May 20, 2022

Via E-Mail

Ms. Linda Hollis
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division (7511P)
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Re: Response to 90-day Preliminary Technical Screening Results of Efficacy Report, Mimikai, Inc. Study ID MIM-007, EPA File Symbol: 93616PA12, Action Case Number 00337930

Dear Ms. Hollis:

Bergeson & Campbell, P.C. (B&C®) is pleased to respond on behalf of Mimikai, Inc. (Mimikai) to the U.S. Environmental Protection Agency's (EPA) 90-day Technical Screening for Mimikai's "Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks under Laboratory Conditions," Study ID MIM-007, MRID No. 517706-01. EPA has concluded that the study report "has not passed the preliminary technical screening" and that additional data and/or information must be provided.

EPA requested various levels of explanations, discussions, and points of clarification for the science and ethical aspects of the study, most of which require amendment to the final report. Technical responses prepared by the testing facility, Carroll-Loye Biological Research (CLBR), are included in Appendix 1 of this letter. The following support documents, as referenced in the technical responses (Appendix 1) are provided as part of the CDX submission:

- Proposed Master Label for Lilly Pilly Repellent, dated January 2022, as provided in registration application 93616-R (document number 353257);
- Subject Participation Chart, unaudited draft (document number 364883); and
- Statistical R Suite analysis output, unaudited draft of amended report Appendix 10 (document number 364949).

For comments prompting explicit clarifications and/or corrections in the final report, language for a report amendment is provided in the enclosed technical responses (Appendix 1) and accompanying documentation, as applicable. Due to the extensive measures

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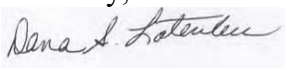
required to "re-open" a completed study to amend and audit a final report in accordance with Good Laboratory Practice Standards (40 C.F.R. Part 160), the amended final report for Study No. MIM-007 will be provided as a separate submission in the coming days. The forthcoming amended final report will replace the originally submitted final report (MRID No. 517706-01).

We look forward to your review. If there are any questions, please contact me at 202-557-3832 or dlateulere@lawbc.com.

Sincerely,

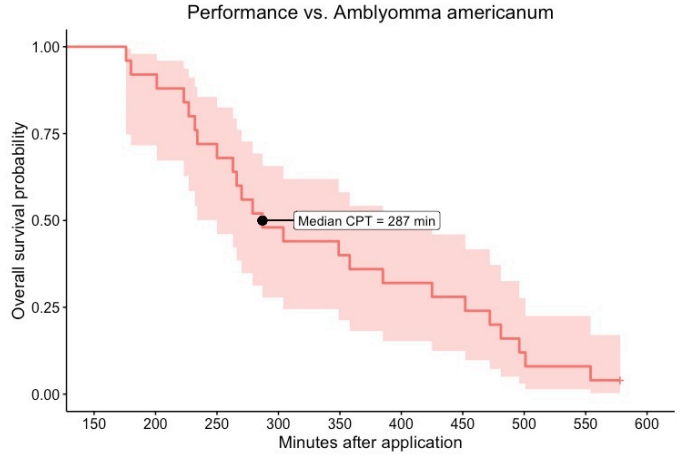
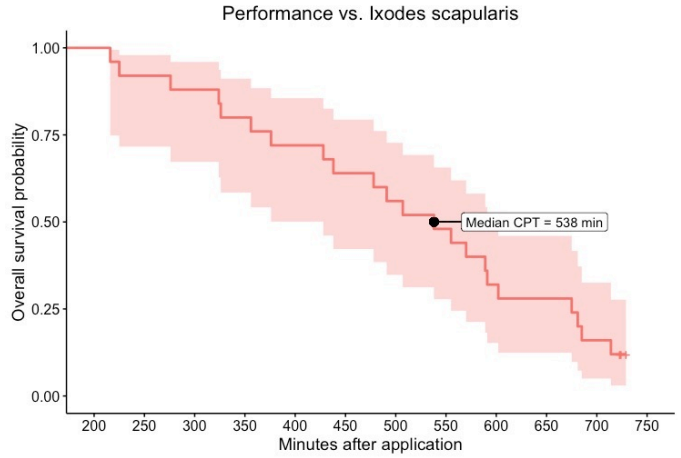


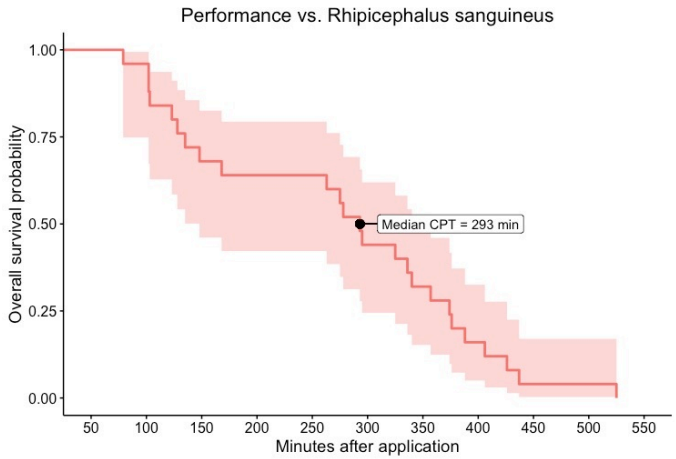
Dana S. Lateulere

Attachments

APPENDIX 1

EPA Comments [1]	***Registrant Responses***
1) You must provide the proposed label for use with the product whose efficacy results were reported in MRID 517706-01.	Please refer to the proposed master label dated January 14, 2022, submitted with registration application EPA No. 93616-R(see attached; document number 353257).
2) You must acceptably address the bulleted points below regarding the statistical analyses and variability: ● The default log transformation (default in the statistical program, R) was used calculate the 95% CI of the estimated median CPT. However, the protocol (Appendix 1, p. 56 of 403) proposed the use of a log-log transformation. This data transformation and the protocol deviation must be clearly stated in the study report. ● Kaplan-Meier survival analyses were performed by Agency statisticians using the log-log transformation to calculate the 95% CI of the estimated median CPT, the results of which are shown below (Table 1 below). The Agency's simulation results in the protocol show that for a sample size of 25, a study would have about 95% power of achieving a precision K > 0.6 if P5MR ≥ 0.3 or about 90% power of achieving a precision 0.7 if P5MR ≥ 0.4 (Appendix 1, pp. 102-105 of 403). A low precision k-value of 0.51 was calculated from the Agency's analysis of the Rhipicephalus sanguineus data, likely due to the variability of the reported CPTs ranging from 1 hour and 19 minutes to 8 hours and 45 minutes (Appendix 7, p. 312). Were there specific experimental factors that may explain the wide variability observed in the R. sanguineus CPT data? You must provide an acceptable explanation.	The report will be amended to include log-log transformed statistical analysis, as specified in the protocol. From the resulting analysis, median CPTs remain the same and confidence intervals change slightly relative to the confidence intervals resulting from the statistical analysis of the default log transformation, as follows: • *Amblyomma americanum*: amended 95% C.I. 250 - 425 • *Ixodes scapularis:* amended 95% C.I. 428 - 602 • *Rhipicephalus sanguineous:* amended 95% C.I. 148 - 357 The statistical analysis output, including the R Suite analysis output, coding and analyses of CPTs, will be included in the amended final report (see attached unaudited draft of amended Appendix 10; document number 364949). Following are the resulting plots from the statistical analysis of the log-log transformed data, which will be included in the amended final report (replacing original Figures 1, 2 and 3, respectively):

EPA Comments [1]	Registrant Responses
	 

EPA Comments [1]	Registrant Responses
	 The reason(s) for the wide variability in protection time outcomes in *Rhipicephalus* testing are unknown, but is attributed in part to the intrinsic variability underlying tick behavior. Among tick ethotypes, *Rhipicephalus* express both ambush ('sit-and-wait') and active-hunting tactics ('running'). Considering these behaviors in the context of the present study, running might be expected to hold the sensory receptors of the palps farther above the treated skin surface, and also potentially narrow elements of sensory perception and integration that might involve deliberations on relative attractancy and relative repellency. Lastly, while *Rhipicephalus* repellency failures began earlier than those of other two species tested, a small number of outcome changes with individual ticks would have been sufficient to make the distribution of failures more compact and more akin to those observed in *Amblyomma*, for example. CLBR does not see a basis for presuming that *Rhipicephalus* outcomes will inevitably be more variable than for other species.

<table>
<tr><th>EPA Comments [1]</th><th>Registrant Responses</th></tr>
<tr><td colspan="2">
Table 1. Agency analysis results & MRID 517706-01 (p. 19 of 403) analysis results
<table>
<tr><th rowspan="2">Species</th><th rowspan="2">Time</th><th colspan="3">EPA Analysis Results</th><th colspan="3">MRID 517706-01 Results</th><th rowspan="2">EPA Precision K value (Lower 95%CI/mCPT)</th></tr>
<tr><th>Est. Median CPT</th><th colspan="2">95% CI</th><th>Est. Median CPT</th><th colspan="2">95% CI</th></tr>
<tr><td>Amblyomma americanum</td><td>minutes (hours)</td><td>287 (4. 8)</td><td>250 (4.2)</td><td>425 (7.1)</td><td>287 (4.8)</td><td>263 (4.4)</td><td>452 (7.5)</td><td>0.87</td></tr>
<tr><td>Ixodes scapularis</td><td>minutes (hours)</td><td>538 (9.0)</td><td>428 (7.1)</td><td>602 (10.0)</td><td>538 (9.0)</td><td>438 (7.3)</td><td>675 (11.2)</td><td>0.80</td></tr>
<tr><td>Rhipicephalus sanguineus</td><td>minutes (hours)</td><td>293 (4.9)</td><td>148 (2.5)</td><td>357 (6.0)</td><td>293 (4.9)</td><td>168 (2.8)</td><td>374 (6.2)</td><td>0.51</td></tr>
</table>
</td></tr>
<tr><td>3) You must revise data entries for subjects with right-censored data as “N/A” under the column heading “Time of FCC” in the treated subject summary tables in Appendix 7 of study report. Time to First Confirmed Crossing (FCC) does not apply to right-censored subjects ending a test day without experiencing FCC.</td><td>The treated subject summary tables in Appendix 7 of the final report will be amended to more clearly denote data entries for subjects with right-censored data, in the column entitled “Time of FCC”, which will read as “NA” (and “NA” will be defined in an accompanying footnote).</td></tr>
<tr><td>4) You must adequately explain the hand-written notes regarding the following corrections in the raw data tables: 4 [sic]
• Corrections 2-4 under the Amblyomma americanum raw data table (p.298 of 403).
• Corrections 1-2 under the Ixodes scapularis raw data table (p.306 of 403).
• Corrections 2-3 under the Rhipicephalus sanguineus raw data table (p.309 of 403).</td><td>Following are the requested clarifications regarding the hand-written corrections in the raw data tables on page 298 of the original final report:
• Correction 2: The researcher recorded an incorrect value. In accordance with Good Laboratory Practice (GLP) Standards, this entry corrects a type of data recording errors in which a researcher records a value that is not the actual value, realizes contemporaneously that they have made an error in recording the datum, then corrects that value promptly, notating the type of correction, and initialing and dating the annotation. In this case, a notation of ‘EE’, defined in CLBR Standard Operating</td></tr>
</table>

EPA Comments [1]	Registrant Responses
	Procedure (SOP) as "entry error", is the appropriate record of the action without need of further elaboration. • Correction 3: The researcher was looking at the time recorded from the last interval (13:00) when writing the time, and accidentally wrote 13:0 when she meant to record 13:15. • Correction 4: Like correction 3, explained above, the researcher was looking at the previous time when recording the current time and made an error based on that previous cell. Following are the requested clarifications regarding the hand-written corrections in the raw data tables on page 306 of the original final report: • Correction 1: The researcher recorded an incorrect value, wrote another value over the top of that value, then formally corrected it using an Entry Error (EE) annotation. A Write-Over error (WO) annotation was added to indicate both incorrect entry and write-over of the original data entry. • Correction 2: The researcher recorded an incorrect value and corrected promptly the error. Following are the requested clarifications regarding the hand-written corrections in the raw data tables on page 309 of the original final report: • Correction 2: The researcher recorded an incorrect value, then formally corrected it with an Entry Error (EE) annotation. • Correction 3: Zeroes (0) were recorded hastily and could be interpreted as sixes (6) or other characters; the researcher struck them out and re-wrote them for clarity (a 'WO' or "write-over" error, per CLBR SOP). Further clarification by report amendment is not considered necessary.

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5) You must revise the statement, "Median CPTs were calculated as the time elapsed between the Test Material application and the beginning of the exposure period in which a confirmed crossing was recorded" (p. 19 of 403) to say: "CPT for each subject was determined as the time elapsed between Test Material application and the beginning of the exposure period in which a confirmed crossing was recorded." A separate statement on how the median TCPT was calculated may be added.	Section 7, Efficacy Measurement Results, of the final report will be amended to clarify how the median CPT was calculated, as follows (bolded, underlined text is added; bolded, struck through text is deleted): "The following text and figures provide statistical and visual details regarding the efficacy outcomes for the MIMIKAI Lilly Pilly against each of the three tick species tested. ~~**Median CPTs were calculated**~~ **The CPT for each subject was determined** as the time elapsed between Test Material application and the beginning of the exposure period in which a confirmed crossing was recorded. In right-censored cases, in which subjects did not experience a confirmed crossing before the Study Director ended exposures for a given Test Day (4 cases) or a subject withdrew during a Test Day (one case), CPT was calculated as the total time elapsed until the beginning of each subject's final exposure period. For all subjects participating in efficacy challenge test days, data was retained and used in the analysis of efficacy outcomes."
6) The protocol describes a candidate enrollment procedure that proposed to include 22 male and 22 female candidates, each of whom would be assigned a sequential number unique to them and this study (Appendix 1, p. 42 of 403). However, a note to file in Appendix 5 (p. 235 of 403) states that test subjects who participated in the mosquito study evaluating the same repellent product (MIM-006) also participated in the laboratory tick study (MIM-007). Based on study dates and subject numbers provided in the raw data appendices of both study reports, a total of 24 subjects used in the mosquito study were also enrolled for the tick study (as shown in Appendix A). Were the subjects that participated in both studies assigned the same numbers as the numbers used in the mosquito study? If not, what was the randomization process in the tick study for assigning subject numbers to candidates that participated in the mosquito study? You must address these questions.	Yes; the subjects that participated in both studies were assigned the same numbers as the numbers used in the mosquito study, with the exception of number '155' that was assigned to a unique individual who did not participate as subject number '155' in study MIM-006. See further response to EPA Comment #11 below. Subject numbers were randomly assigned to consenting individuals, or carried over from Study MIM-006 for subjects who had previously participated in MIM-006. Those who chose to participate as candidates for the MIM-007 study, which occurred after MIM-006, were not re-assigned random subject numbers. There was no justification to repeat the limb measuring procedures for already-measured subjects, whose limb dimensions would not have changed to any significant degree within the few weeks between studies. Re-measuring limbs would unnecessarily expose those subjects to the risks inherent in the measurement process. Since limb measurements are raw data for deriving repellent application dosages, proper reporting procedure was to provide authentic copies of the MIM-006 limb measurement sheets for the appropriate subjects. Those sheets contain the subject numbers assigned in MIM-006; those numbers were retained for subjects who

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	participated in MIM-006 and subsequently volunteered for MIM-007. The subjects who participated in both studies were consented and trained in accordance with each of the two protocols as independent consenting processes. Subjects that were trained and consented for only MIM-007 were randomly assigned subject numbers during consenting and training for MIM-007. Assignment of these numbers was constrained in that the already-assigned numbers from MIM-006 could not be assigned to subjects consenting to MIM-007 without having participated in MIM-006. Since the numeric values of the subject numbers imported from MIM-006 were already randomized, and assignment to treated or alternate status in study MIM-007 was further determined by generating a ranking set of random numbers in Excel, the net result of the entire process remained properly randomized. The study final report will be amended to provide clarifications to address the concerns raised.

7) You must confirm that the attractiveness criteria proposed in the study protocol (Appendix 1, p. 52 of 403), and shown below, were used for exclusion/removal of unattractive subjects from study participation. Furthermore, if these criteria were followed, they must be included in the section of the study report that stated no subjects were excluded/removed due to lack of attractiveness (§7, p. 18 of 403) • During subject training – during the test for subject attractiveness to ticks, if fewer than three of the five ticks of any tick species move up the subject's arm (see §4.8.3.1), the subject will be excluded from the study. • During repellency trial – If three 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. Subjects were removed if not attractive to two species.	The criteria were applied both in attractiveness screenings and during the repellency trials. No subjects exhibited lack of attractiveness at any point during subject training or during the repellency trial. All subjects for all exposure intervals across all three species qualified ticks on their untreated arms. Therefore no subjects were removed or excluded due to lack of attractiveness to ticks at any time during the study. The scenario of the first bullet did not arise, as all subjects completed and passed being screened for the criteria of at least 3 of 5 ticks of each species moving up the subjects arm The scenario of the second bullet point did not arise during the study for any subject on any test day with any of the three species. Section 2 subsection 'Training' will be amended as follows to clarify these points: *"Training* **All subject training for tick handling was completed within the 30 day period prior to the first efficacy challenge test day.** In accordance with protocol procedures, PHRP-trained staff members (see Appendix 13) screened consented subjects for attractiveness to ticks, and trained the subjects in handling adult ticks of each of the three species, in one of three temperature- and humidity-controlled rooms in the CLBR laboratory facility at 5100 Chiles Road, Davis, CA. **Adult ticks, aged between 3 and 5 weeks, post-eclosion were used in the attractiveness assay. In response to the execution of attractiveness screening procedures per the Study Protocol, in which at least three of five ticks of each species had to move up a subject's arm after placement on the palm of the hand, all subjects displayed sufficient attractiveness to ticks, as noted on the Research Candidate/Subject Checklists (Appendix 3). Similarly, sustained and sufficient tick attractiveness for each subject during each efficacy challenge test day is confirmed by the fact that study results show that all subjects for all exposures on all efficacy challenge test days qualified ticks on their untreated skin.** ~~**Tick attractiveness was demonstrated for a**~~**A**ll subjects ~~**and all subjects**~~ demonstrated sufficient competence in handling ticks. Subject training procedures, as detailed in the training document entitled '§1.b. Handling ticks and observing their movement on the skin (version date 23 Dec 2020)' (Appendix 2), were read by the researcher to the subject during the

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	training activity. This document also provides the procedures **that were** followed by that same researcher."
8) You must provide the following information regarding the tick attractiveness test: • Does the fraction reported for subject tick attractiveness assays (Appendix 3, pp. 200-226 of 403) refer to the number of successful ticks crossing out of 5 attempts? • Why was no crossings data recorded for subject 73 (Appendix 3, p. 209 of 403)? • What was the age of ticks used in the attractiveness testing (Appendix 3, pp. 200-226 of 403)?	• Yes; the fraction for subject tick attractiveness assays refers to the number of ticks crossing out of 5 individual attempts. • The data were inadvertently not recorded. The relevant subject tracking sheet (page 199 of the original final report) shows that the subject completed the tick attractiveness screening. Staff conducting the screenings concur that all subjects passed the screening. Subject 73 participated in efficacy challenge days for each of the three species of ticks used in the study and consistently had ticks qualifying on his untreated arm, indicating sufficient attractiveness was maintained throughout the study. • Ticks used for attractiveness testing were the same age as ticks used for testing, which were adults approximately between 3 and 5 weeks post-eclosion. All ticks used were between 3 and 5 weeks post-eclosion adults. No nymphal or other life stages were used. All references to ticks throughout the report are to adult ticks, as no other stage was present or used, per Study Protocol, and references to eclosion are to eclosion associated with the adult phase.

EPA Comments [1]	Registrant Responses
9) The protocol (Appendix 1, p. 42 of 403) states, "We will create a relatively even ratio of male to female subjects (meaning 12 of one and 13 of the other, randomly derived) by randomly choosing one of the two genders and then alternately and randomly choosing subjects from either gender until we have obtained a total of 25." The report also noted that 17 males and 14 females were enrolled as subjects" (p. 23 of 403). The Agency attempted to deduce gender ratios used for each test day and subject assignments (treated versus alternate) based on the following: subject numbers and gender provided in the repellent application sheets (Appendix 5, pp. 267- 272 of 403), subject numbers listed in the tick crossings data (Appendix 7, pp. 301, 308, 312 of 403), and the use of subject numbers 12 & 132 as alternates in the tick study report (p. 14 of 403. Subjects that participated in both tick and mosquito studies were deduced from limb measurement forms that note "MIM-006" in the upper left corner (Appendix 5, pp. 236-266 of 403). You must verify if the subject tracking details shown in the **Appendix A table (below)** is correct.	CLBR developed a *Subject Participation Chart* (see unaudited draft version attached; document number 364883) to summarize sex, age and roles by subject and test day, and gender ratios by test day. This table will be included in Section 2 subsection 'Participation' of the amended final report. The slightly uneven gender ratio was due to subject availability. This deviation from the intended gender ratios of recruited subjects for the entire study is reported in deviation #2 in Section 9, as well as the Note to File (dated 10 October 2021) in Appendix 3 of the final report. The newly developed *Subject Participation Chart* also clarifies subject participation in MIM-007 relative to prior participation in MIM-006, as applicable (see unaudited draft version attached; document number 364883). See also the detailed response to EPA Comment #11 below regarding the reference to subject #153 in the final report, which will be corrected in the raw data and amended final report. Note subject #18 is clearly identified as female and subject #155 clearly identified as male on the Research Candidate/Subject Checklist in Appendix 3 of the original final report.
10) Your study used 31 subjects in total. The subjects were split into two groups: one containing 25 subjects and the other containing 6 subjects. You must verify if the group containing 25 subjects were always assigned as treated? You must verify if the group containing 6 subjects were always assigned as alternates (as shown in the agency's **Appendix A below**)? This remains unclear.	The referenced groups of 25 and 6 are indeed representing treated and alternate subjects, respectively. In this study, all subjects had one treated forearm and each subject's untreated forearm was used to assess attractiveness and individual tick activity, so all 25 subjects in this group were treated with repellent. The 6 additional subjects were present as alternates, only two of which were treated with repellent and exposed to ticks (subject #12 and #132). These two alternates were at some point used to replace subjects who withdrew or were unable to participate on one or more study days. This will be clarified accordingly with the inclusion of the newly prepared a *Subject Participation Chart* (see unaudited draft version attached; document number 364883) in Section 2, subsection *Participation*, of the amended final report.
11) The protocol (Appendix 1, p. 42 of 403) described a gender-stratified randomization procedure to assign subjects as either treated or	• Subject numbers were entered into Microsoft Excel in two lists, one for male subjects and one for female subjects. Using the "RANDBETWEEN" and

EPA Comments [1]	Registrant Responses
alternate status to achieve a balanced gender ratio (12 of one gender, and 13 of the other). Out of the 31 subjects who consented, 17 were male and 14 were female (p.13 of 403). You must provide clarification for the bulleted points below regarding subject gender below. • Describe the randomization procedure used for assigning 31 subjects as treated versus alternate status. If a balanced gender ratio was not achieved, how did this procedure differ from the gender-stratified approach proposed in the protocol (Appendix 1, p. 42 of 403)? If the randomization procedure deviated from the protocol, then this should be clearly stated in the deviations table of the study report (pp. 23-25 of 403). • Specify the gender of alternate subjects 18 and 153 (Appendix 5, p.267 of 403). • The gender ratio used on each day of testing and the gender of each (and all) subjects, including alternates, should be reported. • If an unbalanced gender ratio was used on any test day, please explain the rationale for this unreported protocol deviation. The deviation and rationale should be clearly stated in the deviations table of the study report (pp. 23-25 of 403). • Explain what happened to subject 155. Limb measurements for subject 155 were provided in Appendix 5 (p.260 of 403), but this subject number does not appear anywhere else in the report. Was subject 153 on page 267 supposed to be listed as subject 155? If so, what was the gender of this subject? • Explain the meaning of the protocol proposal that "all subjects will be assigned to the treated group, and blocked by gender" (Appendix 1, p. 51 of 403). Additionally, specify whether/how this gender blocking was used in the tick study.	"CHOOSE" functions within Excel, subject numbers were chosen randomly, alternating by gender, creating two randomly generated lists of subject numbers, one for females and one for males. We then reached out to subjects, alternating between either list until we reached 25 subjects who were available for testing against all 3 species for a total of 3 study days. The stratification procedure proposed in the Study Protocol was followed to the extent possible in the assignment process, then assignment continued as described. Instead of 13 subjects of one sex and 12 of the other sex for the tests with each of the 3 species, we tested *Amblyomma americanum* and *Rhipicephalus sanguineus* with 15 male subjects and 10 female subjects, and *Ixodes scapularis* with 14 male subjects and 11 female subjects. The resulting gender ratio was more uneven than anticipated due to subject availability (only 10 female subjects were available to participate in testing with all three species, creating the need to complete recruitment with males). This deviation from the intended gender ratios of recruited subjects is addressed in deviation #2 (Section 9) and Appendix 3 (Note to File dated 10 October 2021) of the final report. ● Details on each subject's sex and patterns of participation, including gender ratios for each test day, will be further clarified in the amended final report (see attached unaudited draft *Subject Participation Chart*; document number 364883). ● The reported deviation #2 in Section 9 of the original final report, as well as the Note to File (dated 10 October 2021) provided in Appendix 3 of the original final report, address the deviation from the protocol-directed gender balance for the study as a whole, including any given efficacy challenge test day. The gender ratios on each test day are provided in the *Subject Participation Chart* that will be included in Section 2 of the amended final report (see unaudited draft version attached; document number 364883). ● Subject 18 was female; Subject 155 was male. All references to subject number '153' in MIM-007 are erroneous and will be corrected throughout the raw data and amended report to reference subject number 155, assigned to an individual male subject, who is the sole subject from whom all data ascribed in the records to '153' or '155' was derived. Note this individual is not the same individual assigned the number '155' in study MIM-006. This is recorded via the limb

EPA Comments [1]	Registrant Responses
	measurement forms, which can be compared between the two studies and show the measured forearm dimensions of two different individual persons. Instead of consistent and complete correction in study MIM-007 documents, the mis-assigned number was corrected only on the *Research Candidate/Subject Checklist* (page 101 of original final report). Subject #153, which will be corrected to subject #155, appears in the original final report on the repellent dosage calculation document (page 267 of the original final report) and on the finger cot weight document (page 273 of the original final report). • CLBR notes that the sex of subjects is listed by subject number on the raw data sheets entitled 'Research Candidate/Subject Checklist' found on pages 198 and 199 of the original Study Final Report and is reported as such. • The statement 'all subjects will be assigned to the treated group, and blocked by gender' means that, in this study, all subjects who are not alternate subjects are treated subjects. In the study design, each subject has one treated forearm and one untreated (control) forearm. Since every participating subject who exposed skin to ticks has one arm treated, all are considered treated and described as such. All subjects who were assigned to participate in exposures to ticks were assigned be treated subjects according to the study design. This point will be clarified in Section 2, subsection *Participation*, in the amended final report.
12) The report noted that deviations were evaluated using Advarra IRB's Investigator's Handbook to determine the need to report them to the IRB (p. 23 of 403). You must acceptably clarify this statement by answering the bulleted questions below. • What were the specific criteria used for evaluating the need to report protocol deviations? • Are all deviations from the protocol included in the study report? • Were there any deviations that were evaluated but not reported in Table 6 (p. 23 of 403)? If so, what are these deviations?	In subsequent discussions with the Study Director and research staff regarding the deviation from the one-minute residency time criterion for scoring, it became clear that, for biological reasons, staff concerns about tick mobility that motivated the choice to deviate aimed to address unexpected complications in tick handling and scoring of crossings more than a change in the risk profile of the study. Because ticks take multiple hours to begin the process of biting, the possibility of the ticks moving past a treated area of skin represented a problem for tick handling, and not a change in the risk profile of the study. Ticks that left the treated skin area by passing the elbow were no longer in the treated skin area by definition, and could therefore be removed in compliance with the protocol. There was no requirement to continue allowing the ticks to move upward on the arm during the one-minute residency measure. Subjects were trained and prepared to expect to be able to remove ticks from their own forearms. This is not possible if a tick

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	moves onto the back of a subject's elbow or forearm; such tick behavior is counter to the information provided to subjects in the informed consent form and during the consenting process. Finally and plainly, any tick that managed to cross the entire treated skin area into untreated skin higher up on the subject's arm had not been repelled, but would have to be scored as repelled to comply with the stipulation for a one-minute residency time in the treated skin area. Therefore, in actuality, the one-minute residency time did not provide useful confirmation of repellency failure, but only created problems for tick handling and, potentially, observational interval timing during the test.
13) It was reported that three subjects (62, 12, and 132) were unavailable to participate in one or more test days and were replaced with alternates (p.14 of 403). However, the bulleted points below must be adequately addressed to clarify how alternates were allocated amongst test days and the procedures used for replacing test subjects. • Provide information on which subjects showed up to each test day, subject assignments as treated or alternate subjects on each test day, and which subjects that showed up were dismissed or replaced (if any). This information should be clearly stated and included in the study report. • When did the 3 subjects replaced with alternates (p.14 of 403) provide notice that they were not able to participate? • Explain the procedure/criteria followed for the replacement of test subjects that were unable to participate on one or more test days. • Clarify the following statement in the protocol stop rules (Appendix 1, p. 52 of 403) that states: "If subject withdraws or is removed from testing before completing a test day, his or her data will be retained and treated as right-censored, and they will be replaced with an alternate subject on that day." The data of a replaced subject cannot be retained for statistical analysis, so	The scenario proposed by the stop rule mentioned in bullet #4 of EPA Comment #13 never arose during the study, and thus the stop rule was not invoked. Section 2, subsection *Participation*, in the final report states the following: "Three alternates were used in place of subjects unavailable to participate in one or more study days. Subject 132 replaced subject number 62 for *Amblyomma americanum* test days. Subjects 12 and 132 replaced subjects numbered 163 and 177 for *Ixodes scapularis* test days." The newly developed *Subject Participation Chart* clarifies how subjects and alternates were used and participated in efficacy challenge test days. This chart will be included in Section 2 of the amended final report (see unaudited draft version attached; document number 364883). Language in deviation #7 will be amended in the final report as follows to clarify how treated subjects were replaced by alternates, when required (added text is bolded, underlined; deleted text is bolded, struck through): "The Protocol prescribes three efficacy testing study days, one for each tick species. However, in response to scheduling constraints for study subjects, efficacy was instead tested on five total study days: one day for *Rhipicephalus* ticks and two days each for *Amblyomma* and *Ixodes* ticks. The first study day of each of the latter two species was a 'minimal subjects' test day, scheduled for

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this statement should be clarified and revised. Indicate if this stop rule was invoked during efficacy testing. • Specify the criteria used for replacing treated withdrawn subjects, versus the criteria used for treating the crossings data of withdrawn subjects as right censored. Were these criteria based on the length of time into testing when withdrawal occurred? • Describe the procedures for replacing treated subjects who wished to withdraw after alternates were dismissed near the beginning of each efficacy day (p. 16 of 403).	those unable to attend on a later test day scheduled with the larger group of remaining subjects. Five subjects tested *Amblyomma* and six tested *Ixodes* during the respective 'minimal subjects' test days. The Study Director ~~**arranged for alternates to be 'on-call' rather than physically present on each 'minimal subjects' test days, and reminded subjects prior to repellent applications that they could withdraw at any time and that alternates were readily available take the place of any subjects wishing to withdraw prior to repellent application.**~~ **determined subjects withdrawing from a minimal subject day could be effectively replaced by alternates on the following test day when the same species would be challenged by the Test Material, and alternate subjects were already scheduled to be available. In this way, alternates were used per approved Study Protocol and ICF specifications and intentions, coming to the laboratory, if available, on each of three study dates, and being randomly selected amongst the other alternates present to become a treated subject replacing a subject withdrawing from testing that species, whether the withdrawal was from the minimal subject test day prior, or the withdrawal was from the test day during which most of the subjects participated, and the alternates were physically present. The justification was to effectively address limitations in subject availability during weekdays (versus weekends) in a manner that facilitated timely collection of the data set without compromising an individual subject's opportunities and sense of permission to withdraw.** Because environmental conditions were maintained relatively consistently within the laboratory setting of the study, and ticks continued actively qualifying for all intervals on all days, the Study Director concluded that the deviation to testing on multiple days for two of the three species did not impact study quality. (see Appendix 3, Note to File 24 October 2021, Deviations associated…)" Following is a summary of circumstances regarding the 3 subjects replaced with alternates: Subject 62 was originally scheduled to take part in *Amblyomma americanum* testing on 17 October 2021. She informed CLBR staff she would no longer be

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	available for that day on 11 October 2021 by email, but expressed that she would still be available and still wanted to participate in testing on 24 October 2021 (*Ixodes scapularis*). During the 10 October 2021 study day, subject 163 informed CLBR staff she would not be available for either *Ixodes scapularis* testing day. Subject 177 did not provide notice. On 24 October 2021, Subject 177 did not arrive to the testing facilities and could not be reached by phone; he informed CLBR by phone the Monday after the study day (25 October 2021) that he was unexpectedly busy and unable to inform the staff of his unavailability the day of the study. Alternates were selected to replace subjects on the study day they were present, after subjects and alternates arrived at the test site (to avoid selecting an alternate that did not arrive to the test site) but before any repellent applications occurred. The selection was randomized between present alternates using the RANDBETWEEN and CHOOSE functions in Excel. No subjects were replaced within a testing day, e.g. after repellent applications. The stop rule was not invoked, therefore the cited procedures in the protocol were not implemented, and do not need to be reported or clarified for the final report phase of the investigatory process. No subjects withdrew mid-test after applications were made and exposures were started. No subjects asked to withdraw after alternates were dismissed. All withdrawals happened before applications and before exposures.

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14) You must provide acceptable answers to the questions bulleted below regarding 'minimal subject days' (pp. 25-26 of 403). • What were the directions given to alternates that were on-call but not present on the site during 'minimal subject' test days? • Were any of the on-call alternate subject used to replace subjects on any test day? If so, when did the replacement occur? Provide the number of subjects that participated in each 'minimal subject day' in the protocol deviation table (p. 24 of 403).	The term "on-call" as applied to alternates in the context of this study was intended to describe procedures with alternates, but CLBR understands that this terminology may be confusing. Deviation #7 therefore will be clarified in the amended final report, as indicated above in the response to EPA Comment #13. No alternates were waiting for a call or text communication per se, nor were alternates required to remain available for any study activities during the "minimal subject" days. The term "on-call" will be removed from the final report by amendment. The numbers of subjects on "minimal subject" days are confirmed as follows: 5 subjects on 13 October 2021; and 6 subjects on 20 October 2021. The number of subjects present on each minimal subject day will be specified in the amended final report.
15) You must provide acceptable answers to the questions bulleted below regarding the six alternates who were selected instead of the eight proposed in the protocol (p. 23 of 403). • How many on-call versus in-person alternates were assigned to each test day? • How many alternates arrived at the testing center along with the test subjects on each test day? • Please provide more detail regarding the use of two alternates (subject 12 and 132) that replaced subjects that were not able to participate, including the time when alternates were used to replace the original subjects (e.g. before or after exposures began), the time the repellent was applied to alternates, and whether the original subjects showed up to the test site. • What were the procedures for applying repellent to on-call alternates in case a replacement needed to occur?	• See responses to EPA Comments #13 and #14 above regarding the use of the term 'on call'. • The number of alternates engaged on site each test day is clarified in the newly prepared *Subject Participation Chart* (see unaudited draft version attached; document number 364883), which will be included in Section 2 of the amended final report. • On 10 and 17 October 2021, three alternates were present at the test site. On minimal subject days (13 and 20 October 2021), no alternates were present at the test site. On 24 October 2021, four alternates were present at the test site. • Subject 12 replaced subject 177 on the 24 October 2021. Subject 177 did not arrive at the testing facility and was not reachable by phone or email, so they were removed from the study day and replaced with subject 12 who was present at the test site as an alternate. Subject 177 was replaced by subject 12 prior to repellent application, so subject 12 was able to be applied and start exposures along with all other subjects that day. No alternate was applied with repellent unless the alternate was assigned the role of a treated subject to replace a withdrawing subject, and this process of substitution occurred before repellent application to any subject on a given test day. The time of application of repellent to each participating treated subject is provided by subject number in the raw data, regardless of whether an individual treated subject was assigned

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	treated status at the beginning of the study, or was an alternate who was substituted in for a withdrawing subject, thereby becoming a treated subject. • Subject 132 replaced subject 62 for the *Amblyomma americanum* test day October 17th. Subject 62 informed CLBR staff they would no longer be able to attend that test day on 11 October 2021. • The term 'on-call' is admittedly confusing and will be removed in the amended final report (see responses to EPA Comments #13 and #14 above). If a substitution of an alternate for a withdrawing subject needed to occur, the role of the alternate changed to a treated subject, and that subject was treated with repellent at about the same time and employing the same procedures as the other subjects receiving treatments. The timing of repellent application for any treated subject, whether originally-designated as a treated subject or an alternate that become a treated subject, is recorded on the repellent application raw data sheets accordingly (see pages 268 through 272 of the original final report).
16) You must clarify the procedures for recording tick crossings/ repulsions during test days. • The ICF states that the tick would be transferred onto the treated arm for up to 3 minutes (Appendix 2, p. 178 of 403). How was the exposure period of ticks timed for tick placement on the treated arm, orientation of the tick to an upward-facing position, and the start and end time of the observation period for crossings/repulsions? What was the duration of the observation period? • How many staff members were used to record crossings/repulsion data on each test day? Did subjects call on staff members to record crossings/repulsions?	Section 6, subsection *Exposures*, in the final report will be amended to include the following clarifications: **"All exposure times were measured using the digital chronometer displayed in the testing room. The duration of the observation period varied depending on individual tick behavior. The start of each observation period (*i.e.*, the beginning of the sequence of tick qualification), followed by treated-arm tick placement, orientation, and crossing observations were recorded in the raw data for tick crossings. The total elapsed time was approximately 15 minutes for all intervals in an observation period. The duration of the observation period and the time for each stage of the process within an observation interval varied among subjects. Some subjects completed the procedures sooner than others due to mundane variation in tick behavior. Records were not kept of the timings of individual phases of each exposure by individual subject, but rather of the beginning of each observation period (interval) per the approved Study Protocol. Researchers were available to clarify crossing versus non-crossing events and to note any subject at any interval who did**

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	not qualify a tick in accordance with procedures. Regardless, each observation period (interval) had a beginning time common to all subjects and announced by a researcher, per the Study Protocol. Subjects reported crossing versus non-crossing after completing the specified procedures within each observation (interval) period, and the data were recorded by a researcher." The raw data sheets for crossings indicate the number of researchers actually recording data each day, as indicated by the number of signatures on the raw data sheet for that day, and are reported as such. On non-minimal test days, three research staff members were present in addition to the data recorder. For minimal test days, one or two researchers were present in addition to the data recorder.
17) The main report includes this excerpt: "ticks were scored as crossing after traveling the specified distance into the treated skin area, e.g., 3 cm or more. Crossings were scored without the stipulated one minute for ticks to remain in the treated area" (p. 18 of 403). However, it is unclear if/how this description for scoring crossings aligns with the scorings proposed in the protocol. You must specify which of the crossings and repulsions scoring criteria proposed in the protocol (Appendix 1, pp. 54-33 of 403) and shown below, were used for determining repellency during efficacy testing. If different criteria were used for crossings/repulsions, you must adequately describe them. • 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. • Crossing: Tick travels more than 3 cm past the reference line, reaching the uppermost line, within 3 minutes. • 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	A crossing is defined in the protocol as an actively foraging tick locomoting from the untreated skin surface of a subject's hand and traveling 3 cm or more into the treated forearm skin area for at least 1 minute. In practice, during the study, we found that the adult ticks were much quicker at locomoting and, if left for 1 minute after moving onto treated skin to travel up a treated subject's forearm, the ticks could surpass the treated area and become significantly more difficult to observe and remove. For this reason, the 1-minute stipulation was removed. Otherwise, scoring is as described, excepting the nuance of 'at least' vs 'more than' in the excerpted protocol language, which, in practice, amounted to the same thing. It is not possible to specify an 'equal to' without further defining if, for example, half or all of the tick's body must cross the line, or some other portion, and if the line has thickness (say, $1/16^{th}$ or $1/32^{nd}$ of an inch for a sharpie-made line on the skin), whether it is one edge of the line, or the other, or the middle that is the true determinant of the 'crossing' of that line. In practice, ticks were never exactly on the line at exactly 3 minutes into the observation period, so there was never a judgement call required by exacting timing of a given tick position in a crossing challenge. In practice, the process is more straightforward than the above discussion suggests, and can be clarified.

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	Section 6, subsection *Scoring of crossings*, will be amended in the final report as follows: "*Scoring of crossings* **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.** ~~**Ticks were scored as crossing after traveling the specified distance into the treated skin area, e.g. 3 cm or more.**~~ Crossings were scored without the stipulated one minute for ticks to remain in the treated area, which deviated from the Protocol (see §9 below), due to the researchers' observations that**, if allowed a full minute of locomotion after crossing into the treated skin area of a subject's forearm,** crossing ticks of all three species were so mobile as to likely cross the entire treated area **onto the back of the elbow or** ~~**into the**~~ upper arm **and become difficult to observe and remove, especially for the subject who was trained to and expected to be able to remove a tick from their own arm.**

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18) You must clarify what sections of the forearm constitutes 'the treated area' referenced in the crossings/repulsion criteria above. Was repellent applied to the entire forearm (between the wrist joint and elbow) corresponding with forearm measurements provided in Appendix 5 (pp. 236- 266 of 403), or was repellent applied within certain areas delineated by the forearm boundaries shown in Figure 1 of the protocol (Appendix 1, p. 54 of 403)? If the latter is true, which two lines shown in Figure 1 of the protocol demarcated the treated area of the forearm? This 8 information should be clearly stated in the "Applications of Test Material" section in the study report (p. 17 of 403).	Per the Study Protocol, marking lines were not intended to be used to demarcate treatment area, nor were they used that way in practice. According to the Protocol, repellent was applied to subjects' entire forearms, from the wrist joint to the elbow. The lines shown in Figure 1 are reference lines for tick placement (lowermost line near palm) and scoring a "crossing" (uppermost line on forearm). These lines were marked after repellent application. The middle line (*e.g.*, the one at the wrist joint position) indicates the beginning of the treated area only for the purpose of providing a visual guide for scoring crossing activity, not to provide guidance to a researcher applying repellent to a subject, as the line is added after application, per the Protocol. The end of the treated area in the vicinity of the elbow received no reference line, as no aspect of scoring required a mark for the end of a treated area, only for a distance of crossing into the treated area of skin from a tick starting point on a subject's hand. Section 6, subsection *Applications of Test Material*, of the final report will be amended to provide these details.
19) The report states, "up to three researchers applied repellent to different individual subjects roughly simultaneously" (p.17 of 403). You must provide the following information: How many researchers applied the test materials to subjects on each test day? How were subjects grouped with staff members and/or with each other?	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). Subjects were not purposefully grouped or assigned to staff members. Rather, when it came time to apply, subjects were asked to sit around the room following social distancing procedure, and researchers applied based on proximity.

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20) You must clarify and adequately explain the skin-washing procedures that were conducted prior to repellent applications on test days. • It was reported that forearms were washed, rinsed, sprayed with diluted alcohol, and towel-dried (p. 17 of 403). Ticks were placed on palms just distal to the wrist skin of their untreated forearm (p. 17 of 403). Were hands also washed, rinsed, sprayed with diluted alcohol, and towel-dried? • The protocol noted that skin sanitizer as available for use by subjects (p. 36 of 403). Was hand sanitizer applied to hands in the lab before or at any point during the efficacy testing? If so, were subjects instructed to wash off any sanitizer prior to the application of the repellent product?	All subjects washed both hands and both forearms per the approved protocol. Section 6, subsection *Limb Washing*, of the final report will be amended to reflect this clarification. Hands were not mentioned previously because washing of ones own forearms with ones own bare hands necessarily includes hand washing. However, stating specifically that subjects washed their hands keep use of the term 'forearm' consistent throughout the report, as most mentions of the term 'forearm' in the report are intended to mean the part of the body between the wrist joint and the elbow. Skin sanitizer was available but was not, in practice, used by subjects during the extended period of cyclic exposure. Subjects instead were instructed to speak to a researcher if they were concerned about a bare-skin contamination from inadvertent contact with a surface within the laboratory context of the study, at which point the researcher would assist the subject in washing the suspected area, using the same sequence of procedures and materials as the initial washing prior to application, so that any wash-off of repellent could be avoided. Section 6, subsection *Applications of Test Material*, of the final report will be amended to provide these details.
21) You must provide adequate details on whether/how subjects were directed to avoid the loss of applied repellent. • What precautions were taken for not disrupting the repellent applied to subject forearms throughout the test days? • The protocol noted that face masks and gloves would be available for use (p. 36 of 403). Did subjects wear gloves during the efficacy tests? If so, what measures were taken to not disrupt the applied repellent?	Beginning before application of the test material and continuing periodically (approximately once per hour) for the duration of each repellency challenge day, subjects were clearly and repeatedly reminded to ensure that their treated forearms did not touch, rub, or otherwise come into contact with any surface for the duration of testing. For any subjects wearing long sleeves, sleeves were rolled up at least half-way up the upper arm and secured before repellent application and testing. Section 6, subsection *Applications of Test Material*, of the final report will be amended to provide these details. Gloves available for use were for CLBR staff members when handling test material, tick vials, or food for subjects. Although gloves were available to subjects, subjects did not wear gloves at any time while applied with repellent.

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22) You must report the percentage of ambient light in the exposure room during test days.	Light intensity (lux), as well as air temperature and relative humidity, was recorded in approximately one-hour intervals throughout each exposure period per the Study Protocol (see pages 327 through 331 of the original final report). It is not possible to convert lux to a meaningful percentage of ambient light.
23) You must provide additional information about the ticks used for the study to adequately address the following: • Specify the age of ticks on pg. 15 of 403. Eclosion could refer to emergence as larvae, nymphs, or adults, so the ages of ticks specified in table 3 (p.15 of 403) are unclear. For each species, what were the developmental stage and age (e.g., adults 2 weeks-old) of ticks upon arrival and the stage/age of ticks used for each test day? Were any immature stages used? If so, please specify. • Specify if each individual tick was used once, during one exposure and one subject in the submitted study, as proposed in the protocol (Appendix 1, p. 51 of 403). If not, explain the rationale for reusing ticks. • Specify if the used ticks were killed via freezing or ethanol at the end of each handling training day and each efficacy test day, as proposed in the protocol (Appendix 1, p. 55 of 403). • Specify if the tick handling training occurred 30 days before the first efficacy test for all subjects, as proposed in the protocol (Appendix 1, p. 35 of 403)?	All ticks used in this study were between 3 and 5 weeks post-eclosion adults. No nymphal or other life stages were used. All references to ticks throughout the report are to adult ticks, as no other stage was present or used, per Study Protocol, and references to eclosion are to eclosion associated with the adult phase. 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." Ticks were killed by being placed in vials of ethanol during the study, and immediately after the study day was concluded, all vials were placed in a freezer. The protocol states 'Within 30 days before repellent efficacy testing, subjects will be trained by researchers in handling ticks in the laboratory'. 'Within' means '30 days or less' in this context. Therefor subjects needed to have completed training no more than 30 days (and at any time within the 30 day period) before the first efficacy test day. All subjects were trained within 30 days before the efficacy challenges (this is true for all the efficacy challenge days). The preceding four statements in this response will be reflected in the amended final report.

APPENDIX A

EPA-Deduced subject tracking details for 31 subjects enrolled in the tick repellent study (MIM-007)

Subject	Gender	Assignment	Subject in MIM-006?
12	F	Alternate	Yes
103	F	Alternate	Yes
132	F	Alternate	Yes
6	F	Treated	Yes
11	F	Treated	Yes
41	F	Treated	No
62	F	Treated	Yes
66	F	Treated	Yes
76	F	Treated	Yes
131	F	Treated	Yes
147	F	Treated	Yes
163	F	Treated	Yes
171	F	Treated	No
122	M	Alternate	Yes
4	M	Treated	Yes
30	M	Treated	Yes
33	M	Treated	Yes
55	M	Treated	Yes
63	M	Treated	Yes
73	M	Treated	Yes
74	M	Treated	No
129	M	Treated	Yes
134	M	Treated	Yes
142	M	Treated	Yes
150	M	Treated	Yes

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<table><tr><td>167</td><td>M</td><td>Treated</td><td>Yes</td></tr><tr><td>169</td><td>M</td><td>Treated</td><td>No</td></tr><tr><td>177</td><td>M</td><td>Treated</td><td>No</td></tr><tr><td>178</td><td>M</td><td>Treated</td><td>Yes</td></tr><tr><td>18</td><td>Not specified</td><td>Alternate</td><td>No</td></tr><tr><td>153</td><td>Not specified</td><td>Alternate</td><td>No</td></tr></table> Note: Subject tracking details shown above include gender, assignment as treated or alternate subjects, and if subject participated in the mosquito repellent study (MIM-006). Subject genders were determined from repellent application sheets (Appendix 5, pp. 267-272 of 403), except for subjects 103 and 122, whose genders were determined from the MIM-006 study report. The Agency attempted to deduce subject assignments (treated versus alternate) throughout the tick study based on the following: subject numbers and gender provided in the repellent application sheets (Appendix 5, pp. 267-272 of 403), subject numbers listed in the tick crossings data (Appendix 7, pp. 301, 308, 312 of 403), and the use of subject numbers 12 & 132 as alternates in the tick study report (p. 14 of 403). Based on these sources of information, the Agency deduced that the same subject assignments (treated/alternate) were made between A. americanum, I. scapularis, and R. sanguineus test days. Subjects that also participated in MIM-006 were determined from limb measurement forms that note "MIM-006" in the upper left corner (Appendix 5, pp. 236-266 of 403). The gender of subjects 103 and 122 were determined from the mosquito study (MIM-006) report.	
24) Regarding Alternates: you must provide a rationale for not having alternates present each time subject testing occurred. The protocol and consent form both noted that alternates would be on site to replace any subjects who withdrew from participation. Other than the verbal statement of alternates being 'on-call', were any assurances given to subjects regarding their rights to withdraw at any time?	See responses to EPA Comments #13, #14, and #15 above. 'Minimum subject' days each occurred prior to the main or 'normal' study day during which most subjects were available to participate. On 'minimal subject' days, withdrawing subjects would not be replaced that day. Rather, an alternate who was present on the 'normal' efficacy challenge test day for the same tick species would be chosen at random to become a treated subject who's data would replace the data of the subject who had withdrawn or was removed on the 'minimum subject' day. Subjects were reminded by the Study Director at the beginning of the 'minimum subject' day that they were free to withdraw at any time for any reason, privately and without needing to provide an explanation, and without any payment penalty. They were also told that an alternate would take their place for data collection on the next test date for that tick species. This was to make it clear to each subject that a withdrawal would not impact

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	study quality, and thus there was no need to feel pressure or obligation to continue participating. Per previous responses to EPA Comments #13, #14, and #15, the final report will be amended to provide clarifications of these matters.
25) Regarding efficacy testing: you must confirm whether or not the skin of all subjects checked again at the end of the test day.	No, the skin of all subjects was not checked again at the end of each test day. This medical procedure is not specified in the protocol or the consent form and was not consented to by the subjects. Excerpted from Paragraphs 1 and 2 Section 1.3.7 of the approved Study Protocol: *'All subjects are asked to contact the Study Director and a physician of their own choice at any time should they develop a skin rash (a delayed hypersensitivity reaction) within 7 days of the conclusion of the test day.* *On the day of any study visit, staff will immediately communicate all subject concerns about health, safety, or comfort to the Study Director for assessment. The Study Director will also assess skin condition of affected subjects should any bites inadvertently occur during efficacy testing, or any subject reports any discomfort in treated areas. Subjects are instructed to inform the Study Director or any other staff member if at any time during the study a subject suffers a skin reaction, such as redness, edema, itching or pain, or feels ill. Such subjects will be immediately removed from testing and arthropod exposure, and medical management (see next paragraph) will be implemented.'* Excerpted from Section 4.8.3 Test Day of the approved Study Protocol: *'Each subject's arms will be briefly examined for skin conditions by a staff member with an Advanced First Aid or higher level of medical training.'*

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	In the list of procedures that appears on pages 9-10 of the approved ICF, there is no mention of a second examination of skin as a medical procedure subjects consent to. The single examination is intended and specified to occur before repellent applications.
26) Regarding <u>recruitment</u>: • The protocol noted that subjects would be recruited through various announcements, but the protocol did not include previous study participation or drawing from a database of previous research participants as methods for recruitment. The study report indicates that many of the subjects in this study also participated in the mosquito study. You must confirm: Did the subjects who participated in both studies respond to each of the recruitment efforts? If not, how were they identified and contacted for their participation in the tick study? You must explain why no protocol deviation was reported for this issue. • Based on subject numbers, provided in the raw data appendices of both study reports, a total of 24 subjects used in the mosquito study were also enrolled for the tick study (shown in Appendix A). Based on test dates and subject numbers, subjects in the tick study were used in mosquito study field tests 7 or 14 days prior to their first test day in the tick study. Provide a table that indicates whether each subject participated in the mosquito testing and their dates of participation in both the mosquito and tick testing. • Provide in the study report a table with subject information that includes gender, age, each tick species tested, test subject/alternate, date of test. <u>Consent</u> • The protocol describes a candidate enrollment procedure that proposed to include 22 male and 22 female candidates, each of whom would be assigned a sequential number unique to them	The newly developed *Subject Participation Chart* summarizes the dates of participation by subject in both studies (MIM-007 and the preceding MIM-006), as well as gender, age, tick species tested, test subject/alternate roles by species and date, the dates of testing for each subject, and the gender ratios of subjects on each efficacy challenge test date. See the attached unaudited draft version of the *Subject Participation Chart* (document number 364883); an audited version will be included in Section 2 of the amended final report. Recruitment was performed according to the Protocol, so no deviation was noted or reported, and the consent of subjects in Study No. MIM-006 to be contacted about future studies was not required or relied upon. Advertisements for both studies were released simultaneously into the same media outlets. CLBR did not contact MIM-006 subjects during or post-study to recruit them for study MIM-007. Candidates expressed interest in one, the other, or both studies according to their inclinations and knowledge of the existence of both studies. It is also possible that some study candidates/subjects for MIM-006 became aware of more than one study due to word of mouth communications with peers in the community and/or with other subjects in the MIM-006 subject pools. This was allowed according to protocol, but not encouraged. Advertisement outreach and call-back procedures detailed in each protocol were followed per each protocol, so there was no need to report a deviation. Candidates who contacted CLBR voluntarily indicated their interest in the particular study for which they had seen an ad or heard about, or indicated their interest in both studies. Since study MIM-007 subjects who participated in study MIM-006 consented to having their limb measurements taken for that study, and consented also to having them taken for the MIM-007 study, then the measurements taken for study MIM-006 and used again in study MIM-007 constituted consented-to data from consented-to procedures for both studies.

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and this study (Appendix 1, p. 42 of 403). However, a note to file in Appendix 5 (p. 235 of 403) states that test subjects who participated in the mosquito study evaluating the same repellent product (MIM-006) also participated in the laboratory tick study (MIM-007). Did subjects consent to being contacted for future studies when enrolling in the mosquito study? • Did the consent process include a demonstration of the product application process and what would happen during an exposure period? The study report only indicates that the researchers conducting the consent meeting read the consent form. • Were consent meetings held one-on-one, or in groups? • Of all consent meetings held, provide the number of meetings held in person, via video conference, and by telephone. • Describe how researchers conducting the screening meeting tracked individuals' responses to the eligibility screening. Was it a checklist or a form for subjects to fill out?	All consent interviews were conducted one-on-one and in private, and included a demonstration of product application in pantomime, without use of actual material, and of what would happen during exposure periods. The final report will be amended to clarify these procedures. Regarding eligibility screening, no form was provided to subjects to fill out, and no data were recorded other than a certification of completion mark on the Research Candidate/Subject Checklist (Appendix 3 of the final report). The telephone script was used during candidate recruitment as a prompt for lab personnel to exercise preliminary screening, and not a written checklist to fill out. A candidate only needed to disqualify on one criterion to be disqualified from the study as a whole; tracking responses to individual criteria therefore was not required. Any candidate who advanced to a full consenting interview passed all screening criteria, by definition. In a one-on-one consenting interview, a researcher read the relevant section of the ICF containing the comprehensive list of inclusion and exclusion criteria and exercised them verbally with the candidate, confirming verbally that all criteria were appropriately met. Section 2, subsection *Screening and Consenting*, of the final report will be amended as follows: *"Screening and Consenting* A total of 31 subjects were consented, 17 male and 14 female. (see Appendix 3 and deviation **#2** detailed in §9 of this report). **All consenting interviews were conducted in person, privately, and one-on-one.** In accordance with protocol procedures, PHRP-trained staff members read Informed Consent Form (ICF) to candidates~~.~~**. Staff exercised screening criteria during the reading of the ICF documents, following exactly the list of criteria provided in the ICF, and allowing time for the candidate to confirm qualification for each criterion. The interviewing researcher also** asked questions to ensure comprehension, **and as directed by the same ICF documents, performed demonstrations of repellent applications by mimicking the process on their own arm (but without applying any repellent to themselves), the position of the marking**

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	lines, the handling and placement of ticks on the subject's skin by the subject (but without using live ticks), and how tick position relative to the marker lines would be used to score a tick as qualified, then crossing or non-crossing. The researcher then provided **the subject with** copies of documents for review and reference (Protocol §3.4). ~~**Most such interviews were completed remotely via internet video and phone conferencing. In those cases, consent documents were initialed and signed near the beginning of the first in-person laboratory visit when the**~~ **Immediately prior to having the subject sign the ICF, the** interviewing researcher asked each candidate if they still wished to participate and reminded each candidate that they are free to ask questions, request more time, or decline to consent. ~~**Staff exercised screening criteria during the reading of the ICF documents.**~~ Once consented, candidates – now subjects – were assigned a unique subject number from a list of random numbers previously generated in Microsoft Excel specifically for use in the study. The Research Candidate/Subject Checklist data sheets (Appendix 3) capture the relevant demographics, showing the balance of male vs female subject numbers, and demonstrating all subjects were screened to fall in the 18–60 age range."
27) Regarding the Reminder Call/E-mail: You must provide the information that was shared with subjects in the reminder phone calls and emails used to communicate with subjects two days prior to each test day (p. 16 of 403).	The study report will be amended to include a new subsection, *Reminders*, immediately following *Screening and Consenting* in Section 2, as follows: ***"Reminders*** **Via email and by phone prior to each in-person visit to the laboratory, including the consenting interview visit, candidates and subjects were reminded or informed of:** • **Time and date of the study visit;** • **Location of the study and giving instructions to reaching the testing location (how to reach the room in the building);** • **To confirm they were still available or inform us if they were no longer available;** • **Not to smoke or drink alcohol after 9pm the night before the study visit and throughout the study visit;**

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	• **Not to apply fragranced products after 9pm the night before the study visit and throughout the study visit;** • **To bring laptops, books, or other preoccupations for downtime between exposures during the efficacy test days;** • **CLBR would be providing lunch, snacks, and drinks during the efficacy test days;** • **To consider wearing light colored clothes, to make it easier to spot a stray tick if one were dropped; and** • **On efficacy test days, to wear short sleeved or sleeveless shirts, or shirts with sleeves that could easily be rolled up to the upper arm area and secured."**
28) Regarding Pathogen Screening: The Agency requires pathogen-free insects in studies using human subjects. The protocol stated that "Our laboratory-reared tick populations are certified disease free. Methods employed for disease exclusion are summarized in Appendix 4 and will be reported" (p. 35 of 403). The consent form notes that "The ticks used in this lab study are from a colony that has been screened for infectious 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 of 403). You must address the bulleted points below to clarify details regarding the pathogen screening documentation provided in Appendix 8 (p.324) • There is no evidence of pathogen screening for the ticks sourced from BerTek. Please provide a rationale for not following the protocol. An assertion from the company is not sufficient to ensure that the ticks are pathogen-free; more rationale as to why no pathogen screening was conducted needs to be provided. • The R. sanguineus ticks obtained from BerTek were fed on dogs or rabbits, and a statement from the company asserted that: "To our knowledge, there are no known pathogens and or resistances	In practice, it is not possible to guarantee that any colony, regardless of colony maintenance procedures, will be certifiably free of any disease-carrying individual ticks. The risk can be reduced, but not eliminated. The fact that in the research industry and amongst scientists who study arthropod-vectored diseases, the phrase 'disease-free' is fairly commonly used in reference to laboratory colonies does nothing to address the present need to understand risks to subjects clearly, and to minimize those risks in practice. By use of the phrase 'certified disease-free' we inadvertently created an expectation that the risk would be guaranteed to be zero, a frank impossibility. It is, however, possible to provide instead a very low risk colony of ticks through the combined use of colony record keeping, periodic pathogen screening, and attention to what is known about transmission of tick-borne diseases through host feeding and through oviposition, where pathogens might be passed from an adult female tick to the offspring in egg form. CLBR believes it provided subjects with ticks consistent with minimized risk of individual disease-bearing ticks being present. CLBR acknowledges that PCR testing of a subsample of individual ticks from a tick colony is a comparatively secure means of evaluating the likelihood that the screened microorganisms will be present in other ticks from that colony to be used in a repellent assay. Despite that relative security, CLBR further acknowledges that in human studies practice, the characterization of any apparently axenic colonies as 'pathogen-free' could be deemed as an overstatement, regardless of the exceedingly low risk of any tick bites afforded by our testing practices.

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(Appendix 8, p. 324).” Provide justification for not performing pathogen testing for R. sanguineus tick used in the study. • Clarify whether the dogs and rabbits BerTek used to feed R. sanguineus ticks (Appendix 8, p. 324) were kept indoors only (in other words, these host animals never went outdoors). • It is not clear if all the A. americanum and I. scapularis ticks sourced from OSU and used in testing were obtained from the same tick lots that molted in September 2021 that were assayed for pathogens (Appendix 8, p. 320, and p. 322). Clarify if the A. americanum and I. scapularis ticks used for testing repellency were verified to be pathogen-free using the PCR tests documented in Appendix 8 (pp. 320, 322). • Wild, engorged I. scapularis females were introduced to OSU colonies on 10/18/2021, after pathogen screening was conducted on 10/13/2021. Clarify if the I. scapularis tick lot used for testing included any introduced females that were not screened for pathogens.	CLBR notes that the Study Protocol does not mention or specify PCR testing for pathogens, that this stipulation was not part of any communication with the IRB of oversight, or part of HSRB Protocol review-based recommendations, nor was the procedure communicated to subjects in subject-facing documents. Considering more specifically the *Rhipicephalus sanguineus* ticks we used in study MIM-007, which were not subjected to PCR microbial screening, we note that sound risk characterization may also be based on inferences drawn from knowledge of the vector-pathogen biology, pathogen prevalence, colony history, and host husbandry. In this case, four lines of evidence together indicate a low probability of tick infection: 1. *R. sanguineus* is not a known vector in the source region; 2. Candidate pathogens are exceedingly rare; 3. Transovarial transmission is low; and 4. Culturing methods act to exclude disease. Following are additional background and supporting references: ***Rhipicephalus* colony history and supplementation with wild-caught ticks.** The BerTek, Inc. *Rhipicephalus sanguineus* research colony was established in 2006 with wild ticks collected from kenneled dogs in Greenbriar, Arkansas. Consistent with FDA requirements to maintain genetic diversity, that colony has since been supplemented with additional wild-caught individuals from the same area at less than or equal to 5-year intervals. For study MIM-007, BerTek indicated the ticks they provided CLBR in September and October 2021, were from cohorts last supplemented with wild individuals in Fall 2017. **Breeding of *Rhipicephalus* and exclusion of most females from reproduction** The 2017 supplementation was with engorged females. Hatchlings from their eggs were reared according to the BerTek SOP, first on rabbits through the larval and nymphal stages. The small fraction of adults utilized in colony perpetuation are then fed on dogs. All BerTek rabbits and dogs are sourced from, and maintained in, rearing facilities with no exposure to outdoor settings or any evident risk of infection by tick-borne pathogens.

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	To introgress novel genes, 25 adult offspring of the wild-collected females were paired with 25 adults from the standing BerTek colony to create a mixed lineage for subsequent tick production. The methods underlying that production, which exclude almost all individuals from breeding, and should act as a severe bottleneck on any extant pathogen populations, particularly if they are rare. Specifically, breeding females produce 3000-4000 eggs of which at least 2500 on average hatch. Of the resulting adults, two females and two males from every ~5000 hatching eggs are selected to breed at 3-4 day intervals, representing approximately 200 breeding pairs annually extracted from a population of about 500,000 individuals (0.4%). With a 90-day life cycle, the BerTek colony produces approximately 4 generations per year, such that approximately subsequent 15 generations of ticks preceded that of the ticks used in study MIM-007. **Spotted Fever Rickettsiae are not known to be vectored by *Rhipicephalus* in Arkansas** Several species of spirochaete bacteria in the genus Rickettsia occur in *Rhipicephalus sanguineus* ticks (Parola et al. 2013). Their occurrence is rare and sporadic (Eisen et al. 2017). One particularly severe North American rickettsiosis, Rocky Mountain Spotted Fever (RMSF), is caused by *Rickettsia rickettsii*. In Arkansas, however, RMSF is conventionally regarded as vectored by a different tick species, *Dermacentor variabilis*. *R. sanguineus* is the known vector only in a few rural areas of Arizona and adjacent Mexico (CDC 2022). In a citizen-science associated screening study of approximately 12,020 wild-caught ticks from throughout Arkansas, *R. sanguineus* were not commonly encountered N = 287; 3.7%). None of those specimens were found to harbor Spotted Fever Rickettsii (Frank et al. 2019; expanded and updated in Dowling et al. 2022). Beginning about a decade ago, the CDC-directed protocol no longer distinguishes among Spotted Fever Group rickettsiae in public health reporting, such that incidence includes relatively abundant but significantly less pathogenic species (e.g., *Rickettsia amblyommii*). Delisle et al. (2016) review those much more ubiquitous *Rickettisii* species in relation to human rickettsioses in nearby Tennessee, where the highly pathogenic RMSF agent R. rickettsia is likewise exceedingly rare. They suggest most contemporary reports of RMSF in the region are caused by those other *Rickettsii* species. In Arkansas, distinct from *Rhipicephalus*, Spotted Fever Group Rickettsia incidence in key *Amblyomma*,

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	Dermacentor and *Ixodes* tick species ranged from approximately 10-50% (Dowling et al. 2022).Infection of ticks from experimental feeding on infected dogs has historically met with little success, and limited transovarial transmission of Spotted Fever Group *Rickettsia* species has been shown in *Rhipicephalus* species. However, using experimentally infected dogs, Piranda et al. (2011) were able to detect that some females that fed on infected dogs as nymphs ultimately produced some eggs that produced infected larvae. They concluded that the frequency of transovarial transmission was insufficient to maintain infected ticks in colony without horizontal transmission from infected host reservoirs. In an important contrast, the authors found that while 100% of 'random' colony females fed on infected dogs for the first time as adults became infected, none produced infected eggs. Moreover, *Rickettsias* are pathogenic in host ticks, an outcome that has been used to explain the extremely low incidence of infected ticks that prevails in nature (Labruna et al. 2008, Socolovschi et al. 2012). Different from Rhipicephalus, in the competent vector *Dermacentor variabilis*, transovarial transmission is regarded as the principal driver of Rickettsia incidence, that than host animal reservoirs of *Rickettsia* (Eisen et al. 2017).Dogs are highly susceptible to RMSF and exhibit a diversity of severe symptoms, with mortality rates up to about 10% (e.g., McQuiston 2018). BerTek indicated no veterinary examinations indicating RMSF in their dog colonies. Rabbits are used for single feedings only. Even in the unlikely event of any colonization by Rickettsia during the 2017 supplementation with wild ticks, extreme culling for reproduction (~1/2500), low to zero vertical transmission, and the absence of an evident source of infected hosts, suggest the likelihood of pathogen-infected *Rhipicephalus sanguineus* ticks in our study was extremely low. *References:* CDC 2022. Rocky Mountain Spotted Fever. https://www.cdc.gov/ticks/tickbornediseases/rmsf.html#:~:text=RMSF%20is%20most%20often%20transmitted,along%20the%20U.S.%2DMexico%20border. Accessed 16 May 2022).

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	Delisle, J., Mendell, N.L., Stull-Lane, A., Bloch, K.C., Bouyer, D.H. and Moncayo, A.C., 2016. Human infections by multiple spotted fever group rickettsiae in Tennessee. *The American journal of tropical medicine and hygiene*, 94(6), p.1212. Dowling, A.P., Young, S.G. and Loftin, K., 2022. Collaborating With Community Scientists Across Arkansas to Update Tick Distributions and Pathogen Prevalence of Spotted Fever Group *Rickettsia* and *Ehrlichia*. *Journal of medical entomology*, 59(2), pp.565-575. Eisen, R.J., Kugeler, K.J., Eisen, L., Beard, C.B. and Paddock, C.D., 2017. Tick-borne zoonoses in the United States: persistent and emerging threats to human health. *ILAR journal*, *58*(3), pp.319-335. Frank, A.D. and Dowling, A.P., 2019. Geospatial analysis of rickettsial species in Arkansas. *Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences*, *20*(1), pp.43-50. Labruna, M.B., Ogrzewalska, M., Martins, T.F., Pinter, A. and Horta, M.C., 2008. Comparative susceptibility of larval stages of Amblyomma aureolatum, Amblyomma cajennense, and Rhipicephalus sanguineus to infection by Rickettsia rickettsii. *Journal of medical entomology*, *45*(6), pp.1156-1159. McQuiston, J.H. 2018. Rocky Mountain Spotted Fever (Tick Fever) in dogs. *Merck Veterinary Manual*. https://www.merckvetmanual.com/dog-owners/disorders-affecting-multiple-body-systems-of-dogs/rocky-mountain-spotted-fever-tick-fever-in-dogs. (Accessed 15 May 2022). Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.E. and Raoult, D., 2013. Update on tick-borne rickettsioses around the world: a geographic approach. *Clinical microbiology reviews*, *26*(4), pp.657-702.

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	Socolovschi, C., Gaudart, J., Bitam, I., Huynh, T.P., Raoult, D. and Parola, P., 2012. Why are there so few Rickettsia conorii conorii-infected Rhipicephalus sanguineus ticks in the wild?. *PLoS neglected tropical diseases*, *6*(6), p.e1697. Regarding the *I. scapularis* ticks sourced by Oklahoma State University (OSU), the ticks used in the study were all shipped prior to the 18 October 2021 exposure of the colony to wild females. See Table 3, 'Key characteristics of the ticks used in the study' in Section 4 of the original final report (will be renumbered as Table 4 in the amended final report).
29) Regarding Subject safety: • You must describe the circumstances of the withdrawal of subject 147, including how many test days did subject 147 participate in prior to their withdrawal. • You must confirm that all female subjects and alternates took pregnancy tests on each of the 3 test days and on the day of the attractiveness test/tick handling training (how many, which instances). Describe the steps taken to maintain the privacy of female subjects during this process. • You must describe the COVID-related precautions taken during the consent, attractiveness testing/tick handling training, and on the test days. • You must confirm that all applicable COVID-related restrictions at the local, state, and federal level were followed during the conduct of the study. • You must confirm - did any subjects receive bites during any of the tick handling training days or product testing days? • You must confirm - were any adverse events/reactions reported by subjects after the test day? • You must explain why the deviation related to tick scoring (deviation 10), which was related to the safety of subjects, was not submitted to and reviewed by Advarra IRB. The study report notes that "the deviation was made to protect subjects from	Regarding the last bulleted point, please refer to our response to EPA Comment #12 where we address this issue. Responses to remaining points in EPA Comment #29 are as follows: ● Subject 147 was mistakenly reported as having withdrawn when in fact a stop rule had been invoked. Subject 147 participated in testing until the Study Director chose to conclude the study day due to reaching the limit of consented duration. Subjects gathered at the laboratory at approximately 0800. The last testing interval was recorded as commencing at 2136 hours. The testing interval that would have followed after, if it had been conducted, would have concluded more than 14 hours after the initial arrival time, potentially exceeding the limit of the consented duration ('up to 14 hours' per the Informed Consent Form). The Study Director ended the testing for all subjects that day per Study Protocol section 4.7.6, first stop rule under the 'All Subjects' category. Thus subject 147 did not withdraw. Raw data records (page 304) clearly show no clock times for intervals were recorded on that date for any interval after the 45th, nor were any data taken. This is because there were no exposures after the 45th that day; the Study Director stopped the test at interval 45, for the reason of having reached the limit of consented time. The data for subject 147 was classified as right-censored for the purposes of analysis and in accordance with the Study Protocol. ● Pregnancy tests were administered to all female subjects at the start of any lab or study visit where subjects were potentially to be exposed to repellent or ticks. No

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having ticks get under clothing where they were likely to be difficult to recover and more likely to bite." (p. 25). • The protocol states that "unless required as modifications to immediately ensure subject safety, any changes that may affect the health or safety of study participants … must be reviewed and approved by the Study Director, the approving IRB…" (p. 57 of 103). Even immediate changes to ensure subject safety must be submitted to the IRB within a certain timeframe. You must confirm that this occurred.	records were made to maintain subject privacy. Checkmarks on the Research Candidate/Subject Checklist and the Study Director's Note to File (Appendix 3 of the final report) certify completion of pregnancy testing for each female subject, but do not record the outcome of the test. In Section 2 of the final report, a new section will be added by amendment as follows: ***"Pregnancy testing*** **All female subjects took pregnancy tests in a private restroom prior to their participation in the attractiveness testing and tick training, as well as on each test day they participated as a treated test subject or alternate (see also Note to File, Appendix 3). Results of a female subject's pregnancy test were observed by only one female CLBR staff member. No record of the result was made, only the notation on the Research Candidate/Subject Checklist and the Study Director's Note to File that the testing had been completed by the subject (Appendix 3). Used pregnancy tests were disposed of in an opaque plastic bags provided by the female CLBR staff member assisting. Each female subject of child bearing potential self-checked for pregnancy using the over-the-counter test kit provided by CLBR, taking a test on the day of each study visit in which repellent was applied or in which the subject was exposed to ticks. After the subject self-administered the pregnancy test, the subject was asked if she wanted to continue participating as a subject. If the subject's answer was yes, the female CLBR staff member attending confirmed the test result by visual inspection."** • The Note to File in Appendix 4 (page 233 of the original final report) states the list of screening symptoms and the fact that the list was the same across local, state, and federal levels. Recommended precautionary procedures were also uniform across jurisdictions. The final report will be amended to clarify these points by adding a new subsection to Section 2, as follows:

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	*"**COVID precautions*** **A summary of COVID-19 precautions taken throughout the study and records of the screening of subjects for COVID-19 symptoms are provided in Appendix 4. Prior to entry to the laboratory on each study day, each subject was asked individually if they had experienced any symptoms according to the Center for Disease Control's COVID-19 symptom list (provided in a Note to File found in Appendix 4). Each subject was physically handed in hardcopy to review prior to entry. Once subjects confirmed they had not experienced any of the listed symptoms, they were allowed to enter the facility.** **No subjects screened positive for any symptoms on any of the study days. Subjects wore masks at all times while indoors, except when actively eating or drinking. Approved skin sanitizers and sanitizers intended for plastic and/or metal surfaces were present in sufficient quantity and distributed within laboratory spaces. Surfaces were sanitized before subject arrival on any visit day. At the time of the study, current California and Yolo County regulations stated that masks should be worn at all times while indoors and social distancing should be maintained when possible. Both of these precautions were taken during all study days and laboratory visits by all subjects, excepting during short duration study activities that required closer proximity of a researcher to a subject, such as limb measurements, repellent applications, and limb inspections for skin conditions prior to repellent applications."** ● No adverse events or reactions were observed by staff or reported by subjects after the test day. No subjects received tick bites. Section 6, subsection *Events Reported*, of the final report will be amended accordingly.

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30) Regarding <u>Compensation</u>: • You must explain the compensation provided to the subjects that were unable to participate on test days and were replaced with alternates. • You must explain the compensation provided to the alternate subjects who were on-call and those that arrived in-person for test days. • You must confirm that subjects were compensated for attending the consent meeting, regardless of whether they consented.	The final report will be amended to clarify the compensation provided to alternate subjects. See also responses to EPA Comments #13, #14, and #15 for clarification of the use of the term 'on-call'. Payment was to each subject regardless of role and for each hour of participation for every phase, rounding up to the nearest hour, including the time spent in the consenting interview, for which each subject was paid at the end of the first site visit. All candidates completed consenting and were paid for that time. Alternates are subjects and were paid for the time they were on site, rounded up to the nearest hour, whether they were called upon to replace a treated subject or not.