistical analyses should use log-log transformation instead of only log transformation.

2. Gender (M/F)

   While the study reported "a lack of evidence in the literature indicating gender as a consistent predictor of host attractiveness to ticks", **testing for significant gender differences in survival functions for each tick species, and especially in *A. americanum* provides information. Supporting evidence for the recommended additional statistical work are:**

   - Unreported protocol deviation to recruit equal number of male/female (22 male and 22 female candidates). The gender ratio (M:F) used for *R. sanguineus* and *A. americanum* testing was 15:10, and a gender ratio of 14:11 was used for *I. scapularis* testing.
   - Study raw data related to the *R. sanguineus* showing that female gender tends to cluster on minimum CPT values. E.g. Pt 41(F) has CPT = 79 (outlier), Pt 76 (F) CPT = 102 Pt 163(F) CPT =103, Pt 11(F) CPT-128 Pt 6(F) CPT =135, while male gender is clustered on maximum CPT values: e.g. pt 169 (M) CPT=525, pt 73(M) CPT=437 pt 178(M) CPT=406 etc.
   - A low precision k-value indicating high level of variability in the CPTs against *R. sanguineus*.
   - A significantly different CPT distribution between species.

   Example: A simple way to assess gender significance related to CPT by using SAS is using Proc LIFETEST and adding a "STRATA" statement after "Time" statement
   e/g.

```
ods select Quartiles SurvivalPlot ;
Proc lifetest data = MIM_007 plots=survival (cl) CONFTYPE=log;
by species;
Time TimeFCC*censor(1);
Strata gender;
run;
```

Recommendations (continued)

2. Addressing potential variability related to Tick Rearing and Maintenance Conditions:
   - Reported deviation #5: In addition to what has previously been mentioned, there are significant differences between the CPT values for *R. sanguineus*, so **the significance of potential differences related to quality/viability of the ticks should be at minimum explored**.
   - Reported Deviation #6: The age of ticks used for test days ranged from 2.5-week-old adults to 4.5-week-old adults. While the study reported that "the ticks used were qualified to be sufficiently active during all exposure periods, and thus, this deviation was not expected to compromise the validity of the study," **sensitivity analyses should explore at minimum if the ticks' age has any statistical impact on CPT within same tick group and/or between ticks' groups.**

3. (Minor issue) Tick attractiveness assay

   Data for Subject 73 (page 209 MIM vol 1 report) appears to miss the tick attractiveness assay. The results show that Participant 73 (M) had CPT=437 for *R. sanguineus*; CPT= 491 for *R. scapularis* and CPT=176 (which is the lowest outlier) for *A. americanum*.

   Sensitivity analyses might also clarify if:
   - the variability of the Tick attractiveness (scored as 4-5) has any significant impact on the final conclusions and
   - excluding subject 73 from analyses changes in any way the overall results and conclusions of the study.

Charge to the Board – Ethics

Does the available information support a determination that the research was conducted in substantial compliance with the applicable requirements of 40 Code of Federal Regulations (CFR) part 26, subparts K and L?

HSRB Response

Based on its review of the materials provided by EPA and subject to any limitations or recommendations of EPA or HSRB, the HSRB concludes that the "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions" was conducted in substantial compliance with the applicable requirements of 40 CFR part 26, subparts K and L.

Ethics review:

The study titled "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions" was a laboratory study of a skin-applied insect repellent based on Oil of Lemon Eucalyptus and Methyl Nonyl Ketone designed to determine the efficacy of this repellent against three species of ticks.

The EPA science and ethics reviews provide excellent summaries of the design and conduct of this study, so this review does not include a third summary and instead focuses on topics relevant to the EPA's charge to the HSRB regarding the ethics of this study.

The EPA ethics review provides the applicable ethical standards on pages 11-12 of that review and these have been reproduced below.

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 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.

Comments regarding §26.1703:

This study had a lower age limit of 18 years of age for participants. The youngest enrolled/consented participant was 20 years of age, which meets the requirement that the study not include children.

This study excluded pregnant women as part of the consent process. The authors confirmed the candidate were not pregnant during screening and further included extensive pregnancy testing (with

appropriate maintenance of confidentiality) throughout the study, including before testing sessions. None of the individuals who participated in testing were pregnant, which meets the requirement that the study not include pregnant women.

Comments regarding §26.1705:

For practical purposes, this section of the regulations references the Federal government's Common Rule for the protection of human research subjects (also referred to as the U.S. Department of Health and Human Services regulations 45 CFR 46), with some modifications primarily due to the fact that these studies are regulated by the EPA, not funded, sponsored or conducted by the EPA. Essentially the Common Rule requires that for a study such as this one that involves human subjects address the following (this list incorporates only the requirements that are relevant to this study):

(1) The research must be scientifically sound (covered separately in the HSRB science review).
(2) The study protocol, informed consent document, and all recruitment materials must be reviewed and approved by an appropriately constituted institutional review board (IRB).
(3) The regulatory requirements for IRB membership, functions and operations, review of research, record keeping and communications with the investigator are all specified in the Common Rule.

For a study such as the one being reviewed, the IRB may approve research covered by the Common Rule if it determines that all of the following requirements are satisfied:

(1) Risks to subjects are minimized:
    a. By using procedures that are consistent with sound research design and that do not unnecessarily expose subjects to risk, and
    b. Whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.
(2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits of therapies subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (e.g., the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.
(3) Selection of subjects is equitable. In making this assessment the IRB should consider the purposes of the research and the setting in which the research will be conducted. The IRB should be particularly cognizant of the special problems of research that involves populations vulnerable to coercion or undue influence, such as children, prisoners, individuals with impaired decision-making capacity, or economically or educationally disadvantaged persons.
(4) Informed consent will be sought from each prospective subject or the candidate's legally authorized representative, in accordance with, and to the extent required by, § 26.116.
(5) Informed consent will be appropriately documented or appropriately waived in accordance with § 26.117.
(6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.
(7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.

At the conclusion of the study the protocol and amendments, study report and associated materials, including protocol deviations, are reviewed against these same standards.

As noted in the EPA ethics review, the protocol for this study, informed consents, and study recruitment materials as well as all similar materials for amendments were reviewed and approved by an Advarra IRB. Advarra is a commercial IRB that is registered with the HHS OHRP and meets the Common Rule regulatory requirements for membership, functions and operations, review of research, record keeping, and communications with the investigator.

In addition to Advarra reviewing and approving this study protocol, informed consent and subject recruitment materials, both EPA and the HSRB reviewed the materials and the sponsor largely addressed the EPA and HSRB recommendations prior to the conduct of the study. These changes were reviewed and approved by the Advarra IRB.

Risks to participants were minimized in the study protocol in several ways, including via the exclusion criteria in the protocol if participation in the research might pose a risk to their health (for example, via an allergic reaction to tick bites), the use of colony-sourced ticks, via testing ticks for viral vectors prior to testing sessions (though see discussion of a significant, unreported protocol deviation further down), COVID precautions, confidential handling of pregnancy testing results, etc. Participants were monitored during the study for adverse events associated with tick bites, as well as other risks due to the environment, such as heat exhaustion (however, there were no follow-up visits/participant contacts in the protocol, a concern given the significant, unreported protocol deviation re: vector testing of ticks described below). The risks associated with participation in this study per the protocol, were reasonable given the knowledge that would be gained (about the efficacy of the tested product). There were eleven reported protocol deviations, none of which impacted the safety or welfare of the study subjects.

However, there were two unreported deviations (see page 10 of the EPA ethics review). One involved the gender ratio of study participants, which was different from the protocol. This did not adversely impact the safety or welfare of subjects, though it does raise questions regarding the representativeness of the population selected. The second unreported protocol deviation involved the use of ticks from BerTek, Inc. that had not been screened for vectors of tick-borne illnesses. This deviation is discussed in much more detail below and is significant and concerning from both an ethics and safety perspective.

Research participants were required to speak and read English. Given that this study was conducted in Northern California, it seems likely that some potential participants may have limited English abilities and preferred Spanish, but a Spanish language consent form was not provided. However, it also seems likely that since this study was conducted in Northern California, some individuals whose preferred language was Spanish also spoke and read sufficient English to qualify for the study. Although the protocol mentions tracking ethnicity, there are no summary statistics regarding ethnicity or minorities for the group of individuals that enrolled/consented, so it is not possible to determine if the selection of participants is equitable in this regard, particularly with respect to the local communities in Northern California where this study was conducted. Of particular concern, during the HSRB review of this protocol in April, 2021, an ethics recommendation was made as follows, “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 by recruiting within targeted communities or other methods,” (page 402 of volume 1 of the study report). This does not appear to have been done, and the protocol includes a scientific (but not ethical) discussion of why ethnicity is not important to the study (page 40 of volume 1 of study report). Additionally, as noted, data on ethnicity were not provided in the study report.

The study report does indicate that of the 31 participants enrolled/consented, 17 were men and 14 were women. Some participants (10 women and 11 men) were also test participants in another study

sponsored by Mimikai and conducted by Carroll-Loye Biological Research. Participants were between 20 and 60 years of age (see appendix 3 of volume 1 of the study report).

One of the methods of recruitment was the University of California (UC) Davis ECOSOCIAL listserve and another was the UC Davis Entomology Club email newsletter (see next section). This suggests that some of the participants may be students, a group that in some cases may be subject to coercion (for example, if their faculty are involved in the testing study in some way). This study was conducted by Carroll-Loye Biological Research of Davis, California, but there was no indication of whether individuals listed in the study report may also have a connection with UC Davis. It would be helpful to have such information if this is the case.

The above observations regarding uncertainties concerning the equitable selection of subjects could be improved by requiring future study reports to contain more information regarding demographics of the individuals selected for the study, including breakdowns according to age, gender, and minorities and ethnicities (while not ideal, the census categories are a good starting point). In some cases this may be important for purposes of scientific analyses, but in all cases it is necessary to adequately evaluate whether the selection of subjects is equitable as required by the justice principle in ethics (regarding the importance of this principle, see the Belmont report and also a recent Secretary's Advisory Committee for Human Research Protections (SACHRP) recommendation on the topic: Consideration of the Principle of Justice 45 CFR part 46 | HHS.gov).

To maintain the privacy and confidentiality of participants, they were assigned a study subject number that was used instead of their names in all study documents and communications. Pregnancy tests were conducted in private and were communicated confidentially to a female member of the study staff to confirm eligibility of female candidates to participate in the testing.

Provided the scientific review finds this study scientifically acceptable, the above review of the materials provided indicates that this study was reviewed, approved and conducted in substantial compliance with the Common Rule as required by §26.1705 with two limitations. The two limitations are that due to insufficient information in the report, a thorough evaluation of the equitable selection of subjects was not possible, and the significant, unreported protocol deviation regarding the use of ticks from BerTek, Inc. that were not screened for tick-borne illness vectors (both an ethical and safety concern, as discussed later).

Comments regarding §12(a)(2)(P) of the FIFRA:

Subjects were recruited from the local communities in Northern California via Craigslist, the UC Davis ECOSOCIAL list serve, the UC Davis Entomology Club email newsletter and word of mouth.

This study, including its informed consents and subsequent amendments, was reviewed and approved three times by an Advarra IRB. The final approval of the protocol and informed consent document was for Amendment 3, the last amendment, which occurred on 13 September 2021. As this amendment was to correct a typographical error and was after the study visits, all participants signed the informed consent approved with Amendment 2 (25 August 2021). While the study appears to have been initiated with the IRB in August, 2019, the actual conduct of the field study occurred over five testing days in October 2021, beginning on 10 October 2021 and continuing on October 13, 17, 20, and 24 (there is note to file dated 24 October 2021 describing the five testing days, however, it inexplicably fails to list the dates of testing, a subsequent note to file indicates the first day of testing, the remaining days of testing were determined by examining the research notes labeled "confirmation of no symptoms"). The study was closed out by the IRB on 23 December 2021.

The IRB-approved informed consent includes “the nature and purposes of the test and of any physical and mental health consequences which are reasonably foreseeable therefrom” thus appearing to meet the regulatory requirement. In addition, the consent process included demonstration of repellent application, tick attractiveness testing, and test day procedures and the study procedures included instruction in safe tick handling and behavioral observations (such as behavior prior to attachment and removal/destruction of ticks) further addressing the regulatory requirements.

However, the significant, unreported protocol deviation re: the use of ticks from BerTek, Inc. that had not been screened for vectors of tick-borne illnesses is inconsistent with the IRB-approved protocol and the consent the subjects signed and created a safety risk to subjects. It also is inconsistent with the protocol and other materials reviewed by EPA and the HSRB in April, 2021. While no tick-borne illnesses were reported as an adverse event for this study, it is important to note that there were no follow-up study visits (for example, at 30 days after the testing days) listed in the protocol or in either volume of the study report. Thus there is no data indicating that there was no adverse event involving a tick-borne illness beyond the testing dates. Given that the incubation period for Rocky Mountain Spotted fever is 3-12 days (Rocky Mountain Spotted Fever (RMSF) | Tick-borne Diseases | Ticks | CDC), this provides essentially no reassurance that one or more participants did not experience a tick-borne illness as a result of this study. While we do have a statement from BerTek, Inc regarding their tick colony rearing procedures that makes it less likely that tick-borne illness vectors were present, this still is not screening to confirm the absence of such vectors. Given the ethical and safety concerns, it is unacceptable that the sponsor and contract research organization proceeded with this study without consulting either the IRB or the EPA when they were required to change their tick supplier to one that did not screen for tick-borne illness vectors.

Participants received $25 per hour for each hour of participation in each phase (consent, training, test day) rounded up to the next hour. Some participants were paid in person at the end of each visit, though there is also a study note that many requested a single check at the end of their participation, which was honored. Compensation was consistent with the IRB-approved protocol and informed consent. Given the time spent in testing and the nature of the testing, the compensation amount was reasonable and is not expected to have unduly influenced the recipients to participate in the study.

All participants in the testing appear to have signed the Amendment 2 informed consent document, either during an in-person outdoor meeting or, for those participants attending an on-line consent meeting, when they presented for their first testing session. The consent document specifically indicated that participation was voluntary and that they could withdraw at any time. The candidates were also informed of this verbally prior to testing. Five participants withdrew or were removed from participation in this study. Four participants withdrew due to scheduling issues and one participant withdrew after partial participation in the study, though apparently not due to an adverse event as there were no adverse events reported in this study.

With the exception created by the unreported protocol deviation concerning screening of ticks for vectors of tick-borne illness, the findings indicate that the subjects freely volunteered and gave informed consent to participate in the testing and were not coerced.

Recommendation
The HSRB offers the following recommendation:

EPA should require all future study reports to contain more information regarding demographics of the subjects selected for the study, including breakdowns according to age, gender, race and ethnicities (while not ideal, the census categories are a possible starting point) and potentially other characteristics to improve EPA and HSRB's ability to evaluate the equitable selection of subjects. The HSRB noted that EPA made some recent requests for this study that were consistent with this recommendation, which were helpful.

Additional Recommendations

Study Report Format and Contents

This study report and additional materials were difficult to review, especially the study report itself. The original report lacked sufficient information regarding the actual conduct of the study and IRB interactions, as well as some important information and data (including some missing information regarding IRB interactions – the materials provided start with a protocol that is up for its annual review). EPA required that the report be amended, and that helped comprehension some, but it would have been helpful if the study report was better organized and more comprehensive.

Recommendation
The HSRB offers the following recommendation:

The HSRB noted recommendations regarding future protocols, study reports format and contents on page 11 of the ethics review and greatly appreciates these and encourages EPA to make use of these with this sponsor and contract research organization, as well as other sponsors and contract research organizations as needed in the future. **The HSRB recommends the EPA consider expanding this guidance so it may provide more general guidance to sponsors and investigators working on EPA regulated studies in this area.**

Respecting the process for protecting human subjects

IRB-review and approval of a protocol, as well as EPA and HSRB-review and approval are intended to protect the rights, health, and safety of individuals participating in human subjects research. Protocols and consents are to be written to accurately reflect intended study procedures for the purposes of such reviews and approvals, again with the goal of protecting the rights, health, and safety of individuals participating in human subjects research. They are NOT just checkbox exercises. The significant, unreported protocol deviation regarding the use of ticks from BerTek, Inc. that had not been screened for vectors of tick-borne illnesses technically violates EPA human research protections and placed participants in this study at risk without their knowledge or consent. The HSRB reprimands Mimikai and Carroll-Loye Biological Research for the significant, undisclosed protocol-deviation re: screening of ticks for vectors of tick-related illnesses and encourages EPA to consider appropriate consequences if it deems warranted. However, taking into account the totality of efforts by Mimikai and Carroll-Loye Biological Research to comply with the applicable requirements of 40 CFR part 26, subparts K and L and the lack of any evidence of harm to subjects, the HSRB has found that this study was conducted in substantial compliance.

Study design if it is not possible to certify that the ticks used in a study are free of vectors of tick-borne illnesses

Even with protocol precautions against tick attachment and biting, there is still a risk that tick bites will occur and that transmission of tick-borne illnesses may occur. A follow-up call at 15 days after a subject's last test date would include the period when most post-test adverse events are likely to occur and is soon enough after the exposure to lessen the chance of recall bias compared to a longer interval.

Recommendation

The HSRB offers the following recommendation:

Protocols should both provide for unsolicited follow-up calls by participants in the event of them experiencing a delayed adverse event after test days (up to 30 days), as well as a scheduled follow-up visit or call at 15 days after the last test day the participant is involved to systematically document post-test day adverse events should they occur.

Public comments by the sponsor and contract research organization concerning challenges applying Good Laboratory Practices (GLP) to human pesticide studies

During public comment, the sponsor mentioned difficulties they were having applying some aspects of GLPs to human pesticide studies. The HSRB noted that human pesticide studies, while not exactly clinical studies, are more similar to specialized clinical trials of pharmaceuticals than animal laboratory studies, primarily due to the involvement of human research participants which creates additional safety and ethical concerns. While clinical trials may involve GLP procedures, for example, modifications of commercial formulations by a research pharmacy for use in the clinical trial, clinical trials are more generally conducted under Good Clinical Practices such as ICH guidelines E6(r2) (https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf and https://database.ich.org/sites/default/files/ICH_E6-R3_GCP-Principles_Draft_2021_0419.pdf) and E8(r1) (https://database.ich.org/sites/default/files/ICH_E8-R1_Guideline_Step4_2021_1006.pdf). The ICH E6(r2) guideline also seems particularly relevant as it addresses the responsibilities of sponsors, investigators and IRBs.

Recommendation

The HSRB offers the following recommendation:

EPA should explore, in collaboration with stakeholders the possibility of utilizing both GLPs and GCPs, as appropriate and perhaps with modifications, to evaluate/guide the development, conduct, reporting and regulation of human pesticide studies.

Suggestion for EPA to consider regarding the GLP/GCP recommendation

The HSRB suggests that EPA convene a small (5-8 members) work group composed of a few HSRB members, a few members of the EPA involved in these studies, and a few sponsors or investigators involved in these studies to review the GLP and GCP guidances/standards in light of the needs of pesticide studies (and possibly other studies that the HSRB reviews) and develop a set of draft recommendations for the HSRB to review, discuss, and vote on at a publicly convened meeting of the HSRB with public comments to be encouraged. EPA could also seek wider review and input of the draft recommendations within EPA, of external experts, OHRP, SACHRP, and the regulated research

community before or after the HSRB review, depending on what EPA determines may be most helpful to the process.

An alternative approach might be to refer this question to SACHRP for a recommendation. The advantage of this option is that SACHRP deals primarily with human subjects research protections, which GCPs fall under, and is very familiar with clinical trials as FDA often consults them. The disadvantage of this option is that the type of specialized studies that EPA brings to the HSRB for review are covered by a separate set of laws and regulations that SACHRP and its subcommittees are not as familiar with, and SACHRP and its subcommittees are also not as familiar with these types of studies. For that reason, the HSRB has listed this as an alternative to the preferred suggestion above.