August 23, 2022

Via Central Data Exchange

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

Re: Action Case Number 00337930, EPA File Symbol Number 93616PA12

Dear Ms. Hollis:

On behalf of Mimikai, Inc. (Mimikai), Bergeson & Campbell, P.C. (B&C®) is responding to the U.S. Environmental Protection Agency's (EPA) June 17, 2022, 75-Day Deficiencies letter. The 75-Day letter (Attachment 1) reflects EPA's remaining comments following its cumulative review of Mimikai's Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray with Ticks Under Laboratory Conditions (Master Record Identification (MRID) 517706-01, Carroll-Loye Biological Research (CLBR) Study Number MIM-007), and CLBR's responses to EPA's May 6, 2022, preliminary technical screen results.

In the 75-Day letter, EPA concluded that Study Number MIM-007 was INCOMPLETE due to a lack of clarity regarding specific experimental details and methodologies used for testing repellency. In response to EPA's conclusions, technical responses prepared by the testing facility, CLBR, are provided in Attachment 2 (enclosed). Based on CLBR's responses, Mimikai believes that Study Number MIM-007, with amendments as indicated, is sufficient to support the proposed tick label claims for registration of its Lilly Pilly Repellent. We are pleased to discuss any outstanding questions and the path forward for study acceptance and Office of Pesticide Programs consultation with the Human Studies Review Board.

We look forward to your review. If there are any questions, please contact Dana Lateulere, B&C. at 202-557-3832.

Sincerely,

Dana S. Lateulere

Attachments

ATTACHMENT 1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

17 June 2022

E-MAILED

Ms. Dana Lateulere
Bergeson & Campbell PC
Agent for Mimkai, Inc.
2200 Pennsylvania Ave.
Washington, D.C. 330031-1701

Deficiencies: Review of the response to the Agency's 10-day letter dated 05/6/22
PRIA Code: M002
Product Name: Field Test Efficacy Report, Mimikai, Inc., Study MIM-007 -Ticks
EPA File Symbol: 93616PA12
EPA Receipt Date: 01/06/2022
PRIA due date: 10/27/2022
Action Case Number: 00337930

Dear Ms. Lateulere:

The U.S. Environmental Protection Agency (EPA) has received and begun its in-depth review of the subject application and has determined that it is incomplete, and that further information is needed. This letter is a written notification of the and identifies your options under 40 CFR § 152.105.

The U.S. Environmental Protection Agency (EPA) has received and begun its in-depth review of the subject application and has determined that it is incomplete, and that further information is needed. This letter is a written notification of the deficiencies and identifies your options under 40 CFR § 152.105 and Section 33 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Your options under 40 CFR § 152.105 and Section 33 of FIFRA are addressed separately because each involves a different time frame and set of options for responding to this letter. Please ensure that you consider each of the sections below in determining how and when you respond to this letter.

40 CFR § 152.105

In accordance with 40 CFR § 152.105, you are allowed 75 days from the date of this letter, ending August 31, 2022, to provide a response concerning the deficiencies listed in this letter. Your response may include making the corrections or additions (additional information to supplement or complete the studies that have already been submitted) to complete the application, or notifying the EPA of the date on which you expect to complete the application or withdrawing your application. If your response includes new studies, the Agency does not expect to prioritize review of those studies or renegotiate

PRIA timeframes in order to do so. If you do not respond to this letter within 75 days or if you respond with a date on which you expect to complete the application but fail to meet that scheduled date, the EPA will treat the application as if you had withdrawn it. Withdrawal concludes the EPA's review of your application. Any subsequent submission of the same application must then be submitted as a new application with a new deadline for the EPA to make a determination on your application and, as applicable, subject to a new registration service fee.

At this time, the EPA has identified the outstanding deficiencies in its review of the subject application. Please refer to the attached deficiency report for questions that must be addressed. Further review of your application and your response to the deficiencies may identify additional deficiencies and you will be so informed.

FIFRA Section 33/PRIA

This application is also subject to a deadline for making a determination on this application under FIFRA Section 33, Pesticide Registration Service Fees, established under the Pesticide Registration Improvement Act of 2003 (PRIA). The time frame for the EPA to make a determination on this application ends on August 31, 2022. To respond to the deficiencies, you have the following three options:

1. **Establish a New Due Date and Resolve the Issues.** You may work with us to establish a new Section 33/PRIA deadline that allows for an appropriate response to the 75-day letter. If you choose this option, you need to contact the EPA no later than August 31, 2022, to discuss a time frame that allows you to address the deficiencies listed above and the EPA to make a regulatory decision.
2. **Withdraw the Application.** Alternatively, you may notify us no later than August 31, 2022, that you are withdrawing your application. As discussed previously in this letter, withdrawal concludes the EPA's review of your application; however, you may resubmit your application after the deficiencies have been addressed. Should you choose to resubmit your application, it *or* they would be subject to a new deadline for making a determination on your application and a new registration service fee. Since a fee was paid, the EPA will provide any applicable refund as soon as practicable.[1]
3. **Not Respond.** If the EPA does not hear from you by August 31, 2022, the Agency, in meeting its obligation under Section 33/PRIA, may issue a determination to not grant your application. While a determination to not grant your application would allow the EPA to have met its obligation under Section 33 of FIFRA to issue a determination by a specified date, this determination is neither a denial f the application pursuant to Section 3(c)(6) of FIFRA nor withdrawal of the application. Thus, the EPA will continue to diligently work on any such application as long as the EPA receives a response to a deficiency notice within the 75 days described previously in this letter.

[1] See https://www.epa.gov/pria-fees/overview-pria-fee-reduction-and-refund-formula for more information on refunds.

Please respond to this letter by August 31 , 2022, by contacting Andrew Bryceland via email at Bryceland.andrew@epa.gov with a response and for any questions concerning this letter. When submitting information or data in response to this letter through the Central Data Exchange (CDX) portal, a copy of this letter should accompany the submission to facilitate processing.

Sincerely,

Linda Hollis, Chief
Biochemical Pesticides Branch
Biopesticides and Pollution
Prevention Division (7511M)
Office of Pesticide Programs

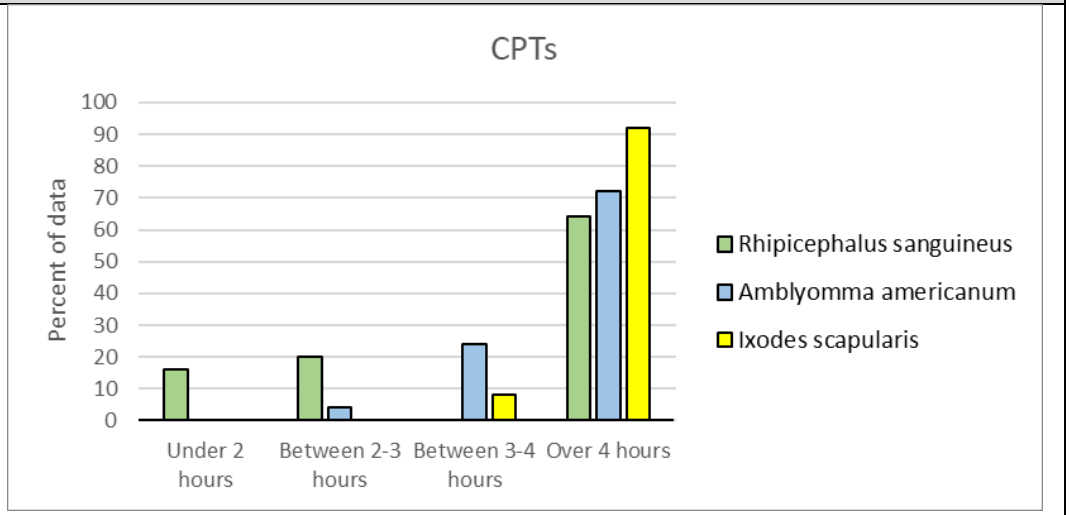
Enclosure(s)

ATTACHMENT 2

EPA Comments	*Registrant Responses*
(A) Outstanding Deficiencies	
Comment #2, 2nd bullet point • The registrant response involved assertions that point to inherent biological behaviors of *R. sanguineus* questing that may play a part in affecting the variability observed in the protection time outcomes. The variability seen in this *R. sanguineus* data is inconsistent with other *R. sanguineus* data recently reviewed by the Agency. Therefore, an argument involving intrinsic biological attributes is **NOT** supported. What are potential methodological explanations (e.g., tick handling procedures and/or holding/maintenance conditions) for the wide variability observed in the *R. sanguineus* data (see Section B questions)? • The registrant response also noted that "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	• In response to the concerns raised by EPA regarding the variability observed in *Rhipicephalus sanguineus* tick behavior in Study No. MIM-007, Carroll-Loye Biological Research (CLBR) re-evaluated statistically the data in two ways: (a) variance for each of the three species in Study No. MIM-007 was lower than the variance of the data in an analogous tick efficacy study reported by ARCTEC for Citrefine International Ltd. (Citrefine) in 2020[1], and reviewed by EPA; and (b) comparisons within the study data show variance as expressed by standard deviation was not significantly different between any two species in Study No. MIM-007. The Citrefine study evaluated the same three tick species using a study design nearly identical to CLBR's. The statistical analyses comparing CPT variance between those studies are provided below in Annex I, entitled, 'Investigating Variance of CPT in Selected Studies of Tick Repellent Efficacy'. Upon review of the study record for MIM-007, CLBR finds no documented aspects of study conduct, including tick handling, that suggest a methods-based source of variance in CPTs for *R. sanguineus* or the other species tested. Based on the results of the comparison of variance of CLBR's *R. sanguineus* data with the data from the

[1] Jones, Robert T. (2020) Single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against three species of ticks. Sponsored by Citrefine International Ltd. Unpublished Report. Study Completed November 14, 2019; Updated and Submitted April 23, 2020. 4562 pages. MRID 511322-01.

EPA Comments	*Registrant Responses*
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." The proposed label claims to have a CPT of 4 hours for ticks. However, *Rhipicephalus* had 9 out of 25 subjects that provided CPTs lower than 3 hours (4 of these CPTS were lower than 2 hours). Nine data points out of 25 is not "a small number of outcome changes". No CPT estimates below 3 hours were observed for *Ixodes*. Only 1 data point for *Amblyomma* provided a CPT of less than 3 hours (2 hr 56 minutes), and 2 data points provided CPTs between 3-4 hours. Therefore, the *Rhipicephalus* data in the study report shows the repellent starts to fail at earlier timepoints for this species than for the other tick species tested. The response quoted above does **NOT** support the assertion that the data for this species would be more akin to the *Amblyomma* dataset with just a small number of outcome changes. Regardless of hypothetical small outcome changes, the Agency can only review the raw data that has been presented to assess product performance.	comparable ARCTEC study, as well as the observed behavior of this species of adult ticks under the conditions of CLBR's study, CLBR concludes that the observed variance in complete protection times (CPT) is to be expected with *R. sanguineus* adult laboratory-reared ticks when tested according to EPA guidelines. • CLBR agrees that 'the Agency can only review the raw data that has been presented to assess product performance'. CLBR maintains that the *R. sanguineus* data are valid and should be included in the data review for regulatory decision making based on its utility in the context of the three-species data set generated during the study, and that the variance of the *R. sanguineus* data was not statistically significantly different than variance of the other species in the study. Comparison with variance in the ARCTEC (2020) study data suggests that the variance is lower in the *CLBR* study (see Annex 1, below). Across pooled species, the variance in *R. sanguineus* data in the CLBR study is seen mainly between less common, shorter CPTs and more common CPTs greater than four hours (figure below; overall median CPT is 5 hours 56 min).

EPA Comments	*Registrant Responses*
	 Further details are provided in Annex I below.
Comment #7 *You must confirm that the attractiveness criteria proposed in the study protocol (Appendix 1, p. 52 of 403) were used for exclusion/removal of unattractive subjects from study participation.* The registrant response was acceptable for this question. However, the response also noted that the training document entitled '§1.b. Handling ticks and observing their movement on the skin (version date 23 Dec 2020)' (Appendix 2)' were followed by the researcher.	• Noted for future studies; the recommendation to revise the training document and/or protocol is no longer relevant to this completed study. To confirm, the version of the training document used in this study (dated 23 Dec 2020) lists forceps and training with them in the goals of the training announced to the subject [item (A)(1)], but the document does not make clear that the procedures described for using the paintbrush, as noted by EPA, were repeated with forceps and tweezers. The following language is proposed for addition to Section 2, subsection 'Training', end of first paragraph, in an Amended Final Report (newly added text is bolded and underlined):

EPA Comments	*Registrant Responses*
Deviation 3 (p. 23 of 403) noted that paintbrushes were only occasionally used for prompting tick movement or correct orientation towards the subject's forearm, and that most tick handling actions were performed with forceps. According to the training manual (Appendix 2, p. 188 of 403), tick transfers onto and off subject forearms were done with a paintbrush. • The text in the training document of the report (p. 188 of 403) should be revised to accurately portray the step-wise procedures used to train subjects in handling adult ticks. • The main report (appropriate sections preceding the appendices) should be revised to clarify the procedures for using forceps and/or paintbrushes for handling ticks during exposures on test days. The revised text should clearly describe, in detail, all tick transfer steps used by subjects in a chronological order.	***Note the training document lists training with forceps and tweezers, but does not clearly state that the procedures described in detail for training in the use of an artist's paintbrush for manipulating ticks were repeated for use of forceps and tweezers during the training session of each subject. During the training process, the use of forceps for tick placement and removal was clearly shown to be superior to the use of artist's paintbrushes or tweezers. Tweezers were not used subsequently, and brushes were only used subsequently for re-orienting ticks***. • The requested report revisions to clarify the procedures for using forceps and/or paintbrushes for handling ticks during exposures on test days will be addressed in an Amended Final Report (see B #5 below). In response to EPA's comment here and in Section B #5, below, the sequence of tick handling procedures followed during study execution of efficacy challenge exposures is summarized as follows: 1. Beginning of observation period was announced; 2. Subject used forceps to pick up a tick from the supply container; 3. Subject placed tick on the palm of the hand of their own untreated arm; 4. Subject oriented arm as instructed and using the angle template to achieve a ~30 degree forearm angle to encourage movement of the tick from the palm of the hand towards the elbow; 5. If the tick moved in any other direction, or stayed stationary, the subject used at their own preference forceps already in hand from placing the tick or an artist's paintbrush to gently reposition or prod the tick in the orientation or direction of movement towards the elbow. To do so, a subject would gently touch the tick without picking it up off of the skin.

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	6. If the tick repeatedly failed to initiate and sustain motion towards the elbow, the subject removed the tick using forceps and placed it in a designated vial labeled for "USED" ticks, then began again with another tick. 7. If the tick showed sustained movement towards the elbow, and passed into the skin area of the forearm more than 3 cm in 3 minutes, the subject then used forceps to move the tick onto the palm of the hand of their treated arm. 8. Subject oriented their arm as instructed (see below) to encourage movement of the tick from the palm of the hand towards the elbow 9. If while in the untreated area of palm skin the tick moved in any other direction, or stayed stationary, the subject might use at their own preference forceps or an artist's paintbrush to gently reposition or prod the tick in the orientation or direction of movement towards the elbow. To do so, the subject gently touched the ticks without picking it up off of the skin. 10. If the tick moved towards then away from the line demarking the treated skin area, or moved laterally along that line without entering the treated skin area, or entered the treated area and turned around or stopped motion without passing more than 3 cm into the treated skin area within 3 minutes, the subject removed the tick with forceps and placed it in a "USED" vial, then placed the forceps in the discard jar for cleaning, and informed the attending researcher to note the tick as non-crossing. If the tick crossed into the treated skin area more than three centimeters within three minutes, the subject removed the tick with forceps and placed it in a "USED" vial, then placed the forceps in the discard jar for cleaning, informing the attending researcher to note the tick as crossing.

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	NOTE: only forceps were used for picking up ticks and relocating them because adult ticks typically did not climb onto and cling to brushes. Both brushes and forceps were effective when used to re-orient a tick or prompt movement without picking the tick up off of the skin surface. 11. The end of an exposure period was announced. 12. Researchers monitored subjects' supplies at the end of each exposure period, removed vials of used ticks and containers of used forceps to the researcher's central station, dispensed the used ticks into kill jars, and replenished fresh tick vials and clean forceps to subject stations as needed. Used paintbrushes were collected and replaced with new ones. 13. After all subjects had completed exposures, the unused ticks that had no contact with subjects or Tests Material, were returned to the insectary and to the environmentally-controlled 'desiccation' jars for sustaining storage. Kill jars were emptied into self-sealing freezer bags labelled with species and test date then placed in a freezer for storage.
Comment #11, 1st bullet point *If a balanced gender ratio was not achieved, how did the procedure for assigning the 31 subjects as treated versus alternate status differ from the gender-stratified approach proposed in the protocol?* - The registrant response noted that the stratification procedure proposed in the Study Protocol for the assignment process was followed to the extent possible. Based on the response to this first bullet point and the *Subject Participation Chart*, the	CLBR proposes the following modifications to EPA's proposed summary (added text is bolded and underlined; deleted text is bolded and struck through): 'Subject numbers were entered into two separate gender-specific lists in Excel. Alternating between these lists, the "RANDBETWEEN" and "CHOOSE" functions in excel were used to create two randomly generated lists of subject numbers for each gender. Alternating between these randomized lists, CLBR staff contacted subjects until 25 subjects were obtained that **initially indicated they** were available for 3 days of testing against 3 tick species. ~~**However, only**~~

EPA Comments	*Registrant Responses*
Agency attempted to write a summary of the subject selection, randomization, and assignment process used for MIM-007 (bullet below). Is the summary accurate? If not, a clear summary of the subject selection, randomization, and treated/alternate assignment process used should be provided. • *'Subject numbers were entered into two separate gender-specific lists in Excel. Alternating between these lists, the "RANDBETWEEN" and "CHOOSE" functions in excel were used to create two randomly generated lists of subject numbers for each gender. Alternating between these randomized lists, CLBR staff contacted subjects until 25 subjects were obtained that were available for 3 days of testing against 3 tick species. However, only the 10 females that participated in MIM-006 were available to participate in testing with all three tick species, resulting in an uneven gender ratio (male: female) of treated subjects (either 15:10 or 14:11) used for testing each tick species (see Subject Participation Chart). Therefore, the gender-stratified randomization procedure described above was followed to the extent possible during the role assignment process,*	**Eight of** the 10 females that participated in MIM-006 **and two additional females (subjects numbered 41 and 171)** were **confident they were** available to participate in testing with all three tick species. **However, just prior to testing on 10 October 2022, the two remaining female subjects (subjects 12 and 18) indicated they were uncertain of their availability for all three testing days. The Study Director prioritized the likelihood of participation of the same subjects across all species over optimizing the sex balance of subjects, and the timely completion of data collection over re-opening recruitment in an effort to secure more reliable subjects. Subjects 12 and 18 were thus directly assigned the role of alternate rather than chosen at random for those roles. These constraints in combination with a** sequence of withdrawals followed by random selection of alternates result**ed**~~**ing**~~ in ~~**an**~~ uneven **sex** ratios (male: female) of subjects (either 15:10 or 14:11) **participating as treated subjects** for testing each tick species (see *Subject Participation Chart*). Therefore, **during the role assignment process,** the gender-stratified randomization procedure described above was followed to the extent possible**, excepting for the selectively-assigned female alternates,** then subject assignments as treated subjects were completed with males. The **overall process resulted in initial role assignments of 4 female and 2 male alternates, 15 male treated subjects, and 10 female treated subject.** ~~**remaining 6 subjects were assigned as alternates.'**~~ Please also refer to Annex VI, 'MIM-007: Explanation of unbalanced sex ratios among treated subjects across efficacy challenge test days' (below), for a detailed explanation of subject selection via randomization, withdrawals, replacement by alternates, and resulting subject sex ratios for each efficacy challenge test day.

EPA Comments	*Registrant Responses*
then subject assignments as treated subjects were completed with males. The remaining 6 subjects were assigned as alternates.'	See also the amended *Subject Participation Chart* (see Annex VII, below), for a detailed accounting by subject number and date.
Comment #11, 4th bullet point *Part 1* *Provide a rationale for unbalanced sex ratios used on the test days. The deviation and rationale should be clearly stated in the deviations table of the study report.* • The registrant response noted that the deviation of an unequal gender distribution was provided for recruited subjects under deviation #2 in the deviations table (p.23 of 403), which states: "Also due to the issue of candidate and subject availability more male subjects were enrolled than female subjects (17 male, 14 female). Because an exact ratio of male and female subjects was not required for the statistical analysis specified in the protocol, and gender was not a variable in the data analysis, the study director determined there was no effect on data quality." The registrant response also mentioned that the deviation from the intended gender ratios of recruited subjects is addressed in a note to file in Appendix 3 (p. 192 of 403), which also states "Because exact balance of male vs. female subjects was not required, and gender was	*RE: Providing a rationale for unbalanced sex ratios used on the test days.* Please also refer to 'MIM-007: Explanation of unbalanced sex ratios among treated subjects across efficacy challenge test days' (Annex VI, below) for a detailed explanation of subject selection via randomization, withdrawals, replacement by alternates, and resulting subject sex ratios for each efficacy challenge test day. See also the amended *Subject Participation Chart* (see Annex VII, below) for a detailed accounting by subject number and date. Deviation from the planned subject sex ratio was justified by the Study Director as the optimal response to the realized shifting and challenging conditions of subject availability, timeliness of completion of data collection with available ticks, and the ongoing impacts of COVID-19 on the ability to conduct the study. High uptake and frequency of COVID testing in our area along with well-organized contact tracing contributed to considerable sudden quarantining in our area, compromising the reliability of our recruitment process. In practice, just prior to testing on 10 October 2022, the two remaining female subjects (subjects numbered 12 and 18) indicated they were uncertain of their availability for all three testing days. The Study Director decided to prioritize the likelihood of participation of the same subjects across all species over optimizing the sex balance of subjects, and the timely completion of data collection over re-opening recruitment in a potentially futile effort to secure more reliable subjects. Subject numbers 12 and 18 were thus directly assigned the role of alternate rather than

EPA Comments	*Registrant Responses*
not a variable in the data analysis, the Study director determined that there was no effect on data quality". There is no need to account for gender effects in the data analysis if a balanced gender ratio was used. More importantly, both sets of quoted text above addresses unequal enrollment by gender, not the use of unbalanced sex ratios on test days. The gender ratios (M:F) used for each R. sanguineus and A. americanum test days were 15:10, and a gender ratio of 14:11 was used for I. scapularis test days (see Subject Participation Chart). The protocol (Appendix 1, p. 42 of 403) proposed that the 25 treated subjects would include 12 subjects of one gender, and 13 of the other gender. Protocol deviations should be justified. In this case, the gender ratios used for test days (15:10 and 14:11) and an evidence-based rationale for why an unbalanced sex ratio does not compromise the scientific validity of the tick repellent study should be provided in the Appendix 3 note to file as well as the deviations table in the main report.	chosen at random for those roles. These constraints in combination with a sequence of withdrawals followed by random selection of alternates resulted in less balanced sex ratios (male: female) of subjects (either 15:10 or 14:11) participating as treated subjects for testing each tick species Subsequent to initial role assignments, study protocol procedures were followed for replacement of withdrawing subjects, including randomized selection of alternates to replace treated subjects. In response to EPA's remarks, CLBR considered the importance of balanced sex ratios for the study outcomes. The possibility that ticks may respond differently to male and female subjects serves as a rationale for testing with equal numbers of each. In a study with 25 subjects, we would aim for 13 of one sex, and 12 of the other. In CLBR Study No. MIM-007, the sex ratio of subjects that participated in data collection missed that optimum ratio by the addition of two male subjects and loss of two female subjects for *Rhipicephalus sanguineus* and *Amblyomma americanum,* and by the difference of 1 additional male and 1 less female for *Ixodes scapularis*. In choosing to deviate from Protocol-specified sex ratios for consented subjects, the Study Director considered, and CLBR here notes for discussion, the absence of clearly demonstrated experimental evidence in the scientific literature that host sex is an important driver of tick foraging behavior. In the published literature, there are conflicting reports of variation in attractiveness to mosquitoes in response to the sex of human hosts, and repellent efficacy against mosquitoes often correlates strongly with efficacy of the same repellent against ticks. An optimally conservative approach to design repellent efficacy studies with human subjects therefore is to employ balanced sex ratios. On the other hand, there is no

EPA Comments	*Registrant Responses*
	explicit scientific basis for making balancing sex ratios a requirement of or a primary constraint for the design or conduct of a repellency study. Accordingly, CLBR chose to test the available subjects in a timely, scheduled manner rather than delay this study in the hope of achieving a more optimally balanced ratio, *e.g.* 13:12 by re-opening recruitment after withdrawals and substitutions altered our original 13:12 treated subject pool resulted in non-target sex ratio for the first study date. While the sample sizes per sex in this study are relatively small, in response to EPA's comments, CLBR considered informally (without intent to include in an amended final report) in response to EPA's comments, comparing repellency outcomes between sexes in a heuristic vein to examine variability by subject sex. CLBR assessed three metrics: CPT, time to first crossing, and the mean crossing time per subject. Together, these metrics permit us to examine a richer picture for potential correlations of these responses with subject sex, as afforded by the study design and data available. No consistent association of subject sex and crossing outcomes is evident from this informal evaluation (Figure 1, below). In summary, the potential benefits of increased conservativism and representativeness that would have been achieved with a 13:12 subject sex ratio for all three tick species were offset by the ultimately suitable practicality of testing with the recruited subject cohort that was available to participate in the scheduled exposures with all three tick species. CLBR further concludes that in future repellent testing for which it is determined that the same group of subjects must participate in a multi-day trial, it may be advised to create a larger candidate pool than was provided for the present study.

EPA Comments	*Registrant Responses*
Comment #11, 4th bullet point *Part 2*	
• The note to file in Appendix 3 mentions that equal recruitment by gender was done, but more male than female candidates responded to being available for 3 efficacy test days. <u>The questions below should be addressed.</u> • Were 22 female candidates recruited, as proposed in the protocol (Appendix 1, p. 42 of 403)? If so, did the same 10 female subjects that participated in MIM-006 (see Subject Participation Chart) happen to be the only female subjects available to participate for all tick species test days?	• The report contains an error in reporting recruitment of 60 candidates rather than 44, the actual number. Of the 44 candidates recruited for the study, more than 22 were female; the Protocol stipulates 'at least' 22 subjects of each sex would be recruited. A total of only 14 female candidates were initially available to participate in testing against all three tick species; these subjects participated in the study in the subject categories treated, alternate, or both in those cases where a female alternate took the place of a withdrawing or removed subject (see '<u>MIM-007: Explanation of unbalanced sex ratios among treated subjects across efficacy challenge test days</u>' (Annex VI, below) and the amended *Subject Participation Chart* (Annex VII, below)). Of those 14 subjects, 10 of them also participated in Study No. MIM-006.
• Provide clarification details on the candidate screening processes that lead to those 10 females being selected out of 60 respondents.	• Staff conducted screening first by the phone script verbatim. All candidates passed initial screening criteria listed in the phone script. When presented with the testing schedule at the end of the phone interview, only 14 female candidates were able to confirm their availability to participate. Those who did and who otherwise indicated interest in continuing to participate were scheduled for a consenting interview. In the consenting interview, researchers used the exclusion and inclusion criterion in the informed consent form (ICF, pp 4-5; see p173-174 of the originally submitted Final Report) to further screen candidates for inclusion or exclusion. During the reading of the ICF with the candidate, each

EPA Comments	*Registrant Responses*
• Were two separate recruitment procedures used to obtain two separate candidate pools for use in the mosquito (MIM-006) and tick (MIM-007) studies? If not, provide a clear description of how candidates were recruited for these studies. • How were the nine subjects that only participated in MIM-007 (see Subject Participation Chart) recruited? Were they part of the MIM-006 candidate pool?	criterion was read out loud to the candidate, and the candidate was asked to confirm that they met the criterion. During this process, all 14 female candidates confirmed their qualification for inclusion. • Separate recruitment advertisements were used for Study Nos. MIM-006 and MIM-007. Both advertisements were disseminated to the same outlets. By this means, many candidates who had expressed interest in participating in study MIM-006 also expressed interest in participating in MIM-007. In addition, it was common for candidates and subjects for MIM-006 to ask if there were other CLBR studies they might be eligible to participate in. Researchers were allowed to mention the upcoming tick study in response to such questions. The MIM-007 candidate list was randomized prior to call-backs according to the amended Study Protocol (pg 14; see pg 42 of the originally submitted final report), as follows: 'Each candidate in order of contact will be assigned a sequential number unique to them and to this study, then the candidate numbers list randomized in Microsoft Excel.' • The subjects that only participated in MIM-007 were recruited in the same manner as all other subjects in the study, as described above. Five subjects who only participated in MIM-007 were also candidates (not necessarily participants) in MIM-006. From this process, female candidates who indicated they were interested in enrolling and would be available for multiple study dates were selected for follow-up interviews for consenting until all female candidates had been contacted via call-backs. By this method, a process of elimination from randomized call-backs, CLBR arrived at a total of 14 female candidates, all of whom became subjects. The remaining 17 subjects were male by default. This selection process did not distinguish candidates on the basis of prior participation

EPA Comments	*Registrant Responses*
	in study MIM-006, or prior appearance as candidates for study MIM-006. In our recruitment efforts, CLBR did not consider whether candidates for study MIM-007 were also candidates for study MIM-006. All such overlaps resulted from the phenomena of advertisement of both studies in the same media combined with word-of-mouth communications among candidates and among subjects, and researcher responses to inquiries by either candidates or subjects of study MIM-006. There was no mechanism in the process which would cause restraining of recruitment for MIM-007 by MIM-006 other than the geographic location of recruitment and placement of advertisements via the same media. CLBR calculates that 81% of subjects in study MIM-007 were also candidates (though not necessarily consenting subjects) in study MIM-006.
Comment #11, 6th bullet point *Explain the meaning of "all subjects will be assigned to the treated group, and blocked by gender" in the protocol (Appendix 1, p. 51 of 403). Additionally, clarify if this gender blocking was used in the tick study.* The registrant response explains how all the subjects were assigned to the treated group but is incomplete in addressing what "blocked by gender" means and clarifying if this gender blocking was used in the study. <u>This comment is still outstanding and must be addressed.</u>	The phrase 'blocked by gender' was used in a broad sense to describe the process, previously detailed, by which the candidate pool was resolved into roughly equal numbers of male and female subjects, and then assigned treated versus alternate status. The study design in this study, by its nature, precludes the use of blocking because all subjects serve as treated and control exposures. Accordingly, the more usual and formal sense of the phrase 'blocking', as applied to study design and statistical analysis, did not apply here.

EPA Comments	*Registrant Responses*
Comment #12 *A statement in the study report states. "All deviations were evaluated by the Study Director in regard to Advarra IRB's Investigator's Handbook to determine the need to report them to the IRB for the IRB's evaluation; none qualified for reporting." 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?* The registrant provided an off-topic response to question #12 and did not clarify any of the points above. The entirety of comment #12 is still outstanding and must be addressed.	• When considering if a deviation merits reporting it to the IRB, the Study Director evaluated the deviation with respect to the guidance provided by the IRB. The Advarra IRB-supplied decision-making flow chart kept on record for all CLBR studies subject to Advarra IRB's oversight. Annex III below, entitled 'Deviation Evaluation for the Purpose of Reporting' includes 'ADVARRA Subject Safety Event Reporting Decision Charts' (Figure III-A) that detail the decision-making pathways the Study Director used in study MIM-007. This Annex III tabulates by numbered deviation (as summarized in Table 6 of the Final Report) the decision-making path that led up to the decision not to report each deviation to the IRB. • All study deviations from the protocol were addressed in Table 6 of the Final Report. Each deviation was evaluated by the Study Director for its potential to increase risks of harm to subjects, its likelihood of recurring, possible impacts on the conduct of the study, and influence on data quality. Those criteria accord with the published guidance by the overseeing IRB of oversight, which expects such evaluation by the Study Director. • There were no deviations that were evaluated, but not addressed in the Final Report.

Comment #14 – *Provide 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?* - The registrant provided a response noting that the term "on-call" is confusing and will be removed from the final report, and that clarification will be provided for Deviation #7 in the amended final report to address comment 13. It was stated that "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." This statement is the opposite of what was originally written for Deviation 7, where it was noted that on-call subjects could make it to the testing site within 20 minutes. The registrant response did not clarify any of the points above. These comments are still outstanding and must be addressed.	• No specific directions were given to alternates in regards to being "on-call", as none were needed. Alternate subjects did not in any way actively participate on 'minimal subject' days, nor were alternates asked to appear for more than the consented number of study visits, which would have resulted in a protocol deviation (*i.e.*, if alternates had participated by showing up to the laboratory on those days). • Subject replacements that occurred on non-minimal, but reduced subject days were by alternates physically present at the lab on those days. - For 'minimal subject' days, the term "on call" was used to indicate a conceptual status of alternate subjects, not a physical one. The concept was that since no subjects consented to being present for more than one efficacy challenge study visit per tick species, any subject withdrawing on a minimal subject day could and would be substituted in the following test day for the same species, rather than on the minimal subject day. Please see proposed Note to File (Annex V) titled, 'Realized Use of Alternates in Study No. MIM-007.' The phrase *"no alternates were waiting for a call or text communication per se"* means that for 'minimal subject' days, the term "on call" was used to indicate a conceptual status of alternate subjects, not a physical one. As noted above, no subjects consented to being present for more than one efficacy challenge study visit per tick species, so any subject withdrawing on a minimal subject day were to be substituted the following test day for the same species, rather than on the minimal subject day.

EPA Comments	*Registrant Responses*
- Clarification is needed for unclear statements made in the registrant response to comment #14 by answering the bulleted follow-up questions below. • Explain the meaning/definition of "on-call alternates" in the context of study procedures with alternates? The term was noted as confusing (see review of response above) but remains to be clarified. A clear definition must be provided, regardless of future report amendments. • Amendments to the term "on-call" were proposed but the text of these amendments should be provided and subject to Agency Review. • Explain the phrase "no alternates were waiting for a call or text communication per se"?	ADDITIONAL DISCUSSION: Existing documentation in the form of Notes to File require clarification. The 11 October 2021 Note to File (Final Report, Appendix 3) was issued to record the timing and scope of decisions regarding the use of alternates based on emerging difficulties with scheduling subjects for the remaining species' efficacy test days. The arrangement intended to be communicated in the 11 October 2021 Note to File, was to allow alternates not to appear on the site on 'minimal subject' test days. This is the meaning of the sentence, *"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."* A withdrawal from a 'minimal subject' day would merely result in the automatic assignment by random selection of an alternate into the group of treated subjects on the second day for that tick species. See Annex V for proposed amendment language to clarify these issues in the Final Report.
Comment 27 *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 response to comment 27 needs additional information regarding the reminder phone calls and emails used to communicate with subjects two days prior to each test day. Specifically, were subjects reminded to avoid the use of repellents during the 48 hours before each test day?	Yes; subjects were reminded to avoid the use of repellents within the 48 hours prior to each test day.

EPA Comments	*Registrant Responses*
(B) Additional Questions Regarding Study Methods	
1. Provide clarification to the points below regarding tick maintenance conditions at CLBR. a. Why are different values (24°C; :88-90% relative humidity) listed in Appendix 8 (p. 318) for tick holding conditions compared to the values in the study report (22-27° C, ~95% relative humidity; p. 15 of 403)? b. Were ticks held in the insectary only, or were there two sets of tick storage/maintenance conditions? c. Describe the vessels in which the ticks were held (materials, dimensions, methods of preventing tick escape). d. How were ticks handled during holding times in the insectary/other environmental rooms?	Clarifications to EPA's questions regarding tick maintenance conditions are provided below: a. The environmental data provided in Final Report Appendix 8 pg. 318 (Research Note to File dated 21 September 2021, 'Tick Viability and Conditions under storage) are discrete measurements at the time of the viability check, and are not min/max values. The values in the study report refer to the overall conditions of the insectary during tick storage, which would include the min/max values as listed (22-27°C). b. Ticks were stored in the insectary only until the test day during which they were used. After use they were discarded without re-use. On each exposure study day, sufficient numbers of ticks for the planned activity involving the use of ticks during that study day were quickly relocated within the same building down a short hallway then down one flight of stairs to the exposure study room, which was separately environmentally controlled (environmental data for the exposure study room is reported separately in Final Report Appendix 9, 'Site Characteristics'). c & d. All ticks were received in film-canister style containers (clear polystyrene vials with white plastic snap-on lids, approximately 1-inch diameter and 2 inches tall). The lids of these containers had a single hole covered in fine mesh to allow airflow but prevent tick escape. The lids were further sealed with a layer of flexible cloth tape around the bottom edge of the lid. This tape was carefully replaced every time the vial was accessed.

EPA Comments	*Registrant Responses*
	Upon receipt and a confirmation of tick viability, these canisters were placed in large (gallon) self-sealing bags that were labeled with the date of receipt and species identity. These bags were loosely folded over, but not sealed (zipped) closed, as to allow airflow but also provide a as secondary containment should any ticks escape from the vials. These bags were then placed in large environmentally-controlled 'desiccation' jars with lids (10 inch diameter by 8 inches tall), each labelled by species. These jars were equipped with copper sulfate/H2O humidifiers, and stored in our insectary. Ticks were then removed from the jars when needed for study activities as described below. On test days, researchers carefully transferred ticks from the polystyrene containers to 20mL scintillation vials (sized similarly to the film canisters, approximately 1 inch in diameter and 2 inches tall), distributing approximately 5 ticks per scintillation vial. Ticks allocated for use on study were quickly relocated within the same building to an adjacent room (for tick training and attractiveness screenings) or down a short hallway then down one flight of stairs to the environmentally-controlled exposure study room. Ticks were not removed from containment canisters (clear, polystyrene canisters as described above) for any use until needed for training or study days, with the exception of colony maintenance measures detailed in the Note to File dated 21 Sep 2021 (Appendix 8 of final report), which were performed in the insectary. e. Section 4.7, end of paragraph in the approved Study Protocol states "At the CLBR laboratory and prior to and between test days, ticks of each of the three species will be maintained under the conditions recommended by the rearing facility at the time of shipment." Note the protocol does not state a standardized

EPA Comments	*Registrant Responses*
e. OPPTS 870.3700 recommends using a 16:9 (light: dark) photoperiod for colony maintenance conditions, but the study used a 12:12 (light: dark) photoperiod (p. 15 of 403). Provide a justification for this guideline deviation.	16/9 (light/dark) regime was to be followed. This above statement from the Protocol was intended to mean conditions that were recommended to sustain tick activity. At our facility, CLBR holds ticks that are both summer-active and winter-active in foraging. CLBR conferred with the source laboratories for the ticks used in this study regarding photoperiods that would support foraging in all species tested by CLBR. Based upon those consultations and CLBR's research experience, CLBR determined that the 12/12 photoperiod supports foraging behavior in both summer-active and winter-active ticks. In the study context, suitability of this photoperiod was confirmed by the fact that there were no issues with sustained tick avidity at any point in the study. In the peer-reviewed, published literature, we found two references confirming that a 12/12 photoperiod specifically is supportive of active foraging (as opposed to any other tick behavior) for the species used in Study No. MIM-007. See Annex IV (below), titled 'Day and Night Cycles for Ticks references by species' for additional details.
2) How were ticks handled just prior to use in efficacy testing? Describe temporary holding conditions in the exposure room (e.g., vials held in pans of water) and the duration of these conditions immediately prior to testing.	On test days and immediately prior to the arrival of subjects, researchers working in the insectary carefully transferred ticks from the polystyrene containers to 20mL scintillation vials (sized similarly to the film canisters, approximately 1 inch in diameter and 2 inches tall), distributing approximately 5 ticks per scintillation vial, each of which was sealed with a cap that was partially screened, rather than continuously solid, to allow air flow. Ticks allocated for use on study were quickly relocated within the same building to an adjacent room (for tick training and attractiveness screenings) or down a short hallway then down one flight of stairs to the environmentally-controlled exposure study room. During these discrete periods, ticks were held in vials (20mL scintillation vials approximately 1 inch in diameter and 2 inches tall), with approximately 5 ticks

EPA Comments	*Registrant Responses*
	per scintillation vial, within the temperature- and humidity-controlled exposure study room. A fine mesh plastic screening was attached across the top of each vial to prevent tick escape.
3) Was tick lot source information, number of days at CLBR, and the number of days that have passed since the last blood feeding recorded for each tick lot prior to their use on each test day? If so, please provide a table with these details for each tick lot used on each test day.	Ticks were not blood-fed at CLBR between time of receipt and use in study MIM-007. Tick colonies were tracked by date of receipt rather than by lot numbers, which were not assigned by the Suppliers. No information regarding the time passed since the last blood feeding was known, although starved ticks were requested. Subsequent inquiries to the source laboratories yielded confirmation of the starved status of the ticks. Details regarding tick lots are provided in the appended document 'Details of Tick Colony Timing Including Feeding Regimes' (see Annex II, below).
4) Describe the exposure station set-up used for each subject in detail, in a manner that addresses questions below. a. What equipment/supplies were used to move/position ticks, position treated or untreated arms, hold ticks in a manner that prevents ticks from escaping, or used as holding containers/surfaces? b. What surface did the subjects perform the exposures on (e.g., trays, tabletop, etc.)? c. Were paintbrushes that contacted the repellent product discarded in a designated area of each exposure station after use? d. Forceps were used during tick transfers onto and off subject forearms (p. 23 of 403).	CLBR provides the following clarifications regarding exposure station set-up used for each subject in Study No. MIM-007, as follows. At the appropriate time, an Amended Final Report will be issued to clarify these points within the report, as well. a. All tick positioning (placement and removal) was accomplished with forceps. Tick orientation was accomplished primarily with artist's paintbrushes. Ticks were held in one glass vial per subject, each vial holding 5 ticks, so subjects could easily keep track of them. At the top of these vials, the glass tapers such that ticks would have to traverse a sharp angle (nearly perpendicular to the bottom of the vial) to reach the mouth of the vial. This angle, combined with the glass surface of the vial, caused ticks to fall to the bottom of the vial before reaching the mouth and prevented ticks from rushing the mouth of the vial when it was opened. The ticks could be readily removed from the vials with forceps for transfer to subjects' skin. Subjects kept vials capped and tightly

EPA Comments	*Registrant Responses*
e. Describe any procedures used to avoid the use of repellent-contaminated forceps for picking up 'fresh' ticks, or in general, to avoid cross-contamination?	sealed at all times when not actively removing ticks from vials. Once a tick was successfully taken from its vial, the vial was immediately recapped. b. Subject exposures were performed on tabletops. At each table, one glass vial per subject, each vial containing five ticks, was provided at the start of exposures and replaced by researchers as needed throughout the day. c. No paintbrushes were used on treated skin areas at any time. Paintbrushes were only used to manipulate ticks on untreated skin. Each subject was supplied with their own paintbrushes to use, so brushes were never shared between subjects. Paintbrushes were collected and cleaned with 70% ethanol and rinsed with water at the end of each study day. d. Confirmed; forceps were used during tick transfers onto and off of subject forearms. In cases where forceps were used to remove ticks from treated skin, they were discarded and replaced by clean forceps provided by research staff. e. After a subject or researcher removed a tick with forceps and placed it in a vial marked "USED", they then placed the forceps in the discard jar. Researchers monitored subjects' supplies at the end of each exposure period, removing vials of used ticks and containers of used forceps to the researcher's central station, dispensing the used ticks into kill jars, and replenishing fresh tick vials and clean forceps to subject stations as needed. Paintbrushes were only used to manipulate ticks on untreated skin. Each subject was supplied with a clean set of paintbrushes for their exclusive use on that test day, so that brushes were never shared between subjects or across test days. Paintbrushes were collected and cleaned with 70% ethanol and rinsed with water at the end of each study day. At the researcher's central station, unused ticks that had no contact with subjects or Test Material were returned to the insectary and to the environmentally-controlled 'desiccation' jars for sustaining storage. Jars filled partially with 70% ethanol served to drown used ticks. These kill jars were

EPA Comments	*Registrant Responses*
	emptied into self-sealing freezer bags labelled with species and test date, then placed in a freezer for storage. Researchers monitored supplies of ticks and clean forceps at subject stations, replenishing as needed. Used forceps were removed from the exposure area, cleaned with mild detergent, water, and 70% ethanol, then allowed to air dry prior to any re-use.
5) All tick handling procedures (see questions 2-4 above) should be described in chronological order in the main report (appropriate sections preceding appendices) including all steps from transfers out of the insectary/environmental rooms to freezing tick vials at the end of the test day.	The tick handling procedures detailed above in response to EPA Comments B/2-4 are summarized as follows; these details will be reflected in an Amended Final Report at the appropriate time: All ticks were received from suppliers in film-canister style containers (clear polystyrene vials with white plastic snap-on lids, approximately 1-inch diameter and 2 inches tall). The lids of these containers had a single hole covered in fine mesh to allow airflow but prevent tick escape. The lids were further sealed with a layer of flexible cloth tape around the bottom edge of the lid. This tape was carefully replaced every time the vial was accessed. Upon receipt and a confirmation of tick viability, these canisters were placed in large (gallon) self-sealing bags that which were labelled with the date of receipt and species identity. These bags were loosely folded over, but not sealed (zipped) closed, as to allow airflow but also provide a as secondary containment should any ticks escape from the vials. These bags were then placed in large environmentally-controlled 'desiccation' jars with lids (10 inch diameter by 8 inches tall), each labelled by species. These jars were equipped with copper sulfate/H_2O humidifiers, and stored in our insectary. Ticks were then removed from the jars when needed for study activities as described below.

EPA Comments	*Registrant Responses*
	Ticks were stored in the insectary until the test day during which they were used prior to being discarded without re-use. On each study day, sufficient numbers of ticks for the planned activity involving the use of ticks during that study day were quickly relocated within the same building down a short hallway then down one flight of stairs to the exposure study room, which was separately environmentally controlled (environmental data for the exposure study room is reported separately in Final Report Appendix 9, 'Site Characteristics'). Ticks that were handled by subjects and discarded were not re-used between days. On test days and immediately prior to the arrival of subjects, researchers working in the insectary carefully transferred ticks from the polystyrene containers to 20mL scintillation vials (sized similarly to the film canisters, approximately 1 inch in diameter and 2 inches tall), distributing approximately five ticks per scintillation vial, each of which was sealed with a cap that was partially screened, rather than continuously solid, to allow air flow. Ticks allocated for use on study were quickly relocated to an adjacent room (for tick training and attractiveness screenings) within the same building or down a short hallway then down one flight of stairs to the environmentally-controlled exposure study room. Once all subjects were ready for exposures, the following sequence of tick handling procedures was executed by each subject: 1. Beginning of observation period was announced; 2. Subject used forceps to pick up a tick from the supply container; 3. Subject placed tick on the palm of the hand of their own untreated arm;

EPA Comments	*Registrant Responses*
	4. Subject oriented arm as instructed and using the angle template to achieve a ~30 degree forearm angle to encourage movement of the tick from the palm of the hand towards the elbow; 5. If the tick moved in any other direction, or stayed stationary, the subject used at their own preference forceps already in hand from placing the tick or an artist's paintbrush to gently reposition or prod the tick in the orientation or direction of movement towards the elbow. To do so, a subject would gently touch the tick without picking it up off of the skin. 6. If the tick repeatedly failed to initiate and sustain motion towards the elbow, the subject removed the tick using forceps and placed it in a designated vial labeled for "USED" ticks, then began again with another tick. 7. If the tick showed sustained movement towards the elbow, and passed into the skin area of the forearm more than 3 cm in 3 minutes, the subject then used forceps to move the tick onto the palm of the hand of their treated arm. 8. Subject oriented their arm as instructed (see below) to encourage movement of the tick from the palm of the hand towards the elbow 9. If while in the untreated area of palm skin the tick moved in any other direction, or stayed stationary, the subject might use at their own preference forceps or an artist's paintbrush to gently reposition or prod the tick in the orientation or direction of movement towards the elbow. To do so, the subject gently touched the ticks without picking it up off of the skin. 10. If the tick moved towards then away from the line demarking the treated skin area, or moved laterally along that line without entering the treated skin area, or entered the treated area and turned around or stopped motion

EPA Comments	*Registrant Responses*
	without passing more than 3 cm into the treated skin area within 3 minutes, the subject removed the tick with forceps and placed it in a "USED" vial, then placed the forceps in the discard jar for cleaning, and informed the attending researcher to note the tick as non-crossing. If the tick crossed into the treated skin area more than three centimeters within three minutes, the subject removed the tick with forceps and placed it in a "USED" vial, then placed the forceps in the discard jar for cleaning, informing the attending researcher to note the tick as crossing. 11. The end of an exposure period was announced. Researchers monitored subjects' supplies at the end of each exposure period, removing vials of used ticks and containers of used forceps to the researcher's central station, dispensing the used ticks into kill jars, and replenishing fresh tick vials and clean forceps to subject stations as needed. Used paintbrushes were collected and replaced with new ones at each station, as were forceps. After all subjects had completed exposures, the unused ticks that had no contact with subjects or Test Material were returned by a researcher to the insectary and to the environmentally-controlled 'desiccation' jars for sustaining storage. Researchers emptied kill jars into self-sealing freezer bags labelled with species and test date then placed them in a freezer for storage.
6) Subject 150 was listed in the raw crossings data for the *R. sanguineus* test day, but the finger cot weight measurements for subject 150 was not included in the	Subject #150's finger cot weight change recorded on 10 October 2021 (p273 of the Final Report) amounts to -0.029 grams, which is an irrational result the Study Director surmises was the result of a data recording error. The error was not caught at the time of recording, and the researcher cannot recall the nature of the error, so correction is not possible. This result was excluded from the summary

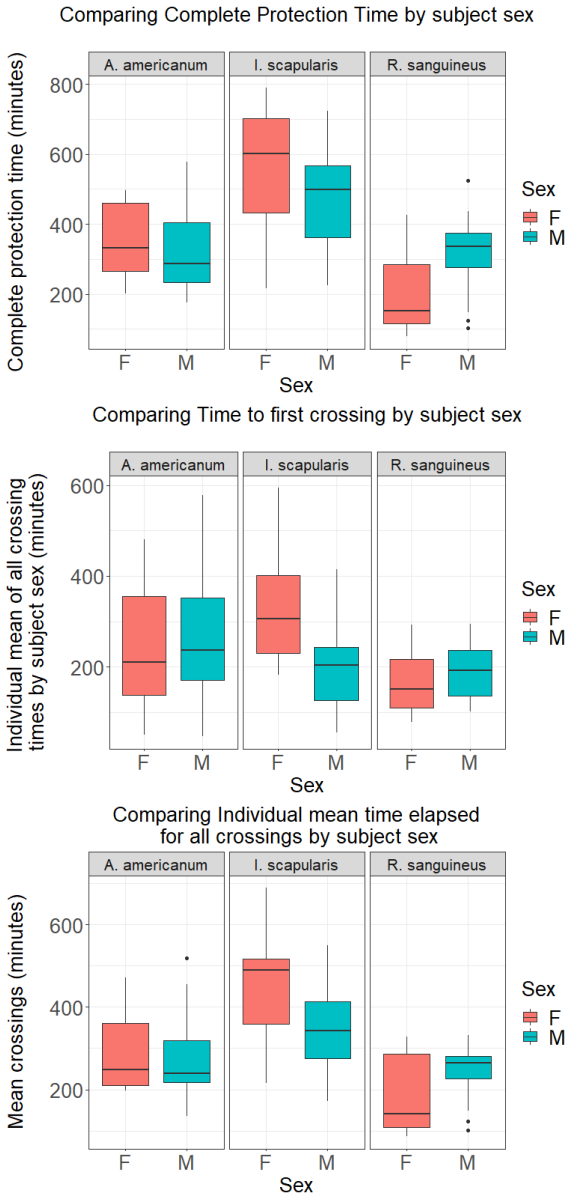
EPA Comments	*Registrant Responses*
summary table in Appendix 5 (p. 278 of 403). Why was subject 150's data not included in this table?	table of finger cot weights (p278 of the Final Report) because the result could not be used in summary statistics data calculations for loss of Test Material during application. The table will be amended to include the finger cot data for Subject #150 with a notation explaining the exclusion of subject 150's finger cot weight data from the summary statistics for the date in question (see Annex VII, below). In regards to the Test Material application to the subject on the efficacy challenge test day, the application of test material to the subject was recorded and initialed by a researcher (p268 of the Final Report), and the subject experienced protection from tick crossings for almost 5 hours (pp 309-310 of the Final Report). Both of these facts strongly indicate that the Test Material was appropriately applied to the subject on that date, and that the presumed error in the finger cot weight record can be isolated to the moment of recording those weight data.

Figure 1.
Comparing three crossing metrics by subject sex.

Top: Confirmed protection time (CPT); **Center**: Time to first crossing; **Bottom**: Means time to all crossings per subject.

Each box-and-whiskers plot shows the minimum and maximum values after removing outliers at the whisker tips, below and above the box, respectively; the box displays the respective 25% quartile, median, and 75% quartile values. As plotted, ostensible 'outliers' are shown as individual dots; for survival study data sets, however, they may better be regarded as indicators that normally distributed values are not anticipated, and that expectations based normal assumptions should be avoided.

The three metrics evaluated by sex were broadly consistent within tick species. Non-parametric Wilcoxon Rank-sum tests *P*-values are provided. One of the nine tests comparing female and male subjects was statistically significant at $p<0.05$ (*Ixodes scapularis*, time to first crossing). There are no clear indications of strong directional subject sex biasing of repellency outcomes in these data.



Annex I
Investigating Variance of CPT in Selected Studies of Tick Repellent Efficacy

Standard deviation is a measure of the amount of variation of a set of values. A high standard deviation indicates that the values tend to be far from the mean. The standard deviation is lower for each tick species in CLBR Study No. MIM-007 compared to the standard deviations calculated from data from similar study performed by ARCTEC for Citrefine[2] (Table I-A).

Table I-A: Summary statistics for complete protection times (CPT) reported in CLBR and Citrefine studies

	Mean CPT		Median CPT		Standard Deviation		
Species	CLBR	ARCTEC	CLBR	ARCTEC	CLBR	ARCTEC	*p*-value
Amblyomma americanum	5.6	8.6	4.8	10	2.1	2.3	0.65
Ixodes scapularis	8.6	5.4	9	4.8	2.7	3.4	0.10
Rhipicephalus sanguineous	4.5	7.1	4.9	8.5	2.1	3.3	0.14

CPT: complete protection time

In both studies, the CPT data distribution is negatively skewed, with a high proportion of right censored records. The symmetrical confidence interval statistics provided in the EPA science review of the ARCTEC report indicate that modeling assumed normal distribution, however, so the same assumptions were employed in the present evaluation for consistency. Levene's test is appropriate to statistically assess the equality of variance between two different data sets. For each subject, the Levene test first measured the absolute difference between the CPT value for that subject and the CPT mean, and then a one-way analysis of variance (ANOVA) compared those differences. The Levene's test detected no significant differences in the standard deviations (variances) between the CLBR and ARCTEC studies (Table 1).

[2] Jones, Robert T. (2020) Single group trial to determine the complete protection time of an insect repellent formulation containing 30% Citriodiol® (Oil of Lemon Eucalyptus) against three species of ticks. Sponsored by Citrefine International Ltd. Study Completed November 14, 2019. Unpublished Report Updated and Submitted April 23, 2020. 4562 pages. MRID 51132201.

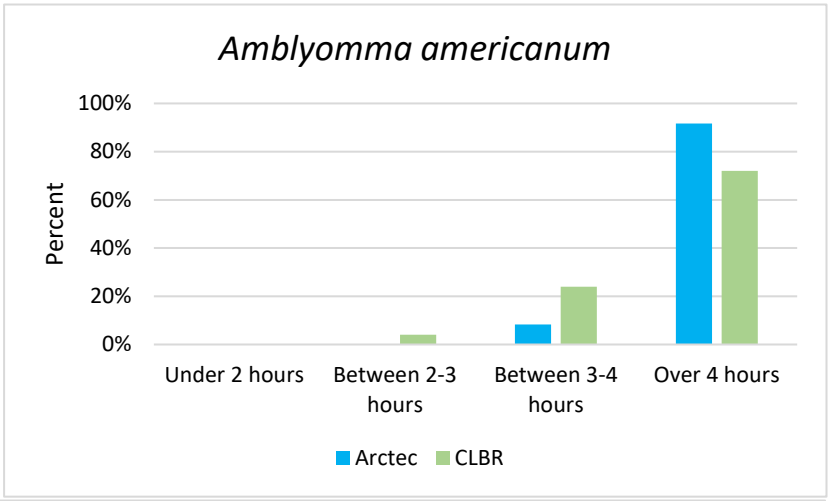
In a second Levene's test, no significant difference was identified in the standard deviations across species within Mimikai Study No. MIM-007 (Table I-B).

Table I-B: Statistical probability outcomes of Levene's tests of variance equality between each tick species within CLBR Study No. MIM-007.

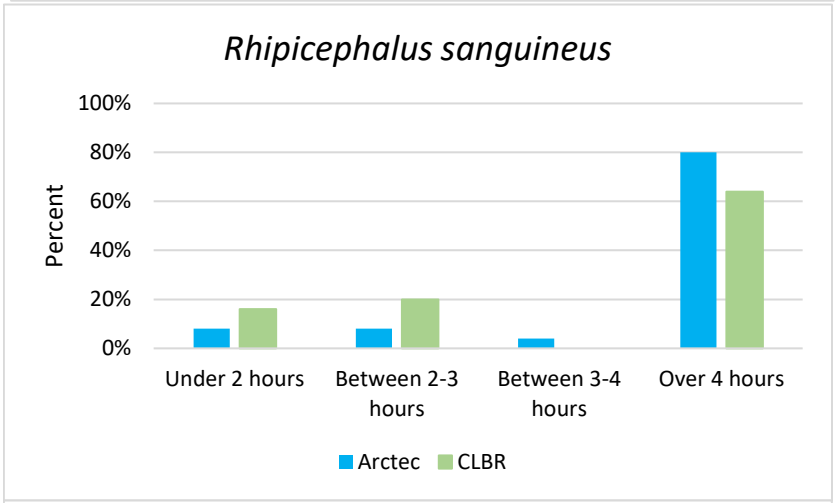
Tick species compared	***p*-value**
Amblyomma americanum vs. *Ixodes scapularis*	0.19
Ixodes scapularis vs. *Rhipicephalus sanguineous*	0.23
Rhipicephalus sanguineous vs. *Amblyomma americanum*	0.32

Between the two laboratories, the observed times of 'early' (<4 hours) versus later CPTs were especially similar for Amblyomma and Rhipicephalus, and somewhat less so for Ixodes (Figure I-A; below).

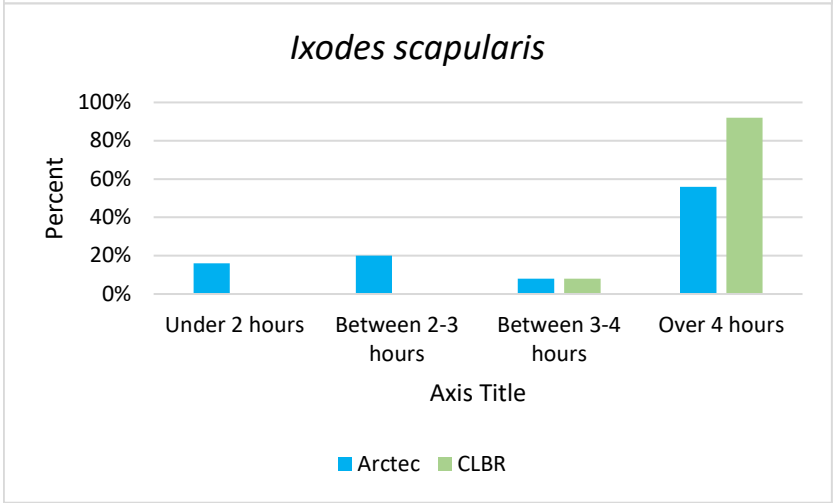
Figure I-A. Visual comparison between early and later (> 4 hrs) CPTs between species and laboratories.



Few crossings before 3 hours



A gap between 3-4 hours



More early crossings at ARCTEC

Statistical analysis method

Standard deviation and Levene's test were computed with the statistical programming language R (4.1.3) and the associated application RStudio. Equations and R code are provided below.

Standard deviation

Equation:

$$\sigma = \sqrt{\frac{\sum_{i-1}^{n}(x_i - \bar{x})^2}{N-1}}$$

Whereas;

x_i = Value of each data point
$\bar{x}$ = Mean
N = Number of data points

R Code for Standard deviation:

```
MIM_007<- read.csv(file.choose())  #Dataset is MIM_007.csv
library(dplyr) #Package for using the data filter function (filter)
library(pastecs) #package for using the descriptive statistic function (stat.desc)
Citrifine_A.americanum <- filter(MIM_007,Species=="Amblyomma americanum" & Study=="Citrifine")
Citrifine_I.scapularis <- filter(MIM_007,Species=="Ixodes scapularis" & Study=="Citrifine")
Citrifine_R.sanguineus <- filter(MIM_007,Species=="Rhipicephalus sanguineus" & Study=="Citrifine")
CLBR_A.americanum <- filter(MIM_007,Species=="Amyblomma americanum" & Study=="CLBR")
CLBR_I.scapularis <- filter(MIM_007,Species=="Ixodes scapularis" & Study=="CLBR")
CLBR_R.sanguineus <- filter(MIM_007,Species=="Rhipicephalus sanguineus" & Study=="CLBR")
stat.desc(Citrifine_A.americanum)
stat.desc(Citrifine_I.scapularis)
stat.desc(Citrifine_R.sanguineus)
stat.desc(CLBR_A.americanum)
stat.desc(CLBR_I.scapularis)
stat.desc(CLBR_R.sanguineus)
```

Levene's test

Hypothesis:

$$Ho: \sigma_1^2 = \sigma_2^2$$
$$Ho: \sigma_1^2 \neq \sigma_2^2$$

Equation:

$$W = \frac{(N-k)}{(k-1)} \cdot \frac{\sum_{i=1}^{k} N_i\,(Z_{i.} - Z_{..})}{\sum_{i=j}^{k}\sum_{j=i}^{Ni}(Z_{ij} - Z_{i.})}$$

$$Z_{ij} = \left|Y_{ij} - \tilde{Y}_{i.}\right|$$

Whereas;

The test statistic, *W*, is equivalent to the *F* statistic, which is used to analyze variance.
k is the number of different groups to which the sampled cases belong
N_i is the number of subjects in the *i*-th group
N is the total number of subjects in all groups
Y_{ij} is the value of the measured variable for *j*-th case from the *i*-th group
$\tilde{Y}$ is the median of the *i*-th group

R Code for Levene's Test:

```
library(car) # #Package for using Levene's Test
A.a <- read.csv(file.choose()) #Dataset is using Amblyomma americanum
I.s <- read.csv(file.choose()) #Dataset is using Amblyomma americanum
R.s <- read.csv(file.choose()) #Dataset is using Amblyomma americanum
leveneTest(min ~ Study, data = A.a, center = "median")
leveneTest(min ~ Study, data = I.s, center = "median")
leveneTest(min ~ Study, data = R.s, center = "median")
```

Annex II
Details of Tick Colony Timing Including Feeding Regimes

Species	CLBR Receipt date	Repellency challenge use date(s)	Days at CLBR prior to use in Repellency challenge	Source	Approx. weeks post-eclosion at time of use	Date of last bloodmeal (if known)
Rhipicephalus sanguineus						
	21-Sep-21	N/A	N/A	Bertek, Inc.	-	Unfed post-eclosion [a]
	7-Oct-21	10-Oct-21	3	Bertek, Inc.	2.5	Unfed post-eclosion [a]
Amblyomma americanum						
	21-Sep-21	N/A	N/A	Oklahoma State	-	12-26 July 2021, *pre*-eclosion
	5-Oct-21	13-Oct-21	8	Oklahoma State	3	12-26 July 2021, *pre*-eclosion
	5-Oct-21	17-Oct-21	12	Oklahoma State	3.5	12-26 July 2021, *pre*-eclosion
Ixodes scapularis						
	21-Sep-21	N/A	N/A	Oklahoma State	-	12-26 July 2021, *pre*-eclosion
	5-Oct-21	20-Oct-21	15	Oklahoma State	4	12-26 July 2021, *pre*-eclosion
	5-Oct-21	24-Oct-21	19	Oklahoma State	4.5	12-26 July 2021, *pre*-eclosion

[a] Bertek, Inc. could not confirm last feeding date *pre*-eclosion.

Annex III
Deviation Evaluation for the Purpose of Reporting to Institutional Review Board (IRB)

Deviation description	Context or explanation	Event and reporting assessment[1]
1. Number of subjects consented: *Seventeen male and 14 female subjects were consented, two fewer than the target of 17 of one sex and 16 of the other.*	Two interested candidates were scheduled to consent then unexpectedly declined to participate shortly before the first test day, due to a change in availability. The Study Director chose not to postpone, but rather to commence assignment of roles with 31 rather than 33 consented subjects.	<u>Evaluation per IRB guidance (see annotated Advarra flowchart, Path I)</u> 1) Event unexpected? Yes 2) Related to participation? No 3) Noncompliant with protocol? Yes 4) Thereby affecting subject rights, safety or data quality or utility? No 5) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
2. Subject sex ratio: *15:10 and 14:11, rather than targeted 13:12*	The pattern of subject withdrawals followed by random selection of replacing alternates yielded an uneven subject sex ratio. CLBR knows of no scientific basis for anticipating a subject sex bias in repellent performance, nor did CLBR observe any clear, consistent sex bias in this study.	<u>Evaluation per IRB guidance (Path II)</u> 1) Event unexpected? Yes 2) Related to participation? Yes 3) Indicative of greater risk? No 4) Noncompliant with protocol? Yes 5) Thereby affecting subject rights, safety or data quality or utility? No 6) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? Unlikely/minor 4) Reported to IRB? No
3. Tick handling: *Forceps used more than brushes*	Superior adult tick control. Determined by Study Director to be noncompliant.	<u>Evaluation per IRB guidance (Path III)</u> 1) Event unexpected? No 2) Noncompliant with protocol? Yes 3) Did the noncompliance issue affect

		a subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study? No 4) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
4. Humidity in test facility: *Briefly ± 3% beyond specified range*	Extra-limital records were fleeting, and within tick tolerance.	<u>Evaluation per IRB guidance (Path III)</u> 1) Event unexpected? No 2) Noncompliant with protocol? Yes 3) Did the noncompliance issue affect a subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study? No 4) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
5. Number of tick sources: *Obtained ticks from more than one source, a possibility the protocol text was meant to communicate.*	Protocol unintentionally implied just one; use of multiple qualified sources permitted testing to be scheduled in a timely and effective manner. Determined by Study Director to be noncompliant.	<u>Evaluation per IRB guidance (Path III)</u> 1) Event unexpected? No 2) Noncompliant with protocol? Yes 3) Did the noncompliance issue affect a subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study? No 4) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No

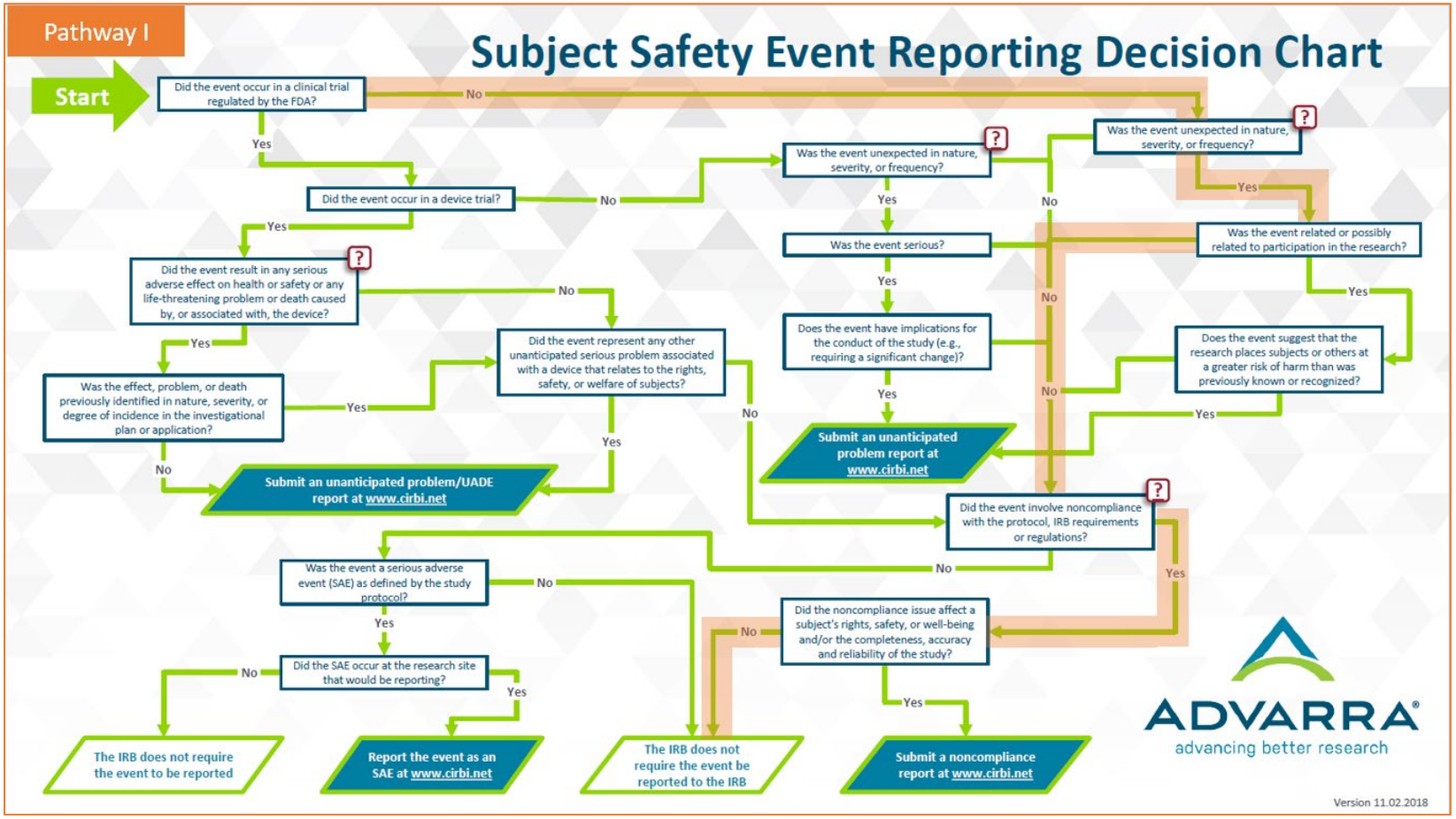
		4) Reported to IRB? No
6. Tick age: *Slightly older at testing than target*	Two-week target was approximate; 3-4 week old ticks still very young and shown to be suitable for use through the avidity screening procedures described in the protocol. Determined by Study Director to be noncompliant.	<u>Evaluation per IRB guidance (Path III)</u> 1) Event unexpected? No 2) Noncompliant with protocol? Yes 3) Did the noncompliance issue affect a subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study? No 4) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
7. Number of test days: *Two test days were added based on subject availability.*	Subject schedules changed more than expected.	<u>Evaluation per IRB guidance (Path II)</u> 1) Event unexpected? Yes 2) Related to participation? Yes 3) Indicative of greater risk? No 4) Noncompliant with protocol? Yes 5) Thereby affecting subject rights, safety or data quality or utility? No 6) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
8. Test material application: *Time lag between subjects*	Differences of a few minutes were inevitable and inconsequential.	<u>Evaluation per IRB guidance (Path III)</u> 1) Event unexpected? No 2) Noncompliant with protocol? Yes 3) Did the noncompliance issue affect a subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study? No 4) Reporting required per IRB? No

		CLBR Summary Assessment 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
9. Test material application: *Finger cots rather than gloves*	Improved efficiency, handling, measurement resolution.	Evaluation per IRB guidance (Path I) 1) Event unexpected? Yes 2) Related to participation? No 3) Noncompliant with protocol? Yes 4) Thereby affecting subject rights, safety or data quality or utility? No 5) Reporting required per IRB? No CLBR Summary Assessment 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
10. Tick behavior: *One-minute criterion too long for fast adult ticks; on a few occasions, subjects were instructed to remove ticks that had entered treated skin less than one minute prior.*	Ticks were more active than anticipated. The original crossing criterion stipulating that ticks remain in the treated lower margin for at least 1 min (rather than quickly retreating to the untreated hand) was rendered moot in cases where ticks crossed the entire treated arm in < 1 minute	Evaluation per IRB guidance (Path II) 1) Event unexpected? Yes 2) Related to participation? Yes 3) Indicative of greater risk? No 4) Noncompliant with protocol? Yes 5) Thereby affecting subject rights, safety or data quality or utility? No 6) Reporting required per IRB? No CLBR Summary Assessment 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
11. Payment schedule: *Changed in response to specific subject requests*	Paid some subjects at end of participation rather than after each visit, per their requests	Evaluation per IRB guidance (Path II) 1) Event unexpected? Yes 2) Related to participation? Yes 3) Indicative of greater risk? No 4) Noncompliant with protocol? Yes 5) Thereby affecting subject rights,

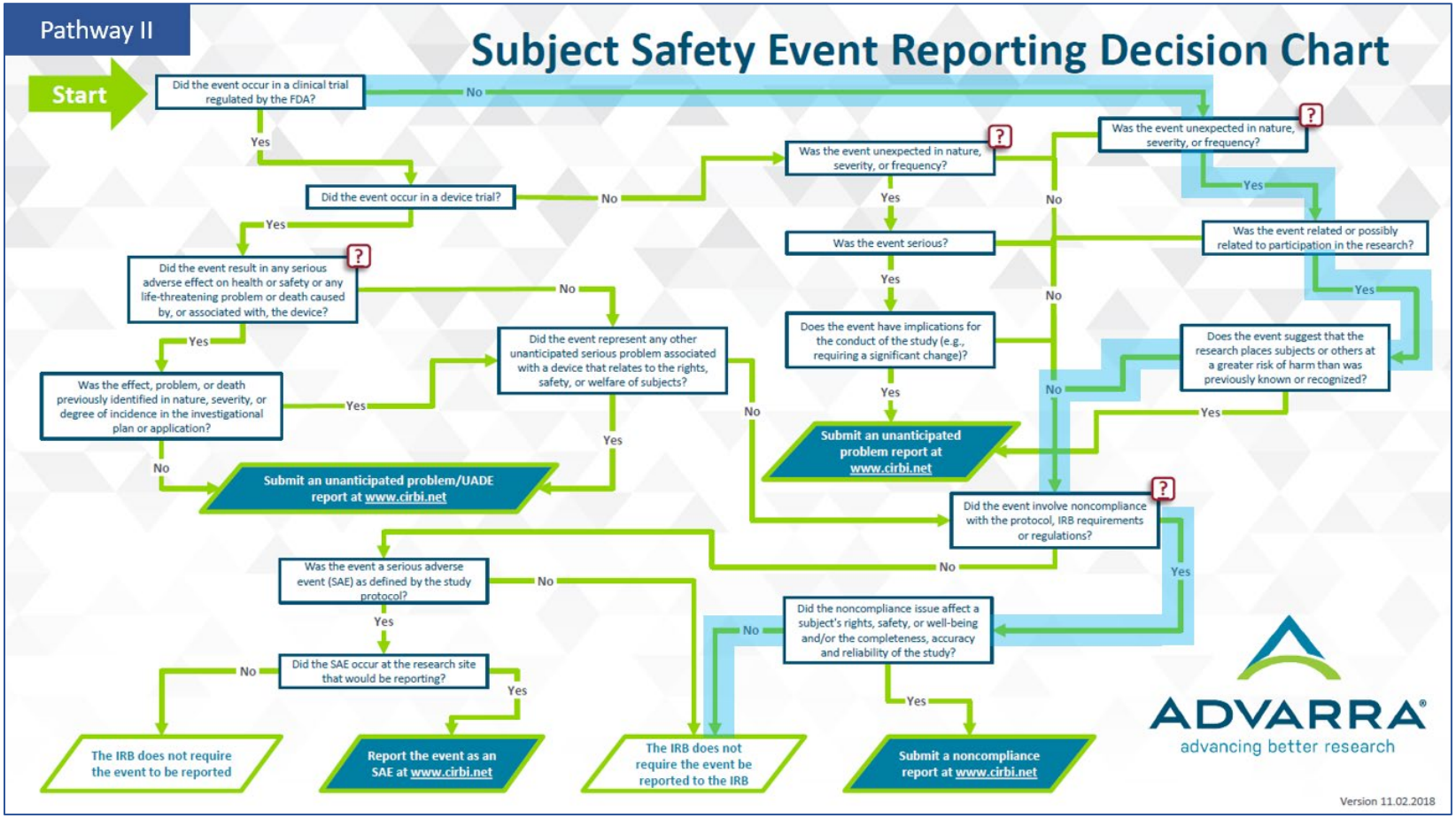
		safety or data quality or utility? No 6) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No
12. Statistical analysis: *Corrected*	Programming error in step at which data were transformed noted by EPA statisticians	<u>Evaluation per IRB guidance (Path I)</u> 1) Event unexpected? Yes 2) Related to participation? No 3) Noncompliant with protocol? Yes 4) Thereby affecting subject rights, safety or data quality or utility? No 5) Reporting required per IRB? No <u>CLBR Summary Assessment</u> 1) Event harmful? No 2) Impact on subject safety? No 3) Impact on data quality? No 4) Reported to IRB? No

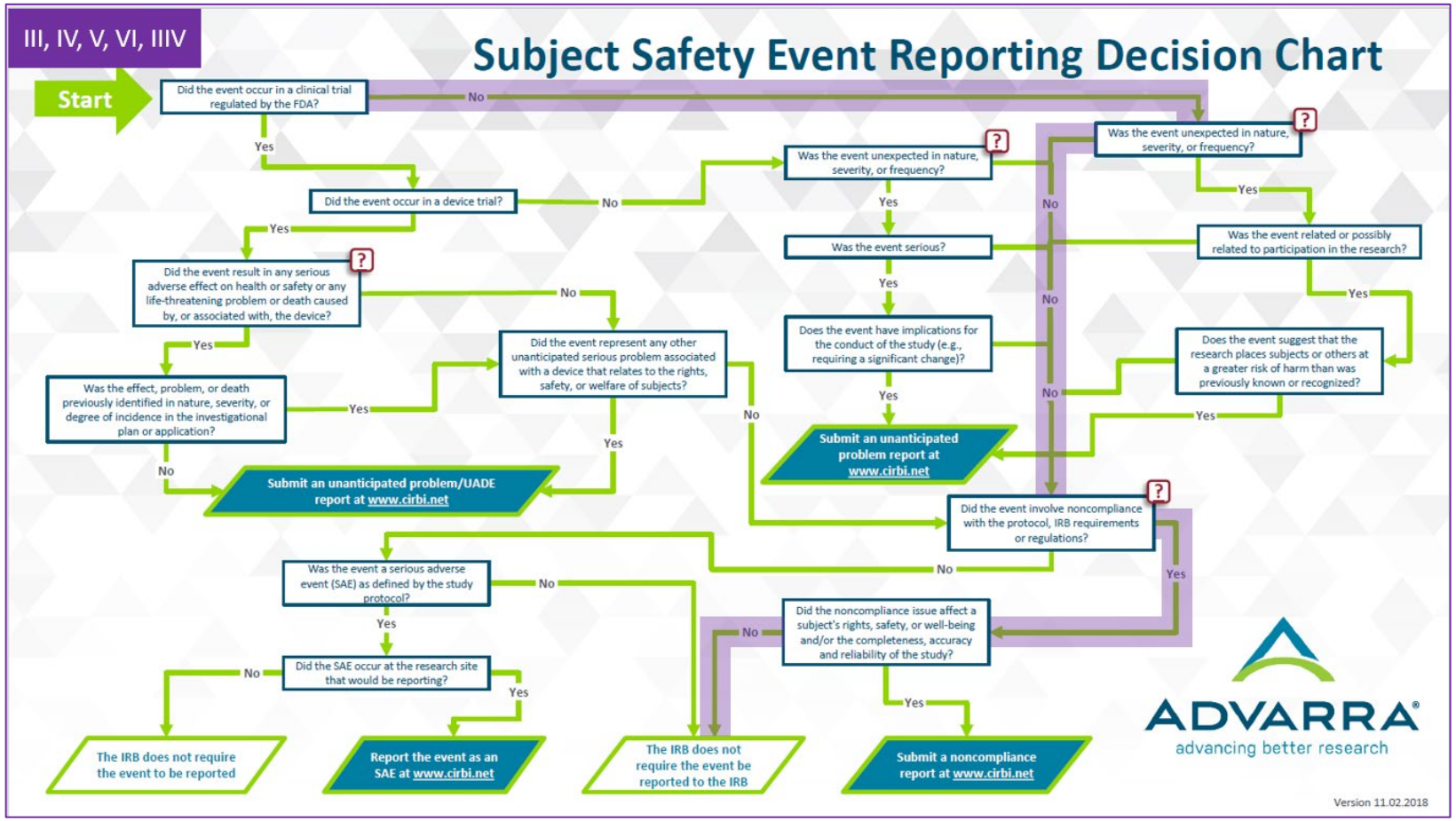
[1] The IRB evaluation criteria in this column refer to specific criteria in Advarra's decision-making flowchart, and are presented here in listed form with abbreviated language. For example, criterion 1, 'Event unexpected', refers explicitly to the following Advarra language in the flowchart: 'Was the event unexpected in nature, severity of frequency?' Please refer to the flowchart for completeness regarding each criterion. The three provided annotated flowcharts (I, II and III) are useful for tracking the path of CLBR assessment for each deviation.

Figure III-A. ADVARRA Subject Safety Event Reporting Decision Charts* – Pathways I, II and II:



*Received by Advarra via direct communication. Advarra has granted CLBR permission to provide this chart to EPA.


Pathway II
Subject Safety Event Reporting Decision Chart
Start
Did the event occur in a clinical trial regulated by the FDA?
No
Yes
Did the event occur in a device trial?
No
Yes
Did the event result in any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, the device?
No
Yes
Was the effect, problem, or death previously identified in nature, severity, or degree of incidence in the investigational plan or application?
Yes
No
Submit an unanticipated problem/UADE report at www.cirbi.net
Did the event represent any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects?
Yes
No
Was the event unexpected in nature, severity, or frequency?
Yes
No
Was the event serious?
Yes
No
Does the event have implications for the conduct of the study (e.g., requiring a significant change)?
Yes
No
Submit an unanticipated problem report at www.cirbi.net
Was the event unexpected in nature, severity, or frequency?
Yes
No
Was the event related or possibly related to participation in the research?
Yes
No
Does the event suggest that the research places subjects or others at a greater risk of harm than was previously known or recognized?
Yes
No
Did the event involve noncompliance with the protocol, IRB requirements or regulations?
Yes
No
Did the noncompliance issue affect a subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study?
Yes
No
Submit a noncompliance report at www.cirbi.net
The IRB does not require the event be reported to the IRB
Was the event a serious adverse event (SAE) as defined by the study protocol?
Yes
No
Did the SAE occur at the research site that would be reporting?
Yes
No
Report the event as an SAE at www.cirbi.net
The IRB does not require the event to be reported
ADVARRA
advancing better research
Version 11.02.2018


III, IV, V, VI, IIIV
Subject Safety Event Reporting Decision Chart
Start
Did the event occur in a clinical trial regulated by the FDA?
No
Yes
Did the event occur in a device trial?
No
Yes
Did the event result in any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, the device?
No
Yes
Was the effect, problem, or death previously identified in nature, severity, or degree of incidence in the investigational plan or application?
Yes
No
Submit an unanticipated problem/UADE report at www.cirbi.net
Did the event represent any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects?
Yes
No
Was the event unexpected in nature, severity, or frequency?
Yes
No
Was the event serious?
Yes
No
Does the event have implications for the conduct of the study (e.g., requiring a significant change)?
Yes
No
Submit an unanticipated problem report at www.cirbi.net
Was the event unexpected in nature, severity, or frequency?
Yes
No
Was the event related or possibly related to participation in the research?
Yes
No
Does the event suggest that the research places subjects or others at a greater risk of harm than was previously known or recognized?
Yes
No
Did the event involve noncompliance with the protocol, IRB requirements or regulations?
Yes
No
Did the noncompliance issue affect a subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study?
Yes
No
Submit a noncompliance report at www.cirbi.net
Was the event a serious adverse event (SAE) as defined by the study protocol?
Yes
No
Did the SAE occur at the research site that would be reporting?
Yes
No
Report the event as an SAE at www.cirbi.net
The IRB does not require the event to be reported
The IRB does not require the event be reported to the IRB
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Version 11.02.2018

Annex IV
Day and Night Cycles for Ticks references by species

Species	Cycle Used (Light:Dark)	Colony Source	Study Topic	Reference
Ixodes scapularis	12:12	Tick Rearing Facility, Dept. of Ent. and Plant Path. Oklahoma State University	Foraging response of adults and nymphs to heat (simulation of human body temperature as attractant)	Otálora-Luna *et al.* (2022)
Ambliomma americanum	12:12	Tick Rearing Facility, Dept. of Ent. and Plant Path. Oklahoma State University	Foraging response of adults and nymphs to heat (simulation of human body temperature as attractant)	Otálora-Luna *et al.* (2022)
Rhipicephalus sanguineus (temperate lineage)	12:12	Tick Rearing Facility, Dept. of Ent. and Plant Path. Oklahoma State University	Foraging response of adults to human vs dogs, a choice test	Backus *et al.* (2021)

Citations:

Otálora-Luna, F., Dickens, J.C., Brinkerhoff, J. and Li, A.Y., 2022. Behavior of Nymphs and Adults of the Black-Legged Tick *Ixodes scapularis* and the Lone Star Tick *Ambylomma americanum* in Response to Thermal Stimuli. *Insects*, *13*(2), p.130.

Backus, L.H., Pérez, A.M.L. and Foley, J.E., 2021. Effect of Temperature on Host Preference in Two Lineages of the Brown Dog Tick, *Rhipicephalus sanguineus*. *The American Journal of Tropical Medicine and Hygiene*, *104*(6), p.2305.

Annex V
DRAFT Note to File: Realized Use of Alternates in Study No. MIM-007

The Study Director has noted that the Note to File dated 11 October 2021, titled 'Regarding: Deviations associated with the number of alternate subjects available each repellent challenge day of the study' (page 195 of the Study Report), which described a plan for the number and use of alternates, was not followed by an additional Note to File detailing actual execution. This Note to File provides that record. The Study Director also notes the prior use of the term 'on-call' to describe certain alternates within the study record was poorly chosen and confusing to readers. This Note to File also provides clarification regarding those alternates.

As already indicated in Note to File dated 24 October 2021, 'Regarding: Deviations associated with subject availability and the use of additional study days for two of the three tick species' (page 194 of the Study Report), efficacy testing against *A. americanum* and *I. scapularis* was divided between two test days each instead of being completed in a single day per species due unforeseen issues with subject availability. For the few subjects who could not attend the originally scheduled test days for the two aforementioned species, we created an additional study day to take place before the originally scheduled testing days, with these few ('minimal') subjects. These minimal subject, additional test days each occurred during the week prior to the originally planned test day for each of the two tick species. Alternates were not physically present for the 'minimal' subject test days of 17 October and 24 October 2021, but were physically present for the originally planned test days. This way, any subject who withdrew during a minimal subject test day could be replaced during the following, originally scheduled test day. Subjects participating in the 'minimal' subject test days were reminded at the beginning of those test days that they were free to withdraw because an alternate was available to take their place, and that the replacement would participate on the following, originally planned test day for the same tick species. Alternates were asked, per Protocol, to appear physically at the laboratory test site on a total of 3 efficacy challenge test days, one for each species, on the originally scheduled test days.

The term 'on-call' used in the Note to File (11 October 2021) referenced above was to describe use of alternates on 'minimal subject' test days and is clarified as follows. After issuing the Note to File on 11 October 2021, and prior to the 17 October 2021 test day with *A. americanum*, the Study Director noted that alternates had only consented to be physically present for three efficacy challenge test days rather than off-site and available to be summoned to participate on any number of days. To accommodate the use of alternates appropriate to consenting, while also allowing the use of two efficacy challenge days per species for two of the three tick species in the study, the Study Director determined that: (a) subjects participating in the 'minimal subject' test days could be reminded that they were free to withdraw because an alternate was available to take their place on the following 'reduced subject' test day for the same tick species; and (b) all available alternates would be present on the 'reduced subject' days, and

could replace one or more subjects withdrawing from a ‘minimal subject’ test day for the same tick species. This practice, internally, continued to be referred to as ‘on call’ even though the term ‘on call’ is normally meant to refer to a person who is not physically present but is ready to be summoned to be physically present. Thus, the term re-appears in the Note to File dated 24 October 2021, regarding deviations associated with subject availability.

Annex VI
MIM-007: Explanation of unbalanced sex ratios among treated subjects across efficacy challenge test days.

Recruitment and consenting were completed with 31 subjects. Instead of the 13 males and 12 females specified in the Study Protocol, 15 males and 10 females were initially assigned the role of treated subjects. This slightly uneven gender ratio was due to subject availability; only 10 female subjects initially indicated they were confident about being available for participation across all three species, creating the need to complete treated role assignment with males. That left 2 males and 4 females to be assigned as the alternate subjects.

In accordance with the Study Protocol, the initial subject role assignments randomly determined, except where constrained as described above, were carried forward through the efficacy challenge test days that followed, excepting where a withdrawing or removed subject had to be replaced by a randomly-selected alternate, and the resulting alternate's sex was opposite that of the replaced subject, as occurred 24 October 2021 when removed male subject 177 was replaced with a randomly-chosen female alternate (subject 12). The result was a more even balance of sexes on that day.

Table VI-A. Numbers of females and males participating as treated and alternate subjects by Study Day and tick species. See text above and the amended the Subject Participation Chart for clarity as to which alternates replaced withdrawing treated subjects and on which dates.

	Treated		Alternate	
Study Day (species)	Female	Male	Female	Male
1 (*R. sanguineus*)	10	15	2	2
2+3 (*A. americanum*)	10	15	2	1
4+5 (*I. scapularis*)	11	14	1	2

Note that efficacy studies such as this one are inherently different than typical clinical trials with a single time stream, where subjects are treated and monitored for effects over time in that single time stream. In that scenario, once a subject withdraws, that subject has withdrawn from the entire time stream of observations, and thus from the entire study. In the scenario of the current efficacy study, this is not the case. Instead, there are three time streams, one for each tick species. As permissible by the study protocol, a subject can withdraw from a single time stream and still participate in the others.

Participation details for subjects who withdrew from a discrete test day, but participated as an alternate and/or treated subject on a later test day are summarized as follows:

- Female subject 18 was assigned to the role of alternate subject, but withdrew from the first efficacy challenge day early on that day, then indicated she would not be available for any of the efficacy challenge study dates that followed.
- Female subject 62 (assigned treated) withdrew from the *Amblyomma americanum* efficacy challenge study date due to scheduling conflicts, but participated on efficacy challenge study dates for the other two tick species.
- Female subject 12 (assigned alternate) withdrew from the *Rhipicephalus sanguineus* efficacy challenge day only, participated as an alternate on one other study day, and participated as a treated subject on final efficacy challenge study day for *Ixodes scapularis*.
- Male subject 155 (assigned alternate) withdrew from the *Amblyomma americanum* efficacy challenge study date due to scheduling conflicts, but participated as an alternate on efficacy challenge study dates for the other two tick species.

Annex VII
AMENDED Subject Participation Chart for Study No. MIM-007
(Updates from version submitted on May 20, 2022, appear in yellow.)

Table 3. Subject Participation Chart

					Participant Roles for Each Efficacy Challenge Study Day and Tick Species [MIM-007]				
					10-Oct-2021	13-Oct-2021	17-Oct-2021	20-Oct-2021	24-Oct-2021
Subject #	MIM-006 Participation	Sex	Age	MIM-007 Initial Role Assignment	*R. sanguineous*	*A. americanum*	*A. americanum*	*I. scapularis*	*I. scapularis*
4	26 Sep 2021	M	22	Treated	Treated	-	Treated	-	Treated
6	3 Oct 2021	F	27	Treated	Treated	-	Treated	Treated	-
11	26 Sep 2021	F	31	Treated	Treated	-	Treated	-	Treated
12	-	F	30	Alternate	Withdrew [e]	-	Alternate (dismissed)	-	Treated [a]
18	-	F	29	Alternate	Withdrew [f]	-	Withdrew [f]	-	Withdrew [f]
30	26 Sep 2021	M	25	Treated	Treated	-	Treated	-	Treated
33	3 Oct 2021	M	20	Treated	Treated	-	Treated	-	Treated
41	-	F	28	Treated	Treated	-	Treated	-	Treated
55	26 Sep 2021	M	30	Treated	Treated	-	Treated	-	Treated
62	3 Oct 2021	F	25	Treated	Treated	-	Withdrew [d]	-	Treated
63	26 Sep 2021	M	32	Treated	Treated	-	Treated	-	Treated
66	3 Oct 2021	F	28	Treated	Treated	-	Treated	Treated	-
73	3 Oct 2021	M	24	Treated	Treated	-	Treated	Treated	-
74	-	M	29	Treated	Treated	Treated	-	-	Treated
76	26 Sep 2021	F	29	Treated	Treated	Treated	-	-	Treated
103	26 Sep 2021	F	25	Alternate	Alternate (dismissed)	-	Alternate (dismissed)	-	Alternate (dismissed)
122	3 Oct 2021	M	30	Alternate	Alternate (dismissed)	-	Alternate (dismissed)	-	Alternate (dismissed)
129	26 Sep 2021	M	28	Treated	Treated	-	Treated	Treated	-
131	26 Sep 2021	F	27	Treated	Treated	-	Treated	-	Treated
132	3 Oct 2021	F	22	Alternate	Alternate (dismissed)	-	Treated [a]	-	Treated [a]
134	-	M	29	Treated	Treated	-	Treated	-	Treated
142	26 Sep 2021	M	22	Treated	Treated	Treated	-	-	Treated
147	26 Sep 2021	F	20	Treated	Treated	Treated	-	Treated	-
150	26 Sep 2021	M	26	Treated	Treated	-	Treated	Treated	-
155	-	M	25	Alternate	Alternate (dismissed)	-	Withdrew [d]	-	Alternate (dismissed)
163	26 Sep 2021	F	21	Treated	Treated	-	Treated	-	Withdrew [d]
167	3 Oct 2021	M	26	Treated	Treated	-	Treated	-	Treated
169	-	M	30	Treated	Treated	-	Treated	-	Treated
171	-	F	21	Treated	Treated	Treated	-	-	Treated
177	-	M	28	Treated	Treated	-	Treated	-	Removed [b]
178	3 Oct 2021	M	35	Treated	Treated	-	Treated	-	Treated
Sex Ratio for Each Efficacy Challenge Test Day:									
				Treated only	15M/10F	2M/3F	13M/7F	3M/3F	11M/8F
				Treated+Alts	17M/12F	NA	14M/9F [c]	NA	13M/9F [c]

[a] Treated subject that was previously an alternate.
[b] Subject was scheduled to participate but did not arrive at test site.
[c] Subject 132 is tallied as a treated subject and not as an alternate for the purposes of reporting the ratio.
[d] Withdrawn subjects are not included in the ratio.
[e] These subjects withdrew from the initial efficacy challenge day prior to that test day
[f] Subject 18 withdrew fully because she was unable to attend any scheduled study date

M: Male
F: Female
Alts: Alternates

Annex VIII
Proposed AMENDED Page 278 of Final Report for Study No. MIM-007

AMENDED - DRAFT

Efficacy* Challenge Date and Species	Weights of Finger Cots in grams				Per subject * prepared dosage expressed in grams at 0.5g/600sq cm rate	Residual on finger cot as % target	Realized application in grams at 0.5g/600sq cm rate	
	Subject #	Before	After	*Delta*				Subject #
10 Oct 2021	4	2.557	2.577	*0.020*	0.445	4.49438202	0.425	4
Rhipicephalus	6	2.563	2.584	*0.021*	0.407	5.15970516	0.386	6
sanguineus	11	2.537	2.568	*0.031*	0.457	6.7833698	0.426	11
	30	2.612	2.638	*0.026*	0.541	4.80591497	0.515	30
	33	2.594	2.596	*0.002*	0.390	0.51282051	0.388	33
	41	2.546	2.569	*0.023*	0.357	6.44257703	0.334	41
	55	2.528	2.563	*0.035*	0.447	7.82997763	0.412	55
	62	2.580	2.615	*0.035*	0.409	8.55745721	0.374	62
	63	2.541	2.621	*0.080*	0.491	16.293279	0.411	63
	66	2.554	2.573	*0.019*	0.383	4.96083551	0.364	66
	73	2.572	2.598	*0.026*	0.431	6.0324826	0.405	73
	74	2.568	2.568	*0.000*	0.541	0	0.541	74
	129	2.594	2.604	*0.010*	0.418	2.3923445	0.408	129
	131	2.607	2.622	*0.015*	0.323	4.64396285	0.308	131
	134	2.613	2.618	*0.005*	0.386	1.29533679	0.381	134
	142	2.556	2.602	*0.046*	0.435	10.5747126	0.389	142
	147	2.668	2.671	*0.003*	0.500	0.6	0.497	147
	163	2.553	2.572	*0.019*	0.443	4.28893905	0.424	163
	167	2.562	2.581	*0.019*	0.496	3.83064516	0.477	167
	169	2.595	2.610	*0.015*	0.528	2.84090909	0.513	169
	171	2.555	2.579	*0.024*	0.447	5.36912752	0.423	171
	177	2.618	2.623	*0.005*	0.471	1.06157113	0.466	177
	178	2.580	2.611	*0.031*	0.560	5.53571429	0.529	178
	76	2.536	2.612	*0.076*	0.381	19.9475066	0.305	76
	150**	2.655	2.626	*-0.029*	0.457	NA**	NA**	150

% residual		
Median	4.88337524	
mean with SD	5.59389879	4.70201397
Realized application		
Median	0.4115	
mean with SD	0.420875	0.06565742

* Typographical errors corrected.

** The change in weight calculated for subject number 150 resulted in a negative value, which was considered an irrational outcome. This error is presumed to have arisen from an erroneous data recording, but since the circumstances of this error nor the corrected value can be resolved, Subject 150's data has been excluded from the summary statistics.