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

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

October 12, 2022

MEMORANDUM

SUBJECT: Laboratory Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Product Against Three Species of Ticks

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REFERENCE: Carroll, Scott P., Study Director. (2020) Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions. Unpublished Document. January 5th, 2022. MRID 517706-01.

ACTION REQUESTED

Conduct a science review of a completed laboratory study testing efficacy of a topical insect repellent spray (MIMIKAI Lilly Pilly Repellent), containing active ingredients of 11% w/w of oil of lemon eucalyptus (OLE, Citriodiol®) and 7.75% w/w of methyl nonyl ketone (MNK, 2-Undecanone), against ticks. The formulation was tested against three species of ticks: *Amblyomma americanum*, *Ixodes scapularis*, and *Rhipicephalus sanguineus.* This product performance test is required to establish the median Complete Protection Time (mCPT) with 95% confidence intervals (CI) against ticks to support registration of the proposed skin-applied repellent product. The protocol (dated February 17, 2020) used to conduct this study was

reviewed by the Environmental Protection Agency (EPA) and Human Studies Review Board (HSRB) on April 21st, 2021. The protocol was amended three times before the study began, on December 23rd, 2020 (Amendment 1); August 25th, 2021 (Amendment 2); and September 5th, 2021 (Amendment 3). The final version of the protocol was approved by the IRB on September 13th, 2021, and adequately incorporated EPA and HSRB recommendations (Attachment 5 in this review). The study was conducted according to OPPTS 810.3700 Guidelines: *Insect Repellents Applied to Human Skin*[1] and the final amended protocol (Appendix 1 in the study report, MRID 517706-01). Protocol amendments and reported protocol deviations are provided in the study report (Appendix 1, pp. 131-168 of 403, and 9, pp. 23-26 of 403; respectively). Appendices referenced throughout this review refer to those included in the study report.

The study report (Attachment 1) and associated attachments are listed in the table below. Following the Agency's technical screen of the study report, two correspondences were made to the registrant to request various points of clarification and discussion regarding the scientific conduct of the study (Attachments 2-3).

Attachment #	Document Date	Document
1	January 5th, 2022	Study report (MRID 517706-01), which includes 14 appendices
2	May 20th, 2022	Registrant response to the first 10-day deficiency letter with EPA technical screen comments (dated May 6th, 2022)
3	August 23rd, 2022	Registrant response to the second 10-day deficiency letter (dated June 17th, 2022)
4	September 27th, 2022	EPA's statistical analysis report (Kaplan-Meier survival analyses)
5	Not applicable	Responsiveness to EPA and HSRB science comments to the study protocol

CONCLUSIONS

The EPA evaluated the scientific validity of the research in relation to recommendations from the EPA, HSRB, and the Product Performance Test Guidelines OPPTS 810.3700 for testing of Insect Repellents to be Applied to Human Skin. Study MRID 517706-01 was conducted in accordance with Good Laboratory Practices (GLP) as described in 40 CFR §160. CPT is defined as the time between product application and repellency failure. The study's CPT data indicates that 20% to 25% of the population may experience repellency failure against *R. sanguineus* two hours post-

[1] EPA. Product Performance Test Guidelines; OPPTS 810.3700: *Insect Repellents Applied to Human Skin*. EPA 712-C-10-001. July 7, 2010. https://www.regulations.gov/document/EPA-HQ-OPPT-2009-0150-0011

application. However, standard policy in the EPA Repellency Awareness Guidance[2] dictates that the CPT used on the product label is based on the most conservative mCPT of the three tick species tested. The most conservative mCPT of 287 minutes (~ 4 hours, rounded down) was provided by *A. americanum*. Therefore, MRID 517706-01 provides scientific data that support a CPT of 4 hours on the product label. The EPA will consult with the HSRB on this study.

SCIENCE REVIEW

Study objective: The objective of this study is to establish the mCPT of a topical insect repellent spray, MIMIKAI Lilly Pilly repellent, containing active ingredients of 11% w/w of OLE (CAS 1245629-80-4; PC Code: 040522) and 7.75% w/w of MNK (CAS 112-12-9; PC Code: 044102), in a laboratory test against lab-reared ticks, and provide repellency data for product registration and labeling purposes. The repellency of the product was tested using human volunteer subjects and three tick species (*A. americanum, I. scapularis,* and *R. sanguineus*).

Endpoints: The scoring of questing tick movement away from (repulsions) or into (crossings) treated forearm sections was used as the endpoint to evaluate the efficacy of the insect repellent product (Appendix 7, pp. 296-312). A repulsion was scored when an active tick moved away from or parallel to the treated forearm area or did not travel more than 3 cm past the reference line within 3 minutes (Figure 1 below; Attachment 2). A crossing was scored when an active tick traveled more than 3 cm past the reference line and reached the forearm line (with any portion of its body overlapping the forearm line) within 3 minutes (Figure 1 below; Attachment 2). A First Confirmed Crossing (FCC) was defined as a crossing followed by another crossing within 30 minutes (Appendix 1, p. 33 of 403). For subjects that experienced FCC, CPT was determined as the elapsed time between product application and FCC (§7, p. 19 of 403); time to FCC indicates the time of repellency failure (see FCCs highlighted in Appendix 7, pp. 301, 308, 312 of 403). The mCPT was calculated for each tick species using Kaplan-Meier survival analyses across a sample size of 25 subjects (§7, p. 19 of 403). The total duration of exposure periods during test days ranged from ~8-12 hours, depending on the species tested (Appendix 7, pp. 301, 308, 312 of 403).

[2] Repellency Awareness Guidance: For Skin-Applied Insect Repellent Producers. https://www.regulations.gov/document/EPA-HQ-OPP-2013-0406-0003

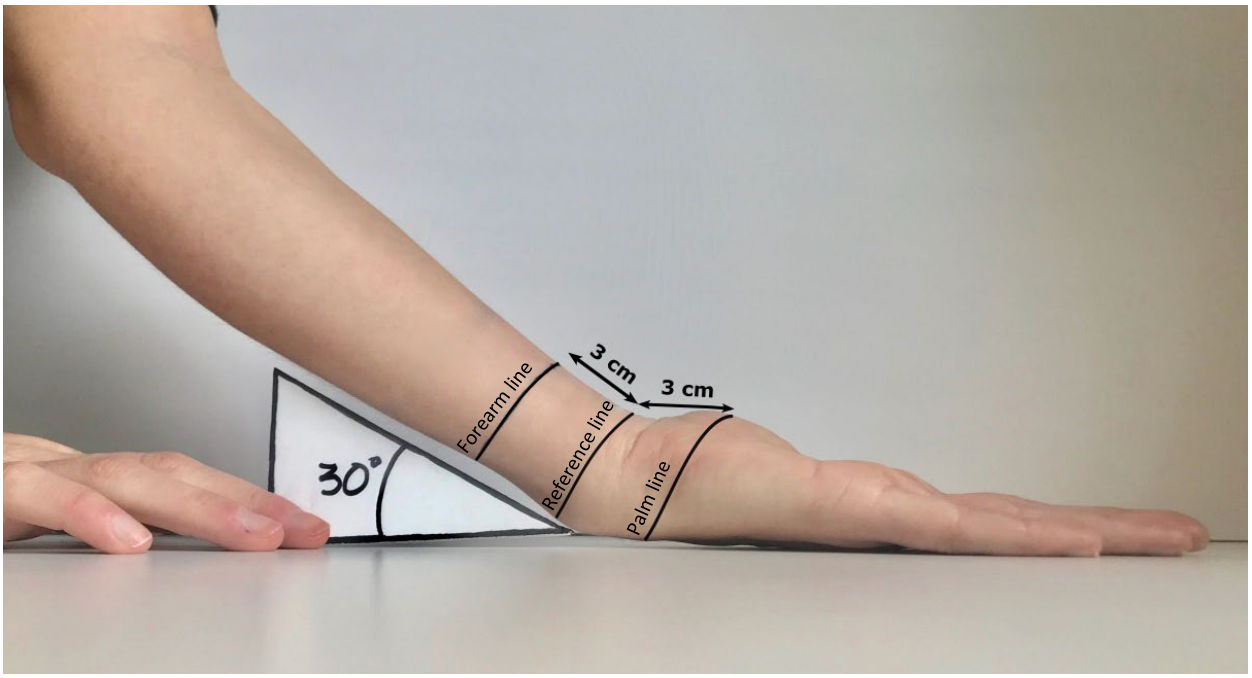

Figure 1. The arrangement of lines for tick placement and crossing determinations, and the use of a degree angle indicator (Figure and text from Figure 1 in MRID 517706-01). The Agency modified this figure to include forearm, reference, and palm line labels based on the description of 'orientation' ink lines provided in the protocol (Appendix 1, p. 53-54 of 403). The treated area consisted of the non-dominant forearm from the wrist to the elbow (§6, p. 17 of 403, Attachment 2), with the reference line marking where the treated area began (Appendix 1, p 53 o 403).

Compliance with Good Laboratory Practice Standards (GLP); 40 CFR, Part 160:
The study is a guideline study designed in conformity with recommendations from the OPPTS 810.3700 product performance guideline for testing of Insect Repellents to be Applied to Human Skin. This study was conducted in accordance with EPA, FIFRA (Federal Insecticide, Fungicide and Rodenticide Act), and (GLP) Standards (40 CFR, Part 160). A Statement of Compliance with Good Laboratory Practice Standards is provided on pg. 3 of the study report in MRID 517706-01. A Quality Assurance Statement, signed and dated on January 5th, 2022, is provided on pg. 4 of the study report in MRID 517706-01.

Identification of the test system: Ticks were the target pest used for repellent product performance testing in MRID 517706-01. The objective of the study was to evaluate the efficacy of the product in repelling three tick species: *A. americanum, I. scapularis,* and *R. sanguineus.* Five species-specific test days were used to evaluate product performance with 25 human subjects treated with the test substance: *R. sanguineus* testing occurred on October 10th, 2021; *A. americanum* testing occurred on October 13th and 17th, 2021 (Appendix 7, p. 301 of 403), and *I. scapularis* efficacy testing occurred on October 20th and 24th, 2021 (Appendix 7, p. 308 of 403). Subjects used in tick repellency trials were selected from a pool of informed and consenting volunteers who were tested for their attractiveness to ticks and trained to handle ticks using forceps and paintbrushes within a 30-day period prior to the first efficacy test day (Attachment 2). Product testing for all three tick species were performed in a single ~5 m × 18 m exposure room located in the same building as the Carroll-Loye Biological Research Laboratory (§3, p. 14

of 403; Appendix 9, p. 338 of 403), with temperature and humidity maintained by an HVAC system and high-output humidifier. Overhead lighting was present to facilitate tick observation, and light intensity was recorded during test days. Environmental data is provided in Appendix 9 (pp. 327-331).

Tick Rearing and Maintenance Conditions: Ticks were obtained from two sources (§4, p. 14 of 403). The *A. americanum* and *I. scapularis* colonies were obtained from the Oklahoma State University (OSU) Department of Entomology and Plant Pathology Tick Rearing Facility, whereas the *R. sanguineus* colonies were obtained from BerTek, Inc (Appendix 8, pp. 319-324 of 403). Ticks sourced from OSU were routinely tested for viral pathogens using polymerase chain reaction (PCR) assays (Appendix 8, pp. 320-323 of 403). BerTek, Inc. provided a statement that their tick colonies, to their knowledge, have no known pathogens or resistances (Appendix 8, p. 324 of 403). Starved ticks were requested from source labs, but ticks were not blood-fed at Carroll-Loye Biological Research (CLBR) (Attachment 3). Records of last bloodmeals provided at rearing sources (if known) are provided in Annex II of Attachment 3.

All tick species arrived at the CLBR lab as ~2-week-old adults in clear polystyrene canisters (~ 1-inch diameter and 2-inch height) with white plastic snap-on lids with a single hole covered in fine mesh and secured with flexible cloth tape (Attachments 2-3). The canisters were placed in gallon self-sealing bags that were loosely folded over and labeled with species identity and date of receipt, and the bags were placed in 'desiccation' jars (10-inch diameter 8-inch height) with copper sulfate/H_2O humidifiers (Attachment 3). Tick jars were maintained in environmental conditions of 22-27° C, ~95% relative humidity, and 12:12 hours (light: dark) photoperiod at the CLBR insectary until the ticks were used in training/attractiveness screening or efficacy tests (§4, p. 15 of 403; §3, p. 14 of 403; Appendix 8: p. 318, Attachments 2-3). Records of tick viability and temperature/percent humidity checks for insectary-held ticks are provided in Appendix 8 (p. 318 of 403).

Subject Procedures Prior to Efficacy Testing

Tick Handling Training and Subject Attractiveness Screening: After consenting, subjects were screened for their attractiveness to ticks and trained in handling adult ticks at the CLBR laboratory facility (§2, p. 15 of 403). Ticks that were 3- to 5-week-old adults were used for this exercise (Attachment 2). The procedures outlined in the CLBR Training Manual §1.b. Handling ticks and observing their movement on the skin were followed (Attachment 2), in which subjects practiced transferring fresh ticks from labeled vials that were held in shallow pans of water onto the wrist line, qualifying 'active' ticks, guiding ticks to move up the forearm, noting the times when the tick passed the reference and forearm lines, discarding ticks into the appropriate vial, and scoring tick movements (Appendix 2, p. 188 of 403; Figure 1 above). These steps were practiced five times with each species to train subjects and assess subject attractiveness to ticks (Appendix 2, p. 188 0f 403). During training, it was noted that forceps were better than paintbrushes/tweezers for tick placement and removal, so procedures deviated from the CLBR training manual by not using tweezers and only using paintbrushes for re-orientating ticks (Attachment 3). All subjects demonstrated competence in handling ticks and passed the attractiveness screening by receiving at least 3 crossings out of 5 attempts for all tick species (§2, p. 13 of 403; Appendix 3, pp. 200-226 of 403, Attachment 2).

Forearm Measurements & Standard Dosage Determinations: For each subject, the surface area of the non-dominant forearm was estimated by the length of the non-dominant forearm (wrist to elbow crease) multiplied by the average circumference of the non-dominant forearm (Appendix 5, page 267 of 403). Subjects' average non-dominant forearm circumference was estimated by four measurements taken with a measuring tape on the upper forearm, lower forearm, and two points spaced equally in between (Appendix 5, page 267 of 403). Most subjects (23 out of 31) participated in efficacy tests of the same repellent product against mosquitoes (study called 'MIM-006[3]') 1-2 weeks prior to their first tick efficacy test day (Attachment 3). Therefore, forearm measurements from MIM-006[3] were used for subjects that consented to participation in both studies (Appendix 5, p. 235 of 403, Attachment 3). The product's specific gravity (0.8874 g/ml) was used to determine the volumetric dosage (milliliters of product) to be applied to each subject's non-dominant forearm on test days to achieve the standard dosage of 0.5 grams per 600 cm^2 of skin (Formula 1 below; Appendix 5, page 267 of 403; Table 1 below).

Formula 1.

$$Dosage\ (\frac{ml}{cm^2\ of\ skin}) = \left[\frac{0.5\ grams}{600\ cm^2\ of\ skin}\right] \times \left[\frac{1\ ml\ product}{0.8874\ grams}\right] = \mathbf{\frac{0.00094\ \ ml\ product}{cm^2\ of\ skin}}$$

Reminder Phone Call: One to two days preceding each test day, subjects were reminded by email and phone call to consider wearing light-colored clothing and to wear short-sleeved, sleeveless, or shirts with sleeves that could be rolled up and secured to the upper arm area (§6, p. 16 of 403; Attachment 2). During this call, a request was also made to confirm that subjects would refrain from smoking, consuming alcohol, or using perfumed products from 9:00 pm the evening before the testing (Attachment 2). Subjects were also reminded to avoid the use of repellents during the 48 hours prior to each test day (Attachment 3). Subject availability was confirmed, and logistical details for participating in efficacy test days were communicated to subjects (Attachment 2).

Test subject selection and randomization: Separate advertisements, disseminated through the same outlets, were used for study MIM-006 and MIM-007 for candidate recruitment (Attachment 3). Forty-four candidates were recruited for the study, including more females than males (Attachment 3). A total of 31 subjects consented, including 17 males and 14 females (§2, p. 13 of 403). Recruitment for this study occurred concurrently with recruitment for a study of this product against mosquitoes (MIM-006), and as a result, some individuals consented to participate in both studies. The same subject numbers were used for the 23 subjects that participated in both studies (MIM-006 and this study, MIM-007). The 9 subjects that only participated in this study (MIM-007) were assigned a unique number from a randomly generated list in Excel (Attachment 2). Subject numbers were entered into two separate gender-specific lists in Excel (Attachment 2). Alternating between these lists, the "RANDBETWEEN" and "CHOOSE" functions in excel were used to create two randomly generated lists of subject numbers for each gender (Attachment 2). Alternating between these randomized lists, CLBR staff contacted subjects until 25 subjects were obtained that initially indicated they were

[3] EPA. Myer and Fuentes. Science Review of Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes. September 22, 2022.

available for 3 days of testing against 3 tick species (Attachment 2). Eight of the 10 females that participated in MIM-006 and two additional females (subjects 41 and 171) were confident they were available to participate in testing with all three tick species (Attachment 3). Just prior to the first test day, two female subjects (subjects 12 & 18) indicated that they were uncertain of their availability for all three test days (Attachment 3). Participation of the same subjects for all species tests was prioritized over obtaining a balanced gender ratio, and timely collection of study data was prioritized over obtaining more reliable subjects (Attachment 3). These considerations, withdrawals, and the random selection of alternates resulted in an uneven gender ratio (male: female ratio of either 15:10 or 11:14) of subjects participating as treated subjects for testing each tick species (Attachments 2-3). Aside from the selectively-assigned female alternates, the gender-stratified randomization procedure described above was followed to the extent possible during the role assignment process, then subject assignment as treated subjects was completed with males (Attachment 3). Initial role assignments included 4 female and 2 male alternates, 15 male treated subjects, and 10 female treated subjects (Attachment 3). Additional details regarding subject selection, role assignment, sex, age, and participation in MIM-006 can be found in Annex VI-VII of Attachment 3.

Subject Procedures during Efficacy Test Days

Reminders during Test Day: The Study Director reminded subjects that they were free to withdraw from the study at any time, privately and without penalty (§6, p. 16 of 403). Subjects were reminded of the exclusion criteria applied to the 48-hour period before the morning of each test day, which included smoking, consuming alcohol, use of performed products after 9:00 pm the night before the test, and the use of repellents 48 hours preceding the test (§6, p. 16 of 403; Attachments 2-3). Before product applications and periodically throughout the test day, subjects were reminded to ensure that their treated forearms did not contact any surfaces during testing (Attachment 2).

Repellent Applications, Orientation Lines, & Dismissal of Alternates: Subjects washed both hands and forearms with a fragrance-free liquid non-soap cleaner, rinsed with clean water, sprayed their forearms with diluted ethanol, and towel dried their skin prior to product applications (§6, pp. 16-17 of 403, Attachment 2). Each subject's pre-determined volumetric dosage was dispensed onto the non-dominant forearm using a syringe and rubbed evenly across the skin with a finger covered with a new, pre-weighed cot (§6, p. 17 of 403, Table 1 below). Two or three gloved researchers applied the product onto subjects at approximately the same time (§6, p. 17 of 403; Attachment 2), with subject application times differing by ≤ 15 minutes (Appendix 7, pp. 301, 308, 312 of 403). An additional researcher verified dosage just prior to applications and recorded application times (§6, p. 17 of 403). The difference in finger cot weight before and after applications was used to quantify the weight of material lost from each application event (§6, p. 17 of 403; Table 2 below). Using these weight loss measurements, the mean actual (realized) dose applied was ~0.4 g/600 cm^2 for each set of species-specific tests (§6, p. 17 of 403; Table 1 below). After product application, subject forearms were marked with 'orientation lines' as shown in Figure 1 above (§6, p. 17 of 403). Alternates not used for substitutions were dismissed after repellent applications and after exposure periods began (§6, p. 16 of 403). No subjects withdrew or were replaced after alternates were dismissed (Attachment 2), except one subject that withdrew but was not replaced after the 45^{th} exposure period (714

minutes total test time before withdrawal) on an *I. scapularis* test day (§7, p. 19 of 403; Appendix 7, p. 308 of 403). October 13th and October 20th were 'minimal subject' test days (§9, p. 24 of 403). 'Minimal subject' test days were days scheduled for the 5 or 6 subjects that were unable to attend later *A. americanum* and *I. scapularis* test days scheduled with the larger group of subjects (Attachment 2). No alternates were physically present or assigned to 'minimal subject' test days (Attachment 3), but it was the Study Director's intention that a subject withdrawing from these days could be effectively replaced by a randomly-chosen alternate subject on the following test day using the same species (Attachment 2).

Exposure Periods: *R. sanguineus* tests occurred on October 10th, 2021; *A. americanum* tests occurred on October 13th and 17th, 2021 (Appendix 7, p. 301 of 403), and *I. scapularis* efficacy tests occurred on October 20th and 24th, 2021 (Appendix 7, p. 308 of 403). On test days, ticks were transferred from the insectary-stored canisters into 20 ml scintillation vials (~ 5 ticks per vial) and quickly transported to the exposure study room for use (Attachment 3). Exposures were performed at tabletops, and each subject station was supplied with a holding vial of unused ticks, paintbrushes, forceps, a vial with 70% ethanol for used/discarded ticks, and a discard jar for used forceps/paintbrushes (Attachment 3). Each holding vial was sealed with a partially screened cap that allowed airflow but prevented prevent tick escape (Attachment 3).

Product applications were made ~1 hour prior to the beginning of the first exposure period on each test day (Appendix 7, pp. 301, 308, 312, of 403). Repellent efficacy was tested using 15-minute exposure periods (Appendix 7, pp. 301, 308, 312, of 403), with exposure times measured using a digital chronometer displayed in the room (Attachment 2). During each exposure period, the start of the observation was announced and each subject used forceps to transfer a tick from the holding vial to the area just below the palm line of their untreated forearm (§6, pp. 17-18 of 403, §9, p. 23 of 403, Attachment 3). Subjects placed their hand of the untreated arm on the flat surface of the table, with the elbow above the wrist and forearm held at an angle of 30º to the table (Attachment 3). If the tick did not initiate and continue movement from the palm towards the elbow, either forceps or a paintbrush was used to gently reposition the ticks or prod the tick into this upward motion (Attachment 3). Ticks were considered actively questing when they moved 3-cm from the reference line to the forearm line within 3 minutes (§6, pp. 17-18 of 403, Figure 1). Any ticks that did not pass this criterion were removed from the arm and placed in the 'used' tick vial (Attachment 2-3). Fresh ticks were used to replace any non-active ticks within the same exposure period to confirm that only active ticks were tested during all exposure periods (§6, p. 18 of 403).

After confirming tick activity on the untreated forearm, the tick was placed just below the palm line of the treated forearm using a pair of forceps and subjects oriented their forearm at a 30º angle as described above (§6, pp. 18 of 403; §9, p. 23 of 403, Attachment 3). If the tick did not initiate and continue movement from the palm towards the elbow, a paintbrush or pair of forceps was used to gently reposition the ticks or prod the tick into this upward motion. A repulsion (non-crossing) was scored when an active tick moved away from or parallel to the treated forearm area or did not travel more than 3 cm past the reference line within 3 minutes (Figure 1 below; Attachment 2-3). In this study, a crossing was scored when a tick traveled more than 3-cm past the reference line and reached the forearm line (with any portion of its body overlapping the forearm line) within 3 minutes, meaning a crossing was scored when a tick crossed 3-cm into

the treated area within 3 minutes (Figure 1, Attachment 2). Each subject informed CLBR staff of the observed scoring, used forceps to transfer the tick from their arm to the 'used' tick vial, and placed forceps into the discard jar before the end of the exposure period was announced (Attachment 3). CLBR staff replenished fresh tick vials as needed and replaced used forceps/paintbrushes with clean forceps/paintbrushes at each subject station (Attachment 3).

Exposure periods were repeated with 1 active tick at 15-minute intervals until FCC or until the end of testing (Appendix 7, pp. 301, 308, 312, of 403). A First Confirmed Crossing (FCC) was defined as a crossing followed by another crossing within 30 minutes (Appendix 1, p. 33 of 403). For subjects that experienced FCC, CPT was determined as the elapsed time between product application and FCC (§7, p. 19 of 403). At the end of each test day, all used ticks were pooled into kill jars containing 70% ethanol, and these jars were emptied into self-sealing bags that were placed in a freezer for storage (Attachment 3). Unused ticks that did not contact subjects or the product were returned to holding jars in the insectary (Attachment 3). Paintbrushes were only used on untreated skin during exposures and were cleaned with 70% ethanol and rinsed with water at the end of each test day (Attachment 3). Used forceps were cleaned with detergent, water, and 70% ethanol, then air-dried before re-use (Attachment 3).

For right-censored subject data, CPT was determined as the time elapsed between product application until the beginning of each subject's final exposure period (§7, p. 19 of 403). Right-censored datapoints included subject 147 that withdrew at the 45th exposure period during *I. scapularis* testing (§7, p. 19 of 403; Appendix 7, p. 308 of 403); subjects 12, 62, and 167 that did not experience FCC during *I. scapularis* testing (Appendix 7, p. 308 of 403); and subject 33 that did not experience FCC during *A. americanum* testing (Appendix 7, p. 301 of 403).

Statistical Analysis and Sample Size Determination:

Sample Size Determination: The sample size determination of 25 subjects per treatment was based on the EPA power analysis calculations in Attachment 3: EPA's Power vs. Sample Size Calculation for Tick Repellency Studies, within EPA's Science and Ethics Review Memo, dated March 25th, 2021, for review of the study protocol dated December 23rd, 2020.

Median Complete Protection time (mCPT): mCPT was estimated using Kaplan-Meier Survival Analyses for each set of species-specific CPT data. The lower 95% confidence intervals (CI) and Upper 95% CI were calculated for each species using *log-log* transformed data (Attachment 2). See Attachment 4 for EPA's Statistical Analysis Report for this completed study.

Protocol Deviations and Amendments:

Deviations:

Protocol deviations that were reported are listed in the study report MRID 517706-01, summarized in Section A below (§9, pp. 23-26 of 403). Additional deviations that were not reported in MRID 517706-01 are discussed under Section B below. Based on the Advarra decision-making pathways, all study deviations were not reported to the IRB (Annex III of Attachment 3). However, EPA notes that all deviations to the protocol

should be included in the study report, regardless of the IRB's requirements for reporting deviations.

A. Reported Protocol Deviations (§9, pp. 23-26 of 403)

- Reported Deviation #1:
  Two fewer subjects (31 subjects out of the proposed 33) were consented due to candidate availability, and 6 alternates were selected out of the subject pool rather than the 8 proposed in the study protocol. The Study Director deemed that these numbers would sufficiently provide 25 treated subjects for all test days and cover withdrawals. Therefore, this deviation was not expected to compromise the validity of the data or deter subjects from withdrawing.

- Reported Deviation #2:
  More male subjects were enrolled than female subjects (17 male, and 14 female). The Study Director deemed there was no effect on data quality since gender was not required or a variable in the statistical analysis. This is not a scientifically sound argument, but an unequal enrollment does not necessarily compromise the validity of the study.

- Reported Deviation #3:
  Rather than using paintbrushes as proposed, forceps were mostly used to handle all 3 species of ticks throughout efficacy testing procedures. Paintbrushes were used only for prompting tick movement or to assist in orienting tick direction but were not used for tick placement or removal. This deviation allowed adult, hard ticks to be handled in a more controlled manner and was not expected to compromise the validity of the study.

- Reported Deviation #4:
  In two separate test days, the relative humidity in the exposure room was temporarily higher (58%) or lower (37%) than the range of 40-55% relative humidity proposed in the protocol. However, 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.

- Reported Deviation #5:
  The protocol language indicated that all ticks would be sourced from one place, but the report (Appendix 8, p. 325 of 403) stated that "*it was our intention to communicate that more than one source may be used*." Two sources, OSU and BerTek, Inc., were used to obtain ticks. 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.

- Reported Deviation # 6:
  Due to subject availability problems, test days occurred by more than a week past the estimated times and resulted in ticks being older than the target age of 2-week-old adults proposed in the protocol. The age of ticks used for test days ranged from 2.5-

week-old adults to 4.5-week-old adults (§4, p. 15 of 403). 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.

- Reported Deviation # 7:
  Efficacy testing occurred over 5 days, instead of the 3 test days (one for each tick species) proposed in the protocol. Due to subject scheduling problems, *R. sanguineus* was tested over one test day, whereas *A. americanum* and *I. scapularis* were each tested over two test days. Minimal subjects test days for these two species were scheduled to accommodate subjects that were unable to attend later test days scheduled with the larger group of subjects. Environmental conditions were similar between test days in this laboratory study, so this deviation was not expected to impact study quality. Additionally, alternates were not physically present at the test site on 'minimal subjects' test days and did not actively participate on these days in anyway (Attachment 3). The Study Director deemed that subjects that withdrew on one of these days could be replaced by alternates on the following test day when the same species would be tested with the larger subject group (Attachment 2). Therefore, these procedures for potential substitutions on 'minimal subjects' test days were not expected to deter subjects from withdrawing (Attachment 2).

- Reported Deviation # 8:
  The time between applications varied between subjects more than was expected, with lag times between application and first exposure that ranged from ~45 – 60 minutes (Appendix 7, pp. 301, 308, 312, of 403). This is only a 15-minute difference in application timing, and application times were recorded for each subject and used to calculate CPT. Therefore, this deviation was not expected to compromise the validity of this study.

- Reported Deviation # 9:
  Pre-weighed finger cots were used to apply the repellent instead of the pre-weighed gloves proposed in the protocol. The mass of product loss represents a greater proportion of the mass of a finger cot compared to the mass of a glove, which likely provides more accurate estimates of product loss. The deviation was not expected to compromise the validity of the study.

- Reported Deviation # 10: Crossing were scored when ticks traveled 3 cm or more into the treated area without the stipulation (proposed in the protocol) that ticks remain in the treated area for at least 1-minute. The Study Director deemed that this deviation helped prevent ticks from escaping. The Agency notes that scoring criteria used was more conservative without the 1-minute stipulation, and thus, it was not expected to compromise the validity of the study.

- Reported Deviation #11: Some subjects requested that a single payment be made at their last day of participation, instead of multiple payments specified in the protocol. The deviation was not expected to compromise the validity of the study.

B. Unreported Protocol Deviations:

- The protocol (Appendix 1, p. of 403) proposed to recruit a minimum of 22 male and 22 female candidates, and that the 25 treated subjects would include 12 subjects of one gender, and 13 of the other gender. More than 22 out of the 44 recruited subjects were female (Attachment 3), so fewer than 22 males were recruited for the study. 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 (Attachment 3). There is a lack of evidence in the literature indicating gender as a consistent predictor of host attractiveness to ticks. Furthermore, CLBR informally assessed the effect of gender on CPT, time to crossing, and the mean crossing time per subject, and found no clear indication of subject gender influencing these repellency outcomes (Figure 1 of Attachment 3). Therefore, this deviation was not expected to compromise the validity of this study.

- The protocol proposed to use a *log-log* transformation in the Kaplan-Meier survival analyses to calculate 95% CI of the estimated mCPT values. The original report (MRID 517706-01) used a *log* transformation, but CLBR re-ran the with a *log-log* transformation in response to EPA statistical recommendations (Attachment 2, 4). The updated values are shown in the results section below (Table 3).

C. Guideline Deviations

- OPPTS 870.3700 recommends using a 16:9 (light: dark) photoperiod for colony maintenance conditions, but the study used a 12:12 (light: dark) photoperiod (§4, p.15 of 403). The study was conducted according to the protocol, which specified that the ticks would be maintained under environmental conditions recommended by the rearing facility at time of shipment (Attachment 3). All ticks used were qualified to be sufficiently active during all exposure periods (Attachment 3). Thus, this minor guideline deviation was not expected to compromise the validity of the study.

Amendments:
Following the HSRB meeting on April 21st, 2021, two amendments were made to the protocol prior to the start of the study (Appendix 1, pp. 148-168 of 403). Amendments made in response to the EPA Science Review and the HSRB recommendations are detailed in the registrant responsiveness tables (Attachment 5). Additional amendments relating to the scientific aspects of study conduct are listed below by protocol section(s).

- Sections 1.1 and 4.7.6: To clarify how data will still be handled when stop rules are invoked. The procedures for handling data of subjects that completed a full test day of exposures or were excluded from testing were appropriate. However, the Agency notes that withdrawn/removed subjects should not be both replaced by an alternate *and* have their data retained for analysis. See additional details provided in

Attachment 1 (Responsiveness to EPA Comments 3, 5, 6).

Additionally, the word 'mosquitoes' should be replaced with 'ticks' in this section.

- Sections 1.3.2 & 4.7.6: To change the attractiveness threshold of subjects during tick handling training, so that a subject would be asked to withdraw if **≥ 3 ticks out of 5 ticks of any species** fails to cross on the forearm. All subjects were found to be adequately attractive to all tick species during subject training (Appendix 3, pp. 200-226 of 403, Attachment 2).

- Section 1.3.5: To include procedural details associated with COVID-19 related risk. Any staff and subjects who are experiencing any of the listed symptoms were to be excluded from participating on that test day and any subsequent test days associated with the contagion period. Face masks, gloves, skin sanitizer, and surface sanitizers were made available for use by the staff and subjects. However, it was clarified that sanitizers and gloves were not used by subjects during exposure periods (Attachment 2). Therefore, gloves did not affect subject repellent applications, nor did alcohol-based sanitizer confound product repellency data.

- Section 3.1: To include a justification as to why recruitment procedures were not guaranteed to include a diverse demographic representation by age, race/ethnicity, and gender and why statistical analyses did not evaluate efficacy by these demographic factors. The Agency notes that the opinionated statements regarding host preference by demographic factors, references to mosquito literature, and the discussion on mosquito host preferences as an analogy for tick host preferences are inappropriate and/or superfluous to the study protocol and should be removed. Following the statement on how these demographic factors were recorded for study completeness, a simple sentence regarding how there are no consistent human demographic predictors for tick host preference would suffice as a justification. Likewise, the statement regarding conclusions drawn from the scientific literature (last sentence in section 3.1) should be removed.

- Section 3.4: To add questions to the bulleted list asked during the consent process to better verify candidate understanding of study participation details outlined in the informed consent form (ICF).

- Section 4.6: To provide clarification on how the margin of exposure (MOE) was calculated for human exposure to the product. MOE was calculated to equal 52 by extrapolating dermal loadings from the NOAEL of the 21-day rabbit dermal irritation study. Justification was also provided as to why the uncertainty factors (UFs) were lowered, resulting in a level of concern (LOC) of 10. Comparing the LOC to MOE indicates there is minimal risks of concern to the participants of this study. In the 7th paragraph of Section 4.6, the statements regarding EPA's *Guidance for Applying Quantitative Data to Develop Data Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* are superfluous and should be removed.

- Section 4.7: To provide clarification on procedures that account for product loss during each repellent application. Pre-and post-application weights of gloves used for application were to be recorded, but the study deviated from this proposal by using finger cots instead of gloves (Reported Deviation #9 in the section above).

- Section 4.8.3.1: To re-word repellency scoring criterion to state the final protocol excerpt italicized below (Appendix 1, p. 54-55 of 403):

  On the treated arm, a crossing is scored if a tick travels at least 3 cm in a vector toward the elbow into the treated area (i.e., at least as far as the forearm line) within 3 minutes of beginning to move up the arm from the palm line and remains within the treated skin area for at least one minute after crossing into that area. A repulsion is scored when a tick changes its orientation away from, or parallel to, the margin of the treated area upon approach, or does not travel more than 3 cm past the reference line toward the elbow within 3 minutes. A crossing (failure of repellency) will be scored if the tick travels more than 3 cm past the reference line, reaching the uppermost line, within 3 minutes, and remains within the treated skin area for at least one minute after crossing into that area.

  However, having two scoring descriptions for 'crossing' was confusing, and the study deviated from protocol by scoring crossings without the stipulation of ticks remaining in the treated area for at least one minute (§9, p. 25 of 403). The registrant later clarified the actual scoring criteria used in the study, shown in the italicized excerpt below (Attachment 2):

  Crossing and non-crossing (e.g., effective repulsion) were scored as follows:
  - *Crossing: Tick travels at least 3-cm toward the elbow into the treated area within 3 minutes of beginning to move up the arm from the palm line, meaning in practice that within three minutes the tick traveled more than 3 cm past the reference (wrist position) line, reaching the uppermost line, and has any portion of its body overlapping the uppermost line marking.*
  - *Non-crossing (effective repulsion): Tick changes orientation away from, or parallel to, the margin of the treated area, or does not travel more than 3 cm past the reference line toward the elbow within 3 minutes.*

  This clarification is acceptable.

- Protocol Appendices/Table of Contents: To exclude documents in the protocol appendices to reflect only those the Study Director deemed necessary for inclusion (Appendix 1, p. 149 of 403). These removals were not based on EPA or HSRB recommendations and are not appropriate. All appendix documents submitted to the Agency for protocol review should be included in the final submission of the study report.

RESULTS

Application of Standard Consumer Dose for Testing Efficacy

The dose applied to each treated subject to achieve the standard consumer dosage rate of 0.5 grams per 600 cm^2 of skin is shown below in **Table 1** (**Formula 1** above). On each test day, finger cots were used to apply the standard consumer dose, with each treated subject's prepared volume (ml) of product adjusted to surface area (SA) measurements of their non-dominant forearm (**Table 1** below). The actual dosage rate applied on species-specific test days were estimated by accounting for pre- and post-application finger cot weights (**Table 1** below).

Table 1. Dose Applied to Individual Subjects to Achieve Standard Consumer Dose

Subject No.	Gender	Forearm SA (cm^2)	Left/Right Forearm	Prepared Volume (ml)	Actual Dose Rate Applied (g/cm^2)		
					R. sanguineus	*A. americanum*	*I. scapularis*
4	M	533.0	L	**0.50**	0.425	0.403	0.404
6	F	487.5	L	**0.46**	0.386	0.385	0.386
11	F	548.2	L	**0.52**	0.426	0.421	0.417
12	F	558.1	L	**0.52**	-----	-----	0.429
18	F	587.8	L	**0.55**	-----	-----	-----
30	M	648.0	R	**0.61**	0.515	0.517	0.503
33	M	467.1	L	**0.44**	0.388	0.359	0.369
41	F	428.4	L	**0.40**	0.334	0.331	0.328
55	M	536.2	L	**0.50**	0.412	0.419	0.401
62	F	490.0	L	**0.46**	0.374	-----	0.356
63	M	588.0	L	**0.55**	0.411	0.462	0.430
66	F	459.0	R	**0.43**	0.364	0.365	0.359
73	M	516.3	L	**0.49**	0.405	0.394	0.409
74	M	649.0	L	**0.61**	0.541	0.511	0.505
76	F	457.1	L	**0.43**	0.305	0.351	0.346
103	F	484.3	L	**0.46**	-----	-----	-----
122	M	647.1	L	**0.61**	-----	-----	-----
129	M	501.2	L	**0.47**	0.408	0.379	0.395
131	F	387.8	L	**0.36**	0.308	0.303	0.275
132	F	527.4	L	**0.59**	-----	0.410	0.398
134	M	462.9	L	**0.44**	0.381	0.350	0.349
142	M	522.0	L	**0.49**	0.389	0.430	0.393
147	F	599.2	L	**0.56**	0.497	0.470	0.471
150	M	548.3	L	**0.52**	N/A	0.407	0.410
155	M	597.4	L	**0.56**	-----	-----	-----
163	F	531.3	L	**0.50**	0.424	0.406	-----
167	M	594.9	L	**0.56**	0.477	0.456	0.451
169	M	632.5	L	**0.59**	0.513	0.506	0.504
171	F	535.5	R	**0.50**	0.423	0.436	0.415
177	M	564.9	L	**0.53**	0.466	0.428	-----
178	M	671.3	L	**0.63**	0.529	0.533	0.525

Data from MRID 517706-01 (Appendix 5, pp. 267, 278-80). The dashed line (-----) indicates subjects that were not included in the 25 treated subject pool associated with each set of species-specific test day(s) (Appendix 7, pp. 301, 308, 312 of 403). 'N/A' indicates a data point that was excluded due to an error with finger cot weights (§6, p. 17 of 403).

Efficacy Testing for Calculation of mCPT

Recorded CPTs or right-censored data resulting from repellent efficacy tests against three tick species (*R. sanguineus*, *A. americanum*, *I. scapularis*) are shown below in **Table 2**. The product was tested with a sample size of 25 subjects using the standard consumer dose. All subjects during *R. sanguineus* test days provided confirmed crossings to calculate CPT, ranging from 79 to 525 minutes (~1 to 8 hours rounded to the lower whole hour). On *A. americanum* test days, 24 out of 25 subjects provided confirmed crossings, with CPT ranging from 176 to 554 minutes (~2 to 9 hours, rounded to the lower whole hour). On *I. scapularis* test days, 21 out of 25 subjects provided confirmed crossings, with CPT ranging from 216 to 685 minutes (~ 3 to 11 hours rounded to the lower whole hour).

Table 2. Recorded CPT or Censored data (CPT or C; respectively) by tick species

R. sanguineus				***A. americanum***				***I. scapularis***			
Date m/d/21	**Subject No.**	**Time (min)**	**CPT or C**	**Date m/d/21**	**Subject No.**	**Time (min)**	**CPT or C**	**Date m/d/21**	**Subject No.**	**Time (min)**	**CPT or C**
10/10	4	376	CPT	10/17	4	180	CPT	10/24	4	225	CPT
	6	135	CPT		6	201	CPT		11	602	CPT
	11	128	CPT		11	425	CPT		12	729	C
	30	336	CPT		30	304	CPT		30	376	CPT
	33	340	CPT		33	578	C		33	356	CPT
	41	79	CPT		41	227	CPT		41	428	CPT
	55	374	CPT		55	263	CPT		55	478	CPT
	62	263	CPT		63	287	CPT		62	723	C
	63	275	CPT		66	264	CPT		63	324	CPT
	66	388	CPT		73	176	CPT		74	589	CPT
	73	437	CPT		129	452	CPT		76	216	CPT
	74	102	CPT		131	496	CPT		131	276	CPT
	76	102	CPT		132	266	CPT		132	675	CPT
	129	278	CPT		134	234	CPT		134	326	CPT
	131	293	CPT		150	501	CPT		142	685	CPT
	134	357	CPT		163	270	CPT		167	724	C
	142	148	CPT		167	554	CPT		169	570	CPT
	147	426	CPT		169	358	CPT		171	591	CPT
	150	295	CPT		177	250	CPT		178	538	CPT
	163	103	CPT		178	232	CPT	10/20	6	681	CPT
	167	325	CPT	10/13	74	349	CPT		66	438	CPT
	169	525	CPT		76	385	CPT		73	491	CPT
	171	168	CPT		142	223	CPT		129	555	CPT
	177	123	CPT		147	481	CPT		147	714	C
	178	406	CPT		171	472	CPT		150	507	CPT

Data from MRID 517706-01 (Appendix 7, pp. 301, 308, 312 of 403). Subject 147 withdrew without a confirmed crossing 714 minutes following application on the *I. scapularis* test day (Table 5 of MRID 517706-01, p. 19 of 403). The other four cases of right-censored data were for subjects that did not experience a confirmed crossing before the end of exposure periods (§7, p. 19 of 403). 'Minimal subjects' test days included 10/13/21 and 10/20/21 (§9, p. 24 of 403).

Results from Statistical Analyses

All treated subject data were used in the Kaplan-Meier survival analyses used to estimate mCPT and 95% CI (§7, p. 19 of 403), summarized in **Table 3** below for each tick species. The resulting mCPT values, in increasing order, included: 287 minutes (~ 4 hours, rounded down) for *A. americanum*, 293 minutes (~ 4 hours, rounded down) for *R. sanguineus*, and 538 minutes (~ 9 hours) for *I. scapularis*.

Table 3. Summary of Kaplan-Meier survival analyses (Results by species)

Species	Time	Kaplan-Meier Survival Analyses			Precision K value (Lower 95%CI/mCPT)
		Est. Median CPT	95% Confidence Interval		
R. sanguineus	minutes (hours)	**293 (4.9)**	148 (2.5)	357 (6.0)	0.51
A. americanum	minutes (hours)	**287 (4. 8)**	250 (4.2)	425 (7.1)	0.87
I. scapularis	minutes (hours)	**538 (9.0)**	428 (7.1)	602 (10.0)	0.80

Estimates of mCPT from MRID 517706-01 (§7, p. 19 of 403). The 95% C.I. values, calculated with *log-log* transformed data, were obtained from the registrant response (dated May 20th, 2022) to the first 10-day deficiency letter with EPA Technical Screen Comments (Attachment 2). Precision *K* values were calculated by the Agency (see detailed statistics report in Attachment 4).

In response to EPA's comments regarding the variability observed in the *R. sanguineus* data (see low precision k-value in **Table 3** above), the registrant provided supplemental variance analyses to: (1) compare the variance observed in this study to an analogous tick study reviewed by the Agency; and (2) compare variance between the species tested in this study (Attachment 3). The Agency concluded that the analyses comparing the variance of CPT data between studies/species are not appropriate (i) due to the nature of censoring in the "survival type" data; and (ii) most importantly, because a single statistic (in this case, variance) cannot characterize the variability of the survival data (or CPT data in this case). In fact, the distribution of survival data (i.e., Kaplan-Meier survival curves) are more important for interpretation of survival data; see the Agency's statistics report for the Kaplan-Meier analyses in Attachment 4. Therefore, the Agency will not consider the registrant's supplemental variance analyses for product labeling.

Furthermore, EPA statisticians performed an additional analysis using the study data, which indicated the distribution of CPT data were significantly different between species (logrank test, p-value < 0.001; Figure 2 below). The distribution of CPT data also visually differs between tick species (Figure 2 below). Specifically, repellency failure occurred for 9 out of 25 subjects exposed to *R. sanguineus* at < 3 hours after product application, whereas repellency failure occurred for 2 or 0 subjects exposed to the other species ≤ 3 hours post-application (Appendix 7, pp. 301, 308, 312 of 403; Figure 2 below).

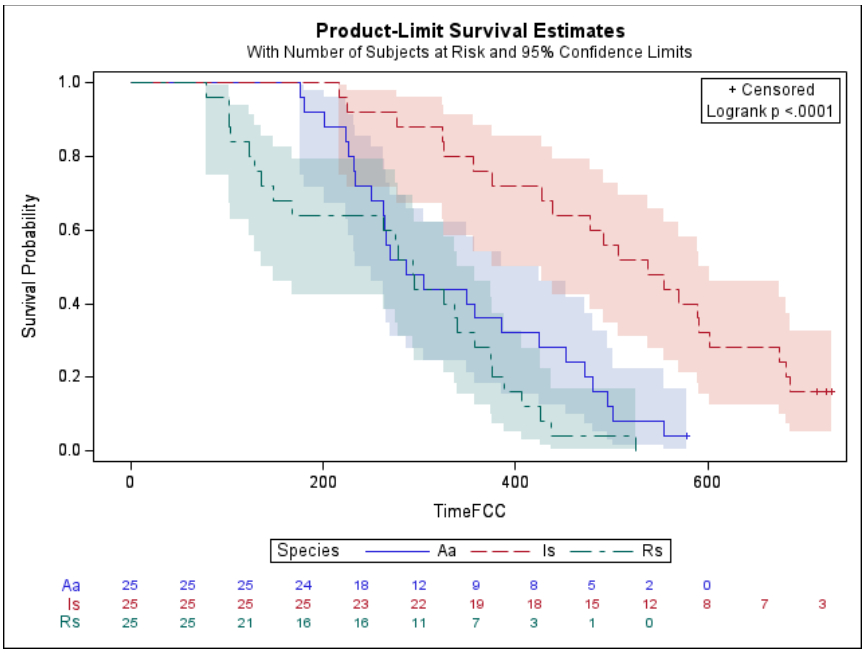

Figure 2. Statistical results of EPA's logrank test.

EPA's Discussion and Conclusions:

Among the 3 species tested, *A. americanum* provided the lowest mCPT of ~4 hours protection time. However, the low precision k-value indicates a high level of variability in the CPTs against *R. sanguineus* (Table 3 above). Nine out of twenty-five subjects provided CPTs lower than 3 hours, and out of these nine subjects, four provided CPTs lower than 2 hours (Appendix 7, p. 312 of 403). Upon review of the registrant responses (Attachments 2-3), the Agency acknowledges that wide variability can occur in real-world data. The Agency can only review the CPT data presented, which in this case, the Kaplan-Meier analysis indicates that the repellent product tested may fail to provide protection against *R. sanguineus* in 20 to 25% of the population after 2 hours (120 minutes), with a lower confidence bound of ~1 ½ hours (Figure 3 below; SAS output in the Appendix of Attachment 4).

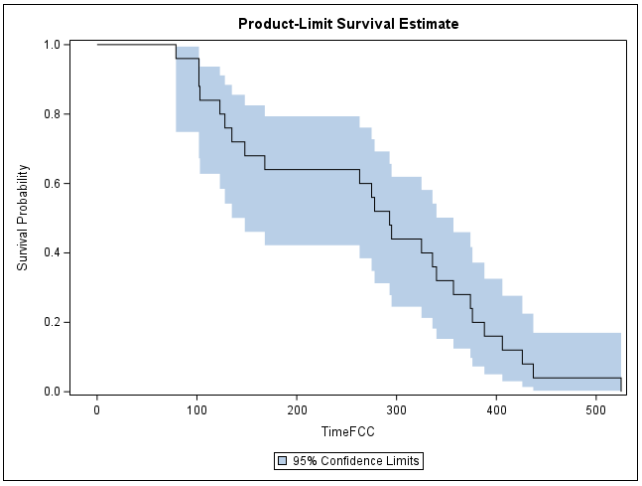

Figure 3 (left). EPA's Kaplan-Meier survival curve of the *Rhipicephalus sanguineus* CPT data (Figure 3 in Attachment 4).

Nevertheless, the current policy (based on the EPA Repellency Awareness Guidance[4]) is to use the most conservative mCPT of the 3 species in determining protection times for labeling purposes. Therefore, the product has been tested on 3 species of ticks and the study results support a claim for "up to 4 hours of tick protection" on the product label.

For future submissions of efficacy data, study reports should describe, in detail, the procedures used to perform product performance testing from subject recruitment through data analysis to avoid delays in the Agency's review process.

Conformity with Protocol and Amendments: The protocol was reviewed by EPA and the HSRB. The protocol was revised to adequately address recommendations from both organizations (see Attachment 5) and the protocol used to conduct the study was approved by the Advarra Institutional Review Board on September 13th, 2021. Overall, the protocol was amended 3 times, on December 23rd, 2020; August 25th, 2021; and September 5th, 2021.

The reported study conformed with the protocol as follows:

- Repellent efficacy tests were used to calculate mCPT on 3 species of ticks, *I. scapularis; A. americanum;* and *R. sanguineus* with a sample size of 25 subjects.
- Application of the standard consumer dose to a sample size of 25 subjects for testing repellency under laboratory conditions.

Conclusion

The methods used in this study are based on the protocol reviewed by the EPA and HSRB, as amended to adequately incorporate EPA and HSRB recommendations before testing began. Study results are acceptable to support a CPT of 4 hours against ticks for the proposed product, either in pump spray or pressurized (bag-on-valve) packaging, containing 11% w/w of the active ingredient OLE (CAS No. 245629-80-4) and 7.75% w/w of the active ingredient MNK (CAS No. 112-12-9). The study report supports the label claim that the product "repels ticks for up to 4 hours," based on the *A. americanum* data that provided the lowest CPT, following standard policy in the EPA Repellency Awareness Guidance[5] for determination of CPT used on the product label.

cc: Michelle Arling

REFERENCES

Attachment 1: Study report (MRID 517706-01), which includes 14 appendices:
App. 1 Amended Study Protocol
App. 2 Subject Facing Documents used in the study
App. 3 Research Subject Tracking Forms

[4] Repellency Awareness Guidance: For Skin-Applied Insect Repellent Producers. https://www.regulations.gov/document/EPA-HQ-OPP-2013-0406-0003
[5] *Id.*

App. 4 COVID-19 screening records
App. 5 Repellent Applications Information
App. 6 Test Material Identity and Storage Conditions
App. 7 Crossings Data
App. 8 Tick colony information
App. 9 Site characteristics* (*Laboratory ambient conditions)
App. 10 Output of R-Suite Analysis
App. 11 Study Specific Facility Records
App. 12 Physical Plan of Laboratory
App. 13 Researchers PHRP training certificates
App. 14 California EPA, IRB final permissions & EPA/HSRB review

Attachment 2: Registrant response to the first 10-day deficiency letter with EPA technical screen comments (dated May 6th, 2022)

Attachment 3: Registrant response to the second 10-day deficiency letter (dated June 17th, 2022)

Attachment 4: EPA's statistical analysis report (Kaplan-Meier survival analyses; dated September 27th, 2022)

Attachment 5: Responsiveness to EPA and HSRB science comments to the study protocol

Attachment 5
Responsiveness to EPA and HSRB Science Comments

Table 1. Responsiveness to EPA Science Review (Protocol review dated March 25, 2021)

	EPA Recommendations	Action Taken by Study Sponsor
1.	One statement reads, "one tick species will be used on each test day for a total of three test days" (§4.7, pg. 26), which is inconsistent with the statement, "multiple species are being tested on a single day" (4.8.3; pg. 29). This inconsistency in the protocol should be revised.	**Section 4.8.3 of the protocol (Appendix 1, p. 53 of 403) was revised to clarify that each tick species will be tested on separate days.** No more than one species of tick was tested on the same subject on the same day. *Rhipicephalus* ticks were tested on a single day by all subjects. The repellent was tested on subjects against *Amblyomma* and *Ixodes* ticks over two days for each species (pg. 16 of 403). Five subjects tested *Amblyomma* and six tested *Ixodes* during the respective 'minimal subjects' test days (Protocol deviation # 7 on pg. 24 of 403). This protocol revision is acceptable.
2.	The list of proposed tick species used for testing should include *Ixodes scapularis*, *Amblyomma americanum*, and either *Dermacentor variabilis*, or *Dermacentor andersoni*, or *Rhipicephalus sanguineus*.	**Sections 1.1 (Appendix 1, p. 33 of 403) and 4.7.2 of the amended protocol (Appendix 1, p. 51 of 403) addressed this comment.** The study report included *Ixodes scapularis*, *Amblyomma americanum*, and *Rhipicephalus sanguineus* as the test species. It was also noted that each subject will test one species per study day and that all subjects would test the same tick species on the same day. This protocol revision is acceptable.

	EPA Recommendations	Action Taken by Study Sponsor
3.	Explain how data from subjects that withdraw (but are not replaced) before completing a day of testing will be treated for statistical analysis.	**Section 4.7.6 of the amended protocol (Appendix 1, p. 52 of 403) states that the data of these subjects will be retained and treated as right censored, and they will be replaced with an alternate subject on that day.** This replacement clause in this protocol statement is inappropriate, and future protocols should specify the time-based criteria (how far into exposure periods the withdrawal/removal occurred) for whether the subject is not replaced with an alternate (data is retained as right-censored values) *or* an alternate is used to replace the subject (data from alternate replaces original subject data). However, the study report (pp. 13-14 of 403) noted, "One subject withdrew from testing prior to completion of exposures and receiving a confirmed crossing for one of the tick species used in the study. While testing the repellent against *Ixodes scapularis* on 20th October 2021, subject number 147 withdrew after completing the 45th interval. Because remaining subjects continued with testing for only 5 more intervals before the Study Director stopped exposures for the day, subject 147's data was retained and used in efficacy data analysis." The data for subject 147 was treated in a manner that is acceptable.
4.	Verify that the same 25 subjects will test all three tick species on multiple days of testing.	**Section 4.7 of the amended protocol (Appendix 1, p. 50 of 403) states that subjects that can participate on all three test days.** The study's *Subject Participation Chart* also shows that the same 25 subjects were originally assigned as treated subjects (Attachment 3). This response is acceptable.
5.	Specify if a subject will be stopped from testing completely or stopped from testing with a specific species when one qualifying tick fails to cross on 5 exposures during tick screening on the untreated arm.	**Section 4.7.6 of the amended protocol (Appendix 1, p. 52 of 403) clarifies that "If 3 exposure periods pass during which ticks of a given species fail to cross on the untreated arm of a subject, the subject will be removed from testing that species and replaced with an alternate."** This protocol revision is more conservative than the recommendation based on 5 exposures, and is thus, is acceptable. This stop rule was not invoked on any test days since "all subjects tested qualifying ticks during all exposure periods" (p. 18 of 403).

	EPA Recommendations	Action Taken by Study Sponsor
6.	Establish a stop rule that a subject will not be used for testing when they fail the screen on two species of ticks.	**Section 4.7.6 of the amended protocol (Appendix 1, p. 52 of 403) states that "A subject that is removed from testing against one species of tick will proceed to testing another species on a subsequent day. A subject that is removed from testing against a second species of tick will be excluded from the study."** This is acceptable, and this stop rule was not invoked on any test days since "all subjects tested qualifying ticks during all exposure periods" (p. 18 of 403).

Table 2. Responsiveness to Human Study Review Board (HSRB) Protocol Comments

	HSRB Recommendations	Action Taken by Study Sponsor
1.	Clarification should be provided on how tick species will be allocated to subjects and test days.	This comment was in agreeance with Agency comments (see EPA comments #1 and #4 in Table 1 above).
2.	The protocol language occasionally refers to how ticks will only be used once on any subject. However, there is language referring to how a tick is placed or used on a subject "up to twice", given that ticks are first tested for questing and then used if questing is successful. This language should be clarified.	**Section 4.7.3 (Appendix 1, p. 51 of 403) states, "Each individual tick used will only be used during one exposure and on one subject". Section 4.8.3.2 (Appendix 1, p. 55 of 403) also clarifies that individual ticks were not re-used (used only once and on only one subject) during efficacy test days, tick handling training, and attractiveness assays.** Additionally, the registrant response to the first 10-day deficiency letter (Attachment 2) stated: "Each individual tick was used during one exposure, and only on one subject. The final report cannot indicate that each tick was 'used only once' because the tick was used first to confirm its own activity (and a subject's attractiveness to it) on a subject's untreated arm, then used again on the same subject in the same observational period (interval) by being placed by the subject on the untreated palm of the hand of his or her treated arm. This could be (mis)understood as being used 'twice.'" This explanation is acceptable.

	HSRB Recommendations	Action Taken by Study Sponsor
3.	Clarify and minimize the number of researchers who will be applying the test material.	**This comment was not addressed in the amended protocol.** However, the registrant response to the first 10-day deficiency letter (Attachment 2) stated, "On 10, 17, and 24 October 2021, three researchers applied repellent. On 13 October 2021, a single researcher applied repellent. On each of two days, 13 October and 20 October 2021, two researchers applied repellent. This was due to the test days on the 13th and 20th having considerably fewer participants (5 and 6 subjects) compared to the other three days (25, 20, and 19 subjects, respectively)." This response is acceptable.
4.	Indicate the amount of test material that is anticipated to be left on the glove and any differences in application versus when the material is sprayed on.	**Section 4.7 proposed to account for product loss due to application procedures, which does not directly address this comment.** However, the study report stated, "To determine the adjusted dose for each subject, i.e., target applied dose less the amount of repellent left behind on an applying researcher's glove (deviation detailed in §9 of this report), finger cots were pre-weighed, placed over the index finger of the applying researcher's already gloved hand, then weighed after application. Each finger cot was used once on one subject and was then returned to its labelled plastic bag. Cot bags were sealed to prevent cot contamination before use and evaporation of repellent after. Raw weight change data for the cots are presented in Appendix 5." The standard consumer dose needs to be measured (such as in a syringe) to be applied, and the methods used to account for product loss is acceptable. The standard dose was pre-determined based on past dosimetry studies and the lower actual dose that gets applied results in a more conservative estimate of efficacy, and thus, is acceptable.

	HSRB Recommendations	Action Taken by Study Sponsor
5.	The Kaplan-Meier method requires the survival time to be recorded precisely, so the time to an event or censorship (known as the "survival time") should be clearly defined and precisely measured.	**Section 4.9 was revised to clarify how repellent failure is scored based on confirmed crossing events, and how censored data will be used in the statistical analysis.** The first crossing would be considered the confirmed crossing if a second crossing occurred in the following exposure period or if the next period is skipped. If a crossing were to occur in the last exposure period, the event will be recorded as a treatment failure, regardless of a confirmatory crossing. Data from withdrawn/removed subjects who are not replaced will be treated as censored data. The mCPT would not be reported if ≥ 50% of subjects' data are right censored. **Additionally, Sections 1.1 and 4.7.6 were revised to clarify data handling procedures when different stop rules were invoked (also described in this review's *Amendments* section).** These changes are adequate.