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

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

November 1, 2022

MEMORANDUM

SUBJECT: Ethics Review of Completed Skin-Applied Tick Repellent Study with Oil of Lemon Eucalyptus and Methyl Nonyl Ketone

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

TO: Charles Smith, Director
Biopesticides and Pollution Protection Division
Office of Pesticide Programs

REF: Carroll, Scott P. (2021) Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions: Volume 1. Sponsored by MIMIKAI and conducted by Carroll-Loye Biological Research. Study Completed January 5, 2022. Unpublished Report. 403 pages. MRID 51170601.

Carroll, Scott P. (2021) Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions: Volume 2 – Institutional Review Board (IRB) Communications File. Sponsored by MIMIKAI and conducted by Carroll-Loye Biological Research. Study Completed January 5, 2022. Unpublished Report. 482 pages. MRID 51170602.

I have reviewed available information concerning the ethical conduct of the referenced research study, "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions". The documents submitted to the Environmental Protection Agency (EPA) describe the implementation and results of a laboratory study based on EPA guidelines OSCPP 810.3700: Insect Repellents to be Applied to Human Skin. The primary objective of the research was to determine the efficacy of this skin-applied repellent against three species of ticks in a lab setting.

After reviewing all available documentation, I have determined that the conduct of this study met applicable ethical standards for the protection of human subjects of research and that

the requirements for documentation of ethical conduct of the research were satisfied. If the research is determined to be scientifically acceptable, I find no barrier in regulation to the EPA's reliance on this study in actions under the Federal Insecticide, Fungicide, or Rodenticide Act (FIFRA) or the Federal Food, Drug, and Cosmetic Act (FFDCA). Research cannot be conducted ethically if the scientific conduct of the study is not sound. Therefore, if the research is not scientifically valid, it would not be ethical to rely on it.

In addition, under 40 CFR 26.1604, EPA is required to seek input from the Human Studies Review Board (HSRB) for intentional exposure human studies covered by EPA's Human Studies rule that are initiated after April 7, 2006. EPA will consult with the HSRB on this study and all associated support documents, as well as EPA's science and ethics reviews of the study. This memorandum and its attachments constitute EPA's ethics review.

Completeness of Submission

The materials provided by Carroll-Loye Biological Research and MIMIKAI satisfied the requirements of 40 CFR 26.1303. A checklist indicating how each requirement has been satisfied is provided in Attachment 1.

Summary Characteristics of the Research

Mimikai sponsored this study in order to determine the complete protection time (CPT) or duration of efficacy of a skin-applied repellent containing 11% Oil of Lemon Eucalyptus (OLE) and 7.75% methyl nonyl ketone (MNK) applied at a typical consumer dose (0.5 g per 600 cm^2) to the skin of human subjects. The study results have been submitted to the EPA in support of product registration. The study involved applying the test substance to the skin of the human subjects and evaluating whether or not the test substance repelled three species of ticks – *Amblyomma americanum, Ixodes scapularis,* and *Rhipicephalus sanguineus*. The testing was conducted at the facilities of Carroll-Loye Biological Research in California.

A total of 44 potential subjects were identified through recruiting, 31 people consented to participate in the study, and 27 individuals participated in at least one testing event. The study was initiated on October 5, 2021. The repellent testing occurred on October 10, 13, 17, and 24, 2021. The study was completed on January 5, 2022.

Human subjects were used because no reliable models or surrogates have been found to adequately predict the duration of efficacy of topically-applied insect repellents. The active ingredients used in this test product (OLE, MNK) have been registered by the EPA in use in other products. Each of the active ingredients has been evaluated by the EPA and found to present little or no risk when used as directed.[1]

Required Reviews and Oversight of the Research

On December 24, 2020, Advarra IRB approved the protocol dated December 23, 2020, informed consent form, and recruitment materials. Advarra IRB is registered with FDA and

[1] Link to EPA protocol science review

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

An IRB-approved draft protocol was submitted to EPA for review. The protocol and EPA's review[2], dated March 25, 2021, were discussed at a public meeting by the HSRB on April 21, 2021. The HSRB concluded that "[t]he research proposed … 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".[3] Attachment 2 describes how the EPA and HSRB comments have been addressed in the protocol used to conduct the study.

In follow-up to the HSRB meeting, the researchers revised the protocol and related materials to address comments, including the EPA and HSRB comments described in Attachment 2, and submitted the revised documents to the California Department of Pesticide Regulation (CDPR) and Advarra IRB for final review and approval of the protocol and materials prior to initiating the study. CDPR approved the study on August 12, 2021 (pp. 384-5) and Advarra approved the Amendment 3 of the protocol, used in the study, on September 13, 2021 (p. 386). Advarra terminated oversight of the study on December 23, 2021.

Documentation regarding final IRB approval of the protocol and subsequent correspondence between the researchers and the IRB is included with the materials provided to the HSRB members in Volume 2 of the study report.

Recruiting

The protocol called for recruiting in the area surrounding Carroll-Loye Biological Research lab through advertisements "on Craigslist™ and on community bulletin boards (virtual and real-world) frequented by members of the larger population that are most active outdoors (e.g., outdoor recreation enthusiasts, college and university students in the life sciences, etc.)" (p. 42) to reach a target of at least 44 candidates willing to attend a consent meeting, with an equal balance of males and females (p. 42). The IRB-approved advertising materials provided a brief explanation of the study, indicated that compensation would be provided, and included phone contact information (p. 185). Recruitment using advertisements in Craigslist, the UC Davis ECOSOCIAL list serve, the UC Davis Entomology Club email newsletter, and word of mouth from other participants and responses from researchers during the consent process for another Mimikai human study yielded interest from 44 candidates, 22 males and 22 females (Attachment

[2] Fuentes, Hull-Sanders, Arling. Science and Ethics Review of a Protocol for Laboratory Evaluation of Skin-Applied Tick Repellent Product Containing Oil of Lemon Eucalyptus (OLE or Citriodiol) and 2-undecanone (Methyl Nonyl Ketone or MNK). March 25, 2021. https://www.epa.gov/sites/default/files/2021-04/documents/2c._epa_science_ethics_review_memo_w_att_mimikai_ticks_mim-007_3-25-21.pdf

[3] Cavallari, Jennifer. April 20-21, 2021 EPA Human Studies Review Board Meeting Report. https://www.epa.gov/system/files/documents/2021-07/042021-hsrb-meeting-report-final.pdf p. 15.

3[4], p. 15; note that there was an error in the study report that stated that recruitment was closed when 60 individuals responded, p. 13). Study staff randomized the list and contacted each interested individual by phone using an IRB-approved script (pp. 186-7). At the end of the screening call, subjects were presented with the schedule of test dates. Subjects who were available for all three test days at the time of the screening were scheduled for a consent interview. As a result, 31 candidates (14 female candidates and 17 male candidates) were invited to attend a consent meeting, a deviation from the protocol which called for enrolling at least 8 additional subjects beyond the 25 needed for the study. The registrant responded to EPA's question about the subject selection, randomization, and assignment process by stating that "[t]he study director prioritized the likelihood of participation of the same subjects across all species over optimizing the sex balance of subjects, and the timely completion of data collection over re-opening recruitment in an effort to secure more reliable subjects" (Attachment 3, p. 11).

This study's timing coincided with another study sponsored by Mimikai and conducted by Carroll-Loye Biological Research. The EPA noted that some individuals participated as test subjects in both studies (10 females, 11 males; see Attachment 3, p. 51). In response to the EPA's questions, the registrant noted that:

> *Separate recruitment advertisements were used for Study Nos. MIM-006 and MIM-007. Both advertisements were disseminated to the same outlets. By this means, many candidates who had expressed interest in participating in study MIM-006 also expressed interest in participating in MIM-007. In addition, it was common for candidates and subjects for MIM-006 to ask if there were other CLBR studies they might be eligible to participate in. Researchers were allowed to mention the upcoming tick study in response to such questions. The MIM-007 candidate list was randomized prior to call-backs according to the amended Study Protocol (pg 14; see pg 42 of the originally submitted final report), as follows: 'Each candidate in order of contact will be assigned a sequential number unique to them and to this study, then the candidate numbers list randomized in Microsoft Excel.'* (Attachment 3, p. 16)

The Study Director indicated in a response to the EPA that candidates who expressed an interest in participating were identified by subject number and added to excel lists separated by gender, and the lists were randomized. Research staff contacted potential subjects according to the randomized lists until 25 subjects indicated that they would be available for all 3 days of testing. No priority or preference was given to subjects who had participated in the previous Mimikai study conducted by Carroll-Loye Biological Research.

[4] Note the references to Attachment 3 are to the page number on the PDF file, rather than the page number on each of the attachments.

Consent and Enrollment

According to the study report, 31 individuals completed the informed consent process and signed the IRB-approved consent form (p. 13).

Consent meetings were held in person or via video/phone call. Subjects were provided with copies of relevant documents (consent form, California DPR Experimental Subjects Bill of Rights, protocol, SDS, toxicology study results) (p. 44). During the consent meeting, a trained member of the study staff member read the consent form. This included an outline of the study, including its purpose, the subjects' potential role, the length of the study on a test day and overall, the pesticide to which subjects would be exposed, risks of participation and how they would be mitigated, compensation for participation, and the eligibility criteria. Female subjects were informed about the prohibition on enrolling pregnant and nursing women, and the study requirement to take a pregnancy test on each study day on which they would be exposed to the test substance or mosquitoes. In addition, subjects received a demonstration of the repellent application, tick attractiveness testing, and test day procedures. During this process, the researchers conducting the session also highlighted that participation was completely voluntary and subjects were free to withdraw at any time.

Subjects were screened during the consent process (p. 13) to confirm that they met the eligibility criteria outlined in the protocol (p. 43). Eligibility was confirmed through verification of age using a government-issued identification, the subject screening, tick attractiveness testing, tick handling training, and pregnancy testing for female subjects on each day ticks were encountered. Subjects were eligible to participate if they were willing to consent, between 18 and 60 years old, and able to speak and understand English. People were not eligible to participate if doing so would pose a risk to their health (allergic or sensitive to tick bites; allergic to the test substance/topical repellents/essential oils; prone to/suffering from rashes or other skin conditions) and if they were unwilling to refrain from using certain products before and during the testing (perfumed products, alcoholic beverages, tobacco). Individuals who were unable to deliver ticks to their arms successfully, deemed unattractive to ticks, unable to see ticks and monitor them on their skin, or who had participated in an interventional study other than a repellent efficacy study were also excluded. Additionally, pregnant or nursing women, and employees of the Study Director or study sponsor, as well as their spouses and immediate family members, and students of the Study Director were not eligible.

Candidates were permitted to ask questions, and then researchers asked the candidates questions to ensure their comprehension of the consent form and study procedures (p. 44). If the person was determined to be eligible, the study staff verified the subject's age with a government-issued identification.

Subjects who attended the meeting and consented to participate virtually were asked again at their first lab visit whether they still wanted to participate, were reminded they were free to withdraw at any time, and were offered the opportunity to ask questions about the research. After confirming a continued desire to participate, they were asked to initial all pages and sign the consent form (p. 13). The sponsor confirmed that "[t]he subjects who participated in both studies were consented and trained in accordance with each of the two protocols as independent

consenting processes" (Attachment 4, p. 9). All subjects received a copy of their signed consent forms.

After completing the consent process, subjects were scheduled for a training visit at the Carroll-Loye Biological Research laboratory facility to be tested for attractiveness to ticks and to be trained on the handling of ticks, and to have their arms measured so the study staff could calculate the appropriate dose of the test substance. Tick attractiveness testing and training on tick handling were conducted according to the protocol, with a deviation that included the use of forceps in addition to paintbrushes for tick handling (p. 23). All subjects were deemed attractive and demonstrated proficiency in handling ticks.

Following successful completion of the screening visit, subjects were eligible to participate in efficacy test days.

Demographics

Of the 31 subjects who consented, 27 participated in at least one test day. The testing of *R. sanguineus* and *A. americanum* included 15 males and 10 females. The testing of *I. scapularis* included 14 males and 11 females. Subjects who participated in at least one test day ranged in age from 20 to 35 years old (Attachment 3, p. 51).

Test Day Procedures

Testing was conducted at Carroll-Loye Biological Research in a room designated for subject exposures (p. 14). All subjects involved in a test day were in the same room. Testing with *R. sanguineus* was conducted with all subjects on a single day (October 10, 2021). Testing with *A. americanum* was conducted on two separate test days (October 13, 2021, 5 subjects; October 17, 2021, 20 subjects). Testing with *I. scapularis* was conducted on two separate test days (October 20, 2021, 6 subjects; October 24, 2021, 19 subjects) (Attachment 3, p. 51).

Prior to the test day, subjects were contacted by phone or email with a reminder about their scheduled test day and the conditions for participation in the study (Attachment 4, pp. 30-31) . Upon arrival at the test site, subjects were reminded about their freedom to withdraw at any time and without penalty, and were asked to confirm that they complied with the study requirement to refrain from using perfumed products, alcohol or tobacco for the 48 hours preceding the test day. At this time, subjects were screened for COVID, their skin was checked for disqualifying conditions, and female subjects completed the pregnancy testing protocol. All subjects were qualified to continue participation in the test day. At this point, they began preparing for the testing.

Subjects began by washing their forearms with a mild, fragrance-free cleanser, spraying them with diluted ethanol, and drying them with a towel. Next, the test material was applied to the skin surface of each subject's non-dominant forearm by a trained member of the research team using a pre-weighed finger cot to spread the appropriate dose over the designated area. Following application of the test substance, subjects' arms were marked with lines for measuring tick progress.

Testing was conducted concurrently for all subjects present on the test day. A researcher announced the beginning of each 15-minute period. Subjects then took a tick and placed it on their untreated arm to evaluate the tick's activity. Ticks that were not sufficiently active were discarded and the subject selected a new tick and evaluated its activeness. A sufficiently active tick was moved to the treated arm, and monitored to see whether it moved 3 cm from the wrist toward the elbow within a 3-minute period. At the end of the test period, the tick was removed for destruction by study staff. Testing continued in this manner, repeating the tick placement with a new tick every 15-minutes until the subject experienced a confirmed crossing (a tick crossing the specified distance into the treated area followed by a second tick doing the same thing within 30 minutes).

At the end of each subjects' testing period, they washed their repellent-treated arm, received their compensation (in some instances), and were free to leave.

Safety Precautions

The protocol identified six types of risks and measures to mitigate them. The risks identified included: exposure to the test material, exposure to biting ticks, exposure to vectors of tick-borne pathogens, physical stress from test conditions, contracting COVID-19, and psychological stress associated with a breach in confidentiality around pregnancy testing results (p. 34). Apart from a deviation related to tick screening, discussed below, the study followed the measures outlined to mitigate risks to test subjects.

The exposure criteria were exercised to eliminate candidates who had know or suspected allergies or sensitivities to the test material or potentially related substances. In addition, the protocol called for checking subjects' skin prior to the start of testing for conditions that could be exacerbated by exposure to the test material and stopping a subjects' participation in the study if they showed signs of reactions to the test material.

Exposure to biting ticks was mitigated in several ways. All subjects were trained in tick handling, which included "how to manipulate ticks with fine paintbrushes, place them on their own forearms, observe and quantify tick movement on their arms, determine if a tick begins to bite them, remove ticks before biting occurs and dispose of used ticks" (p 35). Subjects were trained on observing tick behavior and removing ticks before they could bite. Two deviations to the protocol increased subject safety around exposure to biting ticks. First, forceps were used in addition to paintbrushes for tick handling because they gave subjects greater control (p. 23). Second, the definition for a crossing was changed from traveling a specified distance and remaining in that area for at least 1 minute, to traveling a specified distance in order to minimize the potential for active adult ticks to travel too far up subjects' arms and potentially under clothing (p. 25).

The protocol called for minimizing exposure to vector-borne pathogens through sourcing the ticks for the study from colonies with screening procedures that were certified as disease free. This occurred for two of the three species (*A. americanum* and *I. scapularis*) (pp. 319-323). However, for the third species used (*R. sanguineus*), no adequate documentation of disease-free

status was provided. This unreported deviation from the protocol is discussed in more detail below.

The test location was indoors and measures for reducing subjects' potential risk of physical stress were taken, such as maintaining a comfortable temperature and humidity, providing a rest area stocked with drinks and food, and providing seating for subjects.

The risks of COVID-19 were mitigated through screening of subjects by phone prior to their scheduled appearance at the lab, and in person on each day of testing. Social distancing was maintained to the extent possible (not possible during attractiveness testing and tick handling training), and requiring subjects to wear masks during testing (p. 233).

Risks associated with pregnancy testing were minimized by giving female subjects a private location to take a pregnancy test on each day that they encountered ticks or were exposed to the test substance. They were given the option to share the results with a female member of the research team in private to confirm their eligibility to participate in the study or to withdraw without showing the results. Opaque bags were provided for disposal of pregnancy tests.

Adverse events

No adverse events were reporting during the attractiveness testing and tick handling (p. 13) or on any test day (p. 18).

Female Subject Screening

During recruitment and enrollment, all subjects were instructed that individuals who were pregnant, nursing, or lactating were not eligible to participate in the study, and reminded that on each day of their participation, female subjects would be required to take a pregnancy test to confirm their continued eligibility. Female subjects' continued eligibility to participate was verified at the start of each test day by completing pregnancy testing according to the procedures outlined in the protocol. Pregnancy testing was conducted in by the female subject alone, and the subsequent discussion with a female member of the research team occurred in a private setting.

Confidentiality

The study followed the measures outlined in the protocol regarding confidentiality. Consent meetings were conducted in a one-on-one setting. All records containing names and other personal information are maintained in a locked cabinet with limited access. Subjects were identified with a number throughout the study and in the study report.

Compensation

Each subject received compensation consistent with the protocol, $25 per hour of participation in any of the study-related activities, including consent, training, and testing (p. 38). The protocol called for providing compensation to subjects at the end of each encounter. However, some subjects requested a single payment for their participation on the last day of the

study (p. 197). The Study Director compensated each subject in accordance with their preference with cash or check.

Withdrawal

Subjects were informed that they were free to withdraw from the study at any point, including during the testing period, without forfeiting any benefits to which they were entitled. This was communicated during the consenting process, during the training, and at the beginning of each test day. The consent form indicated that alternate subjects would be available on each test day to replace any subjects who wished to withdraw. A protocol deviation added two test days for subjects who were not available on the scheduled test days. Only subjects were scheduled to attend on the two added test days; no alternates were present to replace withdrawing subjects. However, subjects were reminded on these test days that they could withdraw at any time and that alternates were available to replace them if necessary (p. 25).

There were several withdrawals from the study. Subject 18 was assigned as an alternate and withdrew from the study before it began because she was unable to attend any of the scheduled test dates. Subject 147 withdrew from testing with *I. scapularis* after completing the 45th interval and their data were used in the analysis. Three subjects (62, 163, 177) were unavailable for specific test days, and were replaced by alternate subjects on those days (pp. 13-14).

Protocol Amendments and Deviations

The protocol was amended three times. The first amendment, approved by Advarra IRB on December 24, 2020, updated the original submission to the Advarra IRB and changed the amount of MNK in anticipation of submission of the protocol to the EPA for review (pp. 132-146). Amendment 2 was initiated following review by EPA and the HSRB, and addressed the recommendations following the public meeting held in April 2021 (pp. 148-166). Amendment 3 corrected a typographical error (p. 168).

The EPA noted an issue related to amendment approval dates. The effective dates of some amendments predate the IRB's approval of the amendments. For example, for Amendment 1 the amendment date is listed as December 23, 2020 (p. 146). However, the IRB did not approve the amendment until December 24, 2020 (Volume 2, p. 160). The discrepancies in the effective dates did not affect subject safety or welfare; however, amendments are not effective until the IRB has reviewed and approved them. The EPA recommends that in future studies, the effective dates of amendments and protocol revisions be listed as "IRB approval date" or left blank at the time of submission to the IRB and added after IRB approval.

The study report lists 11 deviations from the protocol during the course of the study (pp. 23-26). Two deviations involved subject recruitment and enrollment: only 31 subjects, rather than 33 as described in the protocol, were enrolled, and the enrollment was not balanced by gender (p. 23). Three deviations were related to the ticks used in the study (tick sourcing from multiple labs, tick age at testing) and the conditions of the test room (relative humidity outside parameters on some occasions) (pp. 23-24). Six deviations related to test days and test

procedures (testing on 5 days instead of 3, using forceps in addition to paintbrushes, varying the time of application between subjects more than anticipated, using pre-weighed finger cots rather than pre-weighed gloves when applying the test substance, scoring of the crossing based on distance traveled rather than time in a specific area, payment at the end of all testing versus at the end each test day) (pp. 24-26).

There is no indication that any of the protocol amendments or reported deviations negatively impacted subjects' health or welfare. EPA found that none of the amendments or deviations impacted the scientific validity of the study.

There were two unreported deviations related to subjects. First, the ratio of male to female subjects during the study differed from the protocol. The protocol called for having a nearly equal ratio of males to females, either 12:13 or 13:12. Based on enrollment, subject availability and the Study Director's discretion, the ratios of males to females was either 15:10 or 14:11. This deviation did not impact the subjects' health or welfare, but should have been reported explicitly as a deviation in the study report.

Second, there was an unreported deviation from the protocol and consent form related to screening of ticks to be used in the testing. The protocol noted that "[t]icks of all three species will be sourced from the Oklahoma State University Department of Entomology and Plant Pathology Tick Rearing Facility, or another source of laboratory-reared ticks *with documented pathogen-free status*" (p. 50) (emphasis added). The protocol also noted that "[o]ur laboratory-reared tick populations are certified disease free" (p. 35), and cited to an example of the certification process that listed the tick species and the diseases for which the ticks had been screened (pp. 117-119). The consent form stated that "[t]he ticks used in this lab study are from a *colony that has been screened for infections diseases* and they have been determined to be free of the pathogens that cause Lyme Disease, Rocky Mountain Spotted Fever, Ehrlichiosis, and Anaplasmosis" (p. 179) (emphasis added). The Study Director obtained ticks from two sources – Oklahoma State University (*A. americanum* and *I. scapularis*) and BerTek, Inc. (*R. sanguineus*). The study report included a record of screening of the ticks from Oklahoma State University for pathogens (pp. 319-323). From BerTek, Inc., all that was included in the study report was an assertion by the company that "[t]o our knowledge, there are no known pathogens or resistances" (p. 324).

There were no adverse events during the study, and no indication that a subject contracted a tick-borne disease as a result of their participation in the study. BerTek, Inc. provided information about their tick colony rearing and feeding as evidence that it was unlikely that the ticks provided would have any pathogens that could be transmitted to the subjects. Further, subjects were trained to remove ticks before they could attach and bite, so the overall risk to subjects from ticks without documented pathogen-free status was low. However, the decision to use in the study ticks that were not procured in compliance with the protocol and consent form, and screened for potential pathogens, is a deviation that could have impacted the subjects' health or safety, and related to the subjects' informed choice to participate. Per the protocol, the IRB's handbook, and EPA's regulation, this change should have been submitted to and approved by Advarra IRB prior to implementation, as it was not a change necessary to eliminate apparent immediate hazards to the subject.

Recommendations

For future studies, EPA recommends the following:

- Include details about subject recruitment, consent process, enrollment and assignment as test/alternate subject, test day participation, and compensation in the study report. This should include the number of individuals contacted at each stage of the recruitment process, the demographics of the enrolled subjects, the rationale (if any) for subject withdrawals, and all other information pertinent to subject participation in the study.
- Recruit broadly from the area surrounding the test location, rather than focusing the recruitment on a small segment of the population in the area.
- Make clear in the protocol and the consent form whether subjects will be recruited to participate in more than one test day and whether enrollment as a test subject will be prioritized for individuals who can participate in all scheduled test days.
- Consider excluding anyone who has participated in another repellent efficacy testing study within the last 3 months in order to avoid using substantially similar pools of subjects.
- Make clear in the protocol, recruitment materials, and phone script whether it is permissible for the research staff contacting potential candidates to mention other potential studies with open enrollment.
- If the protocol calls for enrolling a balanced number of males and females and the initial recruitment and screening process yield an insufficient or imbalanced number of individuals, recruitment should be reopened to allow for enrollment consistent with the protocol.
- The protocol should explain clearly what "documented pathogen-free status" means and how it will be achieved, as well as naming all labs 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.
- 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.
- Ensure that the protocol clearly states that all amendments to the protocol, regardless of whether they are related to subject safety or informed consent, must be reviewed and approved by the overseeing institutional review board prior to implementation. *See* 40 CFR 26.1108(a)(3)(iii) compared with the language on pages 57 of the study report.
- Reduce the use of notes to file; rather, incorporate the relevant information in the appropriate section of the study report.
- Any deviation to the protocol related to subject encounters should be reported to the IRB.

Applicable Ethical Standards

The following provisions of 40 CFR 26 Subpart Q define the applicable ethical standards which are summarized below:

> **§26.1703**: 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.

Because this research was conducted by a third-party rather than conducted or sponsored by the federal government, the relevant provisions of the regulation are subparts K and L. Subpart K outlines specific standards that the research must follow, including: review and oversight of the research by a qualified IRB; obtaining informed consent from all subjects using an instrument approved by an IRB; consent conducted under circumstances to minimize the possibility of coercion or undue influence; consent information presented in a language the candidate understands; and candidates must have an opportunity to discuss the information presented and to consider whether or not to participate. The informed consent must include an overview of the research, a discussion of the risks and how they will be mitigated, the benefits of the research, how confidentiality will be maintained, whether medical treatment for study-related incidents will be provided, who to contact about questions or study-related injuries, a statement that participation is voluntary and subjects may withdraw anytime without penalty, stopping rules for the study and when a subject's participation may be terminated, costs of participation in the study, any consequences of withdrawal and the process, the number of subjects involved, and whether biospecimens will be collected and/or retained. Finally, Subpart K requires the proposed research to be submitted to EPA after being approved by an IRB and prior to initiating the study. Subpart L prohibits research involving pregnant women, nursing women, or children.

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

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

Findings

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

The research was reviewed and overseen by Advarra IRB. 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, and the IRB complies with the requirements in Subpart K for membership and procedures.

All subjects provided consent by signing a written consent form approved by Advarra IRB, and all subjects received a signed copy of the consent form. The consent process was conducted in one-on-one meetings, giving individuals opportunities to ask questions in private about their participation and to consider their options without being pressured by other potential subjects. The consent meetings were held in English, the language spoken by all subjects per the eligibility criteria. Subjects that might feel pressured to participate, such as employees, students, or family members of the Study Director, and employees of the sponsor and their family members were excluded from participation. Subjects were informed that participation was voluntary and that they could withdraw at any time throughout the recruitment, consent, and study processes.

Consent was obtained using an informed consent form that covered all of the elements required by Subpart K.

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

Conclusion

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

Cc: Angela Myer
Clara Fuentes
Shannon Borges

Attachment 1: §26.1303 Completeness Checklist
Attachment 2: Responsiveness to EPA and HSRB Ethics Comments on Draft Protocol
Attachment 3: Registrant's Response to 75-Day Deficiencies Letter (August 23, 2022)
Attachment 4: Registrant's Response to 90-Day Preliminary Technical Screening Results of Efficacy Report (May 20, 2022)

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

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

	Requirement	Y/N	Comments
(a) Copies of all of the records relevant to the research specified by § 26.1115(a) to be prepared and maintained by an IRB	§1115(a)(1): Copies of • all research proposals reviewed, • scientific evaluations, if any, that accompany the proposals, • approved sample consent documents, • progress reports submitted by investigators, and • reports of injuries to subjects.	Y	Volume 1 (Appendices 1 and 2) Volume 2
	§1115(a)(2): Minutes of IRB meetings which shall be in sufficient detail to show • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; • a written summary of the discussion of controverted issues and their resolution.	N/A	
	§1115(a)(3): Records of continuing review activities, including the rationale for conducting continuing review of research that otherwise would not require continuing review as described in §26.1109(f)(1).	Y	Volume 2
	§1115(a)(4): Copies of all correspondence between the IRB and the investigators.	Y	Volume 2
	§1115(a)(5): A list of IRB members in the same detail as described in § 26.1108(a)(2).		Provided separately to EPA
	§1115(a)(6): Written procedures for the IRB in the same detail as described in § 26.1108(a)(3) and (4).	Y	Provided separately to EPA
	§1115(a)(7): Statements of significant new findings provided to subjects, as required by § 26.1116(c)(5).	N/A	
	§1115(a)(8): The rationale for an expedited reviewer's determination under §26.1110(b)(1)(i) that research appearing on the expedited review list described in §26.1110(a) is more than minimal risk.	N/A	
	§1115(a)(9): Documentation specifying the responsibilities that an institution and an organization operating an IRB each will undertake to ensure compliance with the requirements of this subpart.	Y	Provided separately to EPA
(b) Copies of all of the records relevant to the information identified in § 26.1125(a)-(f)	§1125(a)(1): The potential risks to human subjects	Y	Volume 1, Appendix 1
	§1125(a)(2): The measures proposed to minimize risks to the human subjects;	Y	Volume 1, Appendix 1
	§1125(a)(3): The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	Volume 1, Appendix 1
	§1125(a)(4): Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	Volume 1, Appendix 1
	§1125(a)(5): The balance of risks and benefits of the proposed research.	Y	Volume 1, Appendix 1
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.	Y	Volume 1 Appendix 2, Volume 2
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.	Y	Volume 1, Volume 2
	§1125(d): A description of the circumstances and methods proposed for presenting information to potential human subjects for the purpose of obtaining their informed consent.	Y	Volume 1
	§1125(e): All correspondence between the IRB and the investigators or sponsors.	Y	Volume 2
	§1125(f): Official notification to the sponsor or investigator, in accordance with the requirements of this subpart, that research involving human subjects has been reviewed and approved by an IRB.	Y	Volume 2
(c) Copies of sample records used to document informed consent as specified by §26.1117, but not identifying any subjects of the research		Y	Appendix 16.2
(d) If any of the information listed in paragraphs (a) through (c) of this section is not provided, the person shall describe the efforts made to obtain the information.		N/A	

Attachment 2

Responsiveness to EPA and HSRB Ethics Comments on Draft Protocol

EPA Recommendation	Action taken by Study Sponsor
Include information on how adverse events will be evaluated and when they will be reported to the IRB	Information added to the protocol. See pp. 37-38.
Clarify compensation for subjects who withdraw	Clarification added to the protocol to note that all subjects compensated based on the length of their participation at an hourly rate of $25. See p. 38.
Explain the process for verifying subjects' comprehension of the consent form prior to requesting them to sign the consent form.	Questions to confirm comprehension were added to the protocol. See p. 44.
Include a process for a trained medical professional to check subjects' skin for conditions that would impact their eligibility on each day of testing.	This was added to the protocol. See p. 53.
Revise the protocol to address COVID-19 risks that could arise from study participation and how they will be mitigated.	The protocol includes these revisions, see pp. 35-36. The consent form also addresses this comment, see pp. 170-185.
Revise the consent form to align with the requirements at 40 CFR 26.1116, including starting with a concise and focused presentation of key information.	The consent form was revised as requested. See pp. 170-185.
Revise the protocol and consent forms in line with the general and editorial comments made in the EPA's review memo.	The protocol and consent forms were amended as requested.

HSRB Recommendation	Action taken by Study Sponsor
Revise protocol and consent form to acknowledge risks associated with COVID-19 and to describe precautions that will be followed.	The protocol includes these revisions, see pp. 35-36. The consent form also addresses this comment, see pp. 170-185.
Remove spending a significant amount of time outdoors as an inclusion criteria.	This criterion was removed. See p. 43.
Add information on how subjects from a variety of ethnicities will be recruited either within targeted communities or using other methods.	The protocol noted that "subjects will be recruited from the adult human population within 40 minutes-drive [sic] of the Carroll-Loye laboratory site. We will advertise on Craigslist TM and on community bulletin boards (virtual and real-world) frequented by members of the larger population that are most active outdoors" (p. 42). No additional information on recruitment would target a variety of ethnicities.