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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

September 29, 2022

MEMORANDUM

SUBJECT: Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes

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REFERENCE: Carroll, Scott P., Study Director. (2022) Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes. Unpublished Document. MRID 517071-01 and amended report MRID 519127-01, dated May 12, 2022.

ACTION REQUESTED

Conduct a science review of a completed field study testing efficacy of a topical insect repellent spray (MIMIKAI Lilly Pilly Repellent), containing 11% w/w of oil of lemon eucalyptus (OLE) and 7.75% w/w of methyl nonyl ketone (MNK), as active ingredients against mosquitoes. This product performance test is required to determine the median Complete Protection Time (mCPT) with 95% confidence intervals against mosquitoes to support registration of the proposed skin-applied repellent product. The protocol used to conduct this study was previously reviewed and

accepted with recommendations by the Environmental Protection Agency (EPA) and Human Studies Review Board (HSRB) on April 20th, 2021. The protocol used in this study, provided in Appendix 1 of the study report (Attachment 1), was partially amended to incorporate EPA and HSRB recommendations. The portions that were left unamended in the protocol are discussed in Comments and recommendations made to study protocol and study report. However, these portions in the revised protocol that were not amended to conform with EPA and HSRB recommendations are not relevant to the conduct of the study, nor do they compromise the integrity of the study results.

EXECUTIVE SUMMARY and CONCLUSIVE REMARKS

The Agency reviewed the study titled, Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes (dated February 17, 2020, MRID 517071-01), and through multiple correspondences with the registrant, requested various points of clarification and discussion regarding the scientific conduct of the study (Attachments 2-4). The registrant submitted an amended study report MRID 519127-01 (Attachment 1) to provide clarification on study methodologies as requested by the Agency. This amended report replaces MRID 517071-01, so references to the study report hereafter refer to MRID 519127-01 (Attachment 1). The Agency's key concerns regarding the scientific conduct addressed in these correspondences include: (1) the use of mosquito density and landing thresholds in subject attractiveness testing that deviated from guidance in OPPTS 810.3700: *Insect Repellents to be Applied to Human Skin,* (2) the use of proximate test sites that the Agency posited were not distinct habitats where the predominant species differed, and (3) the absence of *Aedes albopictus* and/or *Aedes aegypti* at test sites (Attachments 2-4). The Agency reviewed the registrant responses to these concerns, and found the study methodologies acceptable based on the following:

1) The mosquito density used for arm-in-cage subject attractiveness testing (1 mosquito per 4, 251 cm^3) was more conservative than the density recommended by OPPTS 810.3700 (1 mosquito per 1,160 cm^3), and all subjects demonstrated attractiveness to mosquitoes by receiving 2 landings within 2 minutes as proposed in the EPA-approved protocol (Attachment 2).
2) The registrant used a Principal Component Analysis (PCA) to support that the predominant mosquito species differed between sites in the 4 weeks prior to efficacy test days (Attachment 3). The EPA replicated the registrant's PCA in JMP statistical software and the results were consistent (Refer to Attachment 4, *EPA's review of the Registrant's technical report and rebuttal June 22, 2022* regarding site independence that includes EPA's statistical evaluation of PCA).
3) The product was tested against three mosquito genera (*Anopheles, Culex and Aedes*), as proposed in the EPA-approved protocol. However, testing did not include *Aedes albopictus* and/or *Aedes aegypti* as required in the newly-published EPA rule, *Pesticide Product Performance Data Requirements Claiming Efficacy Against Certain Invertebrate Pests* (87 FR 22464, effective June 14, 2022). *Aedes albopictus* and *Aedes aegypti* are primary vectors of Zika, Dengue and Chikungunya. Therefore, the submitted data do not support efficacy claims against vectors of Zika, Dengue and Chikungunya on the product label. The Agency will not hold this study to the species requirements prescribed in the

new product performance rule because the study commenced before the effective date of these requirements. For future studies, it is required that test sites be selected to include all mosquito species required by the Agency to support general mosquito claims on the product label.

In conclusion, upon review of the amended study report and supporting documents (attachments in bulleted list below), the Agency concludes that the EPA has evaluated the scientific validity of the study and study results based on the information provided and concluded that the data generated by this study is sufficient to support a mCPT of up to eight hours against mosquitoes. However, the data do not support efficacy claims against vectors of Dengue, Zika and Chikungunya because the vectors, *Aedes aegypti* or *Aedes albopictus* are not present at the selected sites and they have not been tested. Additional testing on these species is required for claiming efficacy against these vectors on the product label.

- Attachment 1: Amended study report (MRID 519127-01) that replaces the original study report (MRID 517071-01) with study protocol therein (Appendix 1 in study report).
- Attachment 2: Registrant's updated response (dated May 12, 2022) to EPA's 90-Day Technical Screen
- Attachment 3: Registrant's technical report and rebuttal (dated June 22, 2022) to EPA's letter regarding site independence
- Attachment 4: EPA's review of the Registrant's technical report and rebuttal June 22, 2022) regarding site independence (including EPA's statistical evaluation of Principal Components Analysis (PCA)
- Attachment 5: EPA's statistical report on the Kaplan-Meier survival analyses provided in the study report
- Attachment 6: Responsiveness to EPA and HSRB Science Comments on the Draft Protocol
- Attachment 7: Product Label

SCIENCE REVIEW

Study objective: The objective of this field study is to determine the mCPT of a topical insect repellent spray, containing active ingredients of 11% w/w of OLE and 7.75% w/w of MNK, against species of mosquitoes within the genera *Aedes, Anopheles* and *Culex* using a sample size of 13 informed consenting human volunteers as test subjects, and to provide repellency data for product registration and labeling purposes.

Compliance with Good Laboratory Practice Standards (GLP); 40 CFR, Part 160: This study is conducted in accordance with EPA, Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) , Good Laboratory Practice Standards (GLP) (40 CFR, Part 160) except for the collection of environmental data at one test site. Environmental data associated with the first five exposure periods at Site 1 was not collected due to a miscommunication, so temperature data sourced from a nearby weather station (The Ranch – KCALIVEO2) was used to supplement data at these exposure periods without following GLP. A Statement of Compliance with Good Laboratory Practice Standards is provided on p. 3 of amended study report, MRID 519127-01. A Quality Assurance Statement, signed and dated on October 29th, 2021, is provided on p. 4 of amended report MRID

519127-01. The information provided in MRID 519127-01 is in response to an Agency request for clarifications and complements data submitted in the original report, MRID 517071-01.

Identification of the test system and efficacy endpoint: The first confirmed landing (FCL) of wild mosquitoes on human subjects was used as the endpoint to evaluate the repellency of the insect repellent spray (11 % w/w OLE, 7.75% w/w MNK) applied at the standard dose of 0.5 g/ 600 cm^2 to human skin of 13 test subjects. Two control subjects were used to assess landing pressure against representative mosquito species within the genera *Aedes, Anopheles* and *Culex.*

Recruitment and Randomization: Recruitment yielded a single pool of candidates that later was resolved into two pools of subjects as several candidates were unavailable for testing as proposed in the study protocol. Consequently, subjects from a single pool were not randomly assigned to test days. Instead, two pools of subjects from a single pool of candidates were randomly assigned as treated, controls, and alternates within each test day as follows: two lists, one for males and one for females, were created in Excel to randomize subjects to treatment, controls, and alternates by gender. Treated subjects were randomly assigned by gender to either list. Next, one male and one female were randomly selected as untreated controls, and five subjects, alternating between male and female, were randomly selected as alternates (p. 23 of 380 in MRID 519127-01). This is reported as a protocol deviation unlikely to compromise results since statistical analysis were performed not between test days but rather within each test day.

Test sites selection and qualifications: The test was conducted in two field sites located in adjacent Sacramento Valley counties (Glenn County and Butte County), 15 km apart, where predominant mosquito species (*Aedes melanimon*, *Anopheles freeborni*, *Culex tarsalis,* and *Aedes vexans*) were present (Table 4 on p. 20 in MRID 519127-01) at both sites. The sites are described as having two ecologically distinct habitats. Site 1 was in a seasonally flooded riparian forest habitat where flooded pools, and nearby marshes and cultivated rice fields provided mosquito breeding habitats (p. 17 of 380 in amended MRID 519127-01). Site 2 was a native moist grassland habitat around a small lake, where mosquitoes breed in standing water on site as well as adjacent irrigated pastures and marshes (p. 17 of 380 in amended MRID 519127-01). The sites were selected based on their structural and temporal differences of mosquito habitats, diversity and abundance of mosquito species within the three main genera, *Aedes, Culex* and *Anopheles*, and absence of mosquito-borne pathogens in trapped mosquitoes collected and screened for pathogens during the month preceding field testing (pp. 16 – 17 of 380 in amended MRID 519127-01). Based on confidential latitude and longitude coordinate locations, the Agency created a map using ArcGIS online to show the location of the sites and determine their proximity. The EPA-generated map showing the locations of both sites in Figure 1 is Confidential Business Information (CBI) and is provided in a confidential appendix (CA) not included in this review.

RISK MINIMIZATION

Site monitoring and mosquito processing for identification and detection of mosquito-borne pathogens

Sampling dates: Sites were monitored once in late June 2021, then weekly two months prior to the test dates using carbon dioxide-baited BG-Sentinel-2 traps (p. 17 in MRID 519127-01). Data from collections dates are provided in Appendix 8 in amended MRID 519127-01. Collections started on June 27, 2021, and ended September 27, 2021, at site 1. The first collection date was June 27, and then sampling was performed weekly on Aug. 4, 10, 17, 25; September 2, 8, 16, 21, 27 (pp. 299 - 301 Appendix 8 in MRID 519127-01). September 26, 2021, was efficacy test day at site 1. At site 2, collections started on August 31, 2021, and ended October 3, 2021. The first collection date was Aug. 31, and then trapping was conducted weekly on September 7, 14, 21, 27, and October 3 (pp. 301-303 on Appendix 8 in MRID 519127-01). October 3 was efficacy test day at site 2.

Sampling procedure: Three carbon dioxide-baited BG-Sentinel-2 traps were deployed in each site, with one placed in the three most humid and sheltered areas that were large enough to accommodate study subject pairs during testing (p. 17 in MRID 519127-01). Three of the trap locations were used at exposure sites at site 1 at different times of day, depending on mosquito activity. Two of the trap locations were used as exposure sites at site 2 because the third trap site was unsuitable during the day of testing due to breeze conditions (pp. 17-18 of 380 in MRID 519127-01). The traps were deployed three times per week, from approximately 6:00 pm to 9:00 pm (pp. 18-19 in amended MRID 519127-01). The collection bag from each trap was removed and replaced hourly during this 3-hour period, and the bags were transported to the lab for mosquito species identification and counting (p. 18 in MRID 519127-01).

Viral pathogen screening: The description of virus testing procedures is provided in MRID 519127-01 (p. 18 of 380). Following species identification, mosquitoes were sorted by genus into 50 ml centrifuge tubes and transferred to vials with specimens pooled for pathogen testing by site, collection date, laboratory name, species, and if applicable, subject number (tabulated in Appendix 8, pp. 299-303 of 380 in MRID 519127-01). Appendix 8 includes data from pre-test site monitoring and data from mosquitoes collected from control and treated subjects). Samples were transferred to an insulated box with dry ice and submitted to the University of California, Davis Arbovirus Research and Training laboratory (DART) for pathogen screening via RT-PCR analysis. *Culex* were tested for West Nile Virus, Western Equine Encephalitis, and St. Louis Encephalitis pathogens, and *Aedes* were tested for Chikungunya, Zika, and Dengue pathogens (p. 18 in MRID 519127-01). Varying numbers of female mosquitoes trapped and aspirated by control and treated subjects, ranging from 1 to 916 (see Appendix 8 pp. 299-303 in amended MRID 519127-01), were pooled and analyzed for pathogen screening. There was only one positive case for West Nile Virus at site 1 on August 10 (Appendix 8 in MRID 519127-01). Testing in the field was not initiated until pathogen sampling was negative for at least one month (p. 17 in MRID 519127-01).

All *Aedes* and *Culex* mosquitoes aspirated from control subjects (reported in Table 5 on p. 26 in MRID 519127-01), and 10 mosquitoes aspirated from treated subjects, were screened for pathogens. Mosquitoes collected and tested from subjects at site 1 included five pooled samples: *Aedes* spp. from both control subjects, *Anopheles freeborni* from one control subject, *Culex tarsalis* from the other control subject, and *Aedes* spp. for one treated subject (subject # 30 at site 1). Mosquitoes collected and tested from control subjects at site 2 included 11 pooled samples: *Aedes* spp. combined with *Anopheles freeborni* for one control subject, *Aedes* spp. for the second

control subject, and *Culex tarsalis* for both control subjects. Six *Aedes melanimon* specimens, one *Aedes melanimon*, and one *Aedes vexans* were screened from treated subjects at Site 2. Details of mosquito virology screening process summarized in this paragraph is found on p. 18 in MRID 519127-01 and tabulated in Appendix 8 (pp. 299- 303 in MRID 519127-01). Raw data on landings is provided in Appendix 7 on p. 284 in MRID 519127-01. Landings on control subjects are summarized in Table 5, arranged by species and sites on p. 26 (MRID 519127-01). Landings on treated subjects at sites 1 and 2 (raw data) are provided on pp. 288 and 293 for sites 1 and 2, respectively, in Appendix 7. Total number of mosquitoes collected from treated subjects is 10 for site 1, and 25 for site 2. Raw data tables are provided on p. 288 for site 1 and p. 293, and for site 2, in Appendix 7 (MRID 519127-01).

Pre-testing mosquito species distribution

A total of 8,701 mosquitoes were collected from Site 1 and a total of 5,868 mosquitoes were collected from site 2 (Appendix 8, pp. 283-285 in MRID 517071-01), but a total of 8,112 mosquitoes were reported to be collected and identified at site 1 and a total of 5,645 were reported to be collected and identified at site 2 (Table 4; p. 15 in MRID 517071-01, and Table 4; p. 20 in MRID 519127-01). This discrepancy is explained in MRID 519127-01 (pp. 18 and 19). Appendix 8 includes collection data from June 27 through September 27 for site 1 and collection data from August 31 through October 3 at site 2. Trapping data from September 27 at site 1, and October 3 at site 2, were omitted from Table 4, because only trapping data prior to efficacy testing days were tabulated in Table 4. However, collection values were further corrected (Table 1 below) in the applicant's technical report and rebuttal to the Agency's letter regarding site similarity (Attachment 3 in this review).

Table 1. Mosquito abundance and species distribution at two field sites (pre-test monitoring).

	Aedes melanimon	*Aedes vexans*	*Aedes nigromaculis*	*Aedes sticticus*	*Anopheles franciscianus*	*Anopheles freeborni*	*Anopheles punctipennis*	*Culex erythrothorax*	*Culex pipiens*	*Culex tarsalis*	Grand total
Site 1											
27-Jun-21	33					578				57	668
4-Aug-21	444					363				10	817
10-Aug-21	383	50				423				391	1247
17-Aug-21	150	17				560				21	748
25-Aug-21	250	39				497				19	805
2-Sep-21	**650**	**100**				**312**				**182**	1244
8-Sep-21	**650**	**50**				**128**				**300**	1128
16-Sep-21	**800**	**15**				**13**				**128**	956
21-Sep-21	**750**	**158**		**36**		**97**				**47**	1088
Total per spp.	4110	429		36		2971				1155	8701
Site 2											
31-Aug-21	400	200			11	916	2	7		172	1708
7-Sep-21	**550**	**281**	**50**			**123**		**5**	**4**	**85**	1098
14-Sep-21	**700**	**182**				**76**				**100**	1058
21-Sep-21	**500**	**178**	**158**			**25**			**19**	**108**	988
27-Sep-21	**124**	**32**	**40**			**20**				**800**	1016
Total per spp.	2274	873	248		11	1160	2	12	23	1265	5868

Amended data from response to deficiencies in technical report and rebuttal to the Agency's concerns regarding site independence (Attachment 3 in this review). Numbers in red are corrected values to Table 4 in MRID 519127-01. Blue text represents species total by site for all trapping days. Bolded text indicates values used in the Principal Components Analysis (PCA) conducted in response to the Agency's concerns (Attachment 3). Table 1 replaces data from Table 4 of study report MRID 517071-01 (§3, p. 15 of 362).

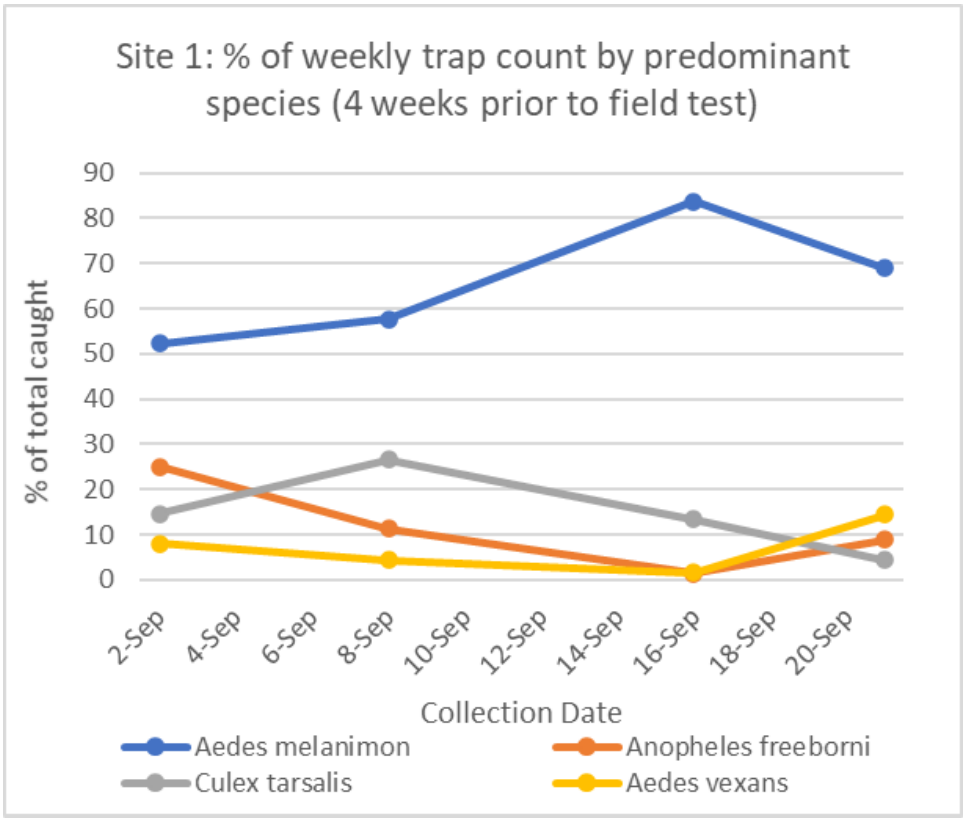
From the total mosquitoes caught at both sites, the most trapped species found included *Aedes melanimon* (Site 1: 47.2% at Site 1, Site 2: 38.7%), *Anopheles freeborni* (Site 1: 34.1%, Site 2: 19.7%), and *Culex tarsalis* (Site 1: 13.3%, Site 2: 21.6 % see Table 2 below). The predominance of *Culex tarsalis* at Site 2 was only observed at the last collection date on September 27th (Figure 2 below). Testing dates were timed for temporal difference in species between sites. Testing at site 2 took place on October 3. Testing at site 1 took place on September 26 when *Aedes melanimon* was the most predominant species at both sites. During the three weeks when collection dates overlapped (September 7/8 through September 20/21), *Aedes melanimon* was the most predominant species at both sites (Figure 2 below).

Table 2. Amended Table 4 in MRID 519127-01**.** Mosquito abundance and species distribution at two field sites (pre-test monitoring)

Mosquito Genera	**Species**	**Total collected at Site 1**	**Total collected at site 2**
Aedes	*Aedes melanimon*	4,110 ~~3,671~~	2,274 ~~2,224~~
	Aedes vexans	429 ~~329~~	873 ~~857~~
	Aedes nigromaculis	~~0~~	248 ~~90~~
	Aedes sticticus	36	0
Anopheles	*Anopheles freeborni*	2,971	1,160
	Anopheles franciscanus	0	11
	Anopheles punctipennis	0	2
Culex	*Culex tarsalis*	1,155 ~~1,105~~	1,265 ~~1,266~~
	Culex pipiens	0	23
	Culex erythrothorax	0	12
	Total	8,701 ~~8,112~~	5,868 ~~5,645~~

Note: Data from amended table 4 in MRID 519127-01: Amendment to Data from Table 4 of study report MRID 517071-01 (§3, p. 15 of 362).

Figure 2 below shows species distributions and predominance at the two sites for the four weeks of trap data prior to efficacy testing at each site.



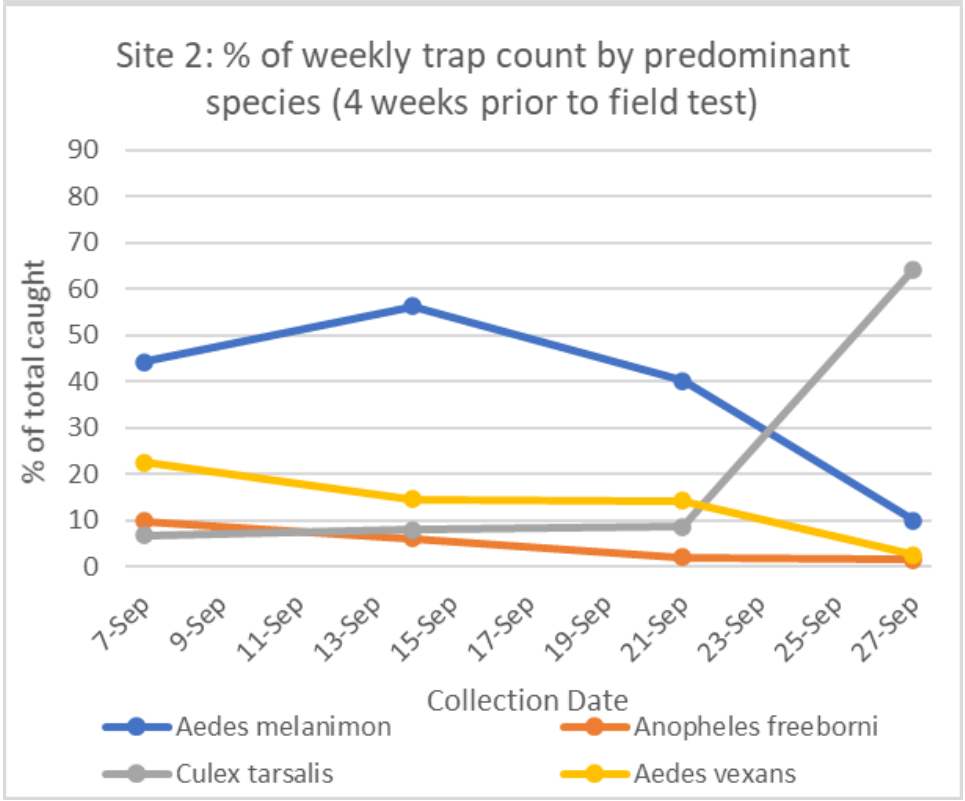


Figure 2**.** EPA-generated plots using the amended species abundance data provided in **Table 1** above, showing weekly trap count percentages by the four predominant species found at both sites 4 weeks prior to field tests. The grey line shows the phenological shift of *Culex tarsalis* on September 27th, 2021, the timepoint when this species

became predominant at site 2.

Experimental design

Efficacy testing**:** This is a field study conducted with human subjects at two sites: a seasonally flooded riparian forest habitat and a native moist grassland habitat (description of habitat structure is provided on p. 17 in MRID 519127-01), located in adjacent counties in California (Figure 1 in Confidential Appendix not included in this review). The first test day took place at Site 1 on September 26, 2021, and the second test day took place at Site 2 on October 3, 2021. At each site, the experimental groups consisted of 13 treated subjects, 2 untreated control subjects (one male and one female), and 5 alternate subjects. No subjects went to the field on more than one test day. Control subjects were used to monitor landing pressure immediately prior to test initiation and throughout the duration of the test. Subjects at each site were selected from a pool of informed and consenting volunteers that were tested for their attractiveness to mosquitoes and trained to catch/handle mosquitoes using aspirators. Exposure of treated subjects was conducted between midday and evening of each test day, for a total duration of 9 hours at Site 1 and 8 hours at Site 2.

Test subject selection and randomization: Subjects were assigned a unique number from randomly generated list in Excel. These numbers were used to designate alternate subjects and assign treatment status (treated or controls) to subject pools within each test day. However, due to a larger number of females than male subjects on the second test day at Site 2, stratified randomization of treatment allocation was used to achieve the relatively even balance of sexes of treated subjects proposed in the protocol. This method resulted in all female alternate subjects. The randomization procedure is explained on p. 23 in MRID 519127-01. Male to female ratios represented on test days were 6:7 in Site 1 and 7:6 in Site 2 (Appendix 5 on pp. 264 and 269, respectively, in MRID 519127-01).

Attractiveness test: The attractiveness test consisted of an arm-in-cage evaluation. Subjects exposed one forearm into a 45.36 × 30.24 × 30.24 cm (~ 18 × 12 × 12-inch cage) containing 10 adult female *Aedes aegypti* mosquitoes (mosquito density of ~1 mosquito /4.15 liters) that were 4-17 days old (p. 16 in amended MRID 519127-01), with exposures lasting until 2 mosquitoes landed per minute. Mosquitoes were sourced from multi-generational laboratory colony that was certified as pathogen-free, ordered from Benzon Research, Inc. (MRID 519127-01, p. 109). Prior to subject exposures, mosquitoes were deprived of food for 18 to 24 hours (p. 16 in MRID 519127-01). The protocol and experimental procedures outlined in the Informed Consent Form (ICF) described having 1 landing/minute (2 landings in 2 minutes) as the attractiveness criteria (Appendix 1, p. 43; Appendix 2: p. 171 in MRID 519127-01), but another section of the ICF described the attractiveness criteria of 2 landings within 1 minute (Appendix 2: p. 167 in MRID 519127-01). All subjects passed the attractiveness test by receiving two landings in ≤ 1 minute (p. 16 in MRID 519127-01). Justification is provided on p. 16 in MRID 519127-01 for assessing attractiveness using 10 mosquitoes in a 30.24 cm x 30.24 cm x 45.36 cm cage (equivalent to 1 mosquito per 4.15 liters, which is 28% of the density recommended by the guideline for arm-in-cage testing of repellent treated arms).

Aspirator training: A separate arm-in-cage test, following the procedure outlined in the CLBR training manual, was performed for aspirator training. Mosquitoes used for aspirator training

were 5 – 12 days old. A trainer demonstrated aspirator use to capture mosquitoes, then used bandaging to cover up the upper half of one forearm and the arm operating the aspirator of each subject. Each subject put on latex or vinyl gloves, placed their arms into a 24 inches cube cage. A larger cage than that used for attractiveness test was used to facilitate movement for practicing use of aspirator to catch landing mosquitoes (p. 16 MRID 519127-01). The cage was equipped with two elastic cloth openings. Subjects practiced operating the aspirator individually until they consistently captured landing mosquitoes before the mosquitoes could take flight to move away from the aspirator tip. Aspirator training was repeated as many times as the subject wished, first with one mosquito, then with two mosquitoes before aspirator proficiency was ascertained by discretion of the attending researcher(p. 16 in MRID 519127-01). The randomization procedure is described on p. 23 of MRID 519127-01, and pp. 264 and 269 in Appendix 5 in MRID 519127-01.

Product application

Application of standard dose: The standard application rate of 0.5 g of product per 600 cm^2 of skin was used for testing efficacy of the proposed product. The amount applied to subjects was adjusted to their skin surface area of the non-dominant forearm. Skin surface area of the non-dominant forearm of each subject was estimated by the length of the non-dominant forearm (from wrist to elbow crease) multiplied by the average circumference of the non-dominant forearm. Average circumference was estimated by four equidistant measurements taken with a measuring tape around the upper forearm and lower forearm. Amount of product applied to each subject, weighed in grams, was converted to volume (milliliters) using the product's specific gravity (0.8874 g/ml) as described in the formula below to achieve the standard dosage of 0.00094 ml product/cm^2 of skin across all subjects.

Formula for dose calculation applied to each subject, adjusted to skin surface area per subject and converted to volume using specific gravity.

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

Application of standard dose is described on pp. 22 and 24 in MRID 519127-01. Raw data are provided in Appendix 5 in MRID 519127-01 and p. 22 in MRID 519127-01.

Application Procedure: On test days, subjects used screened stations to wash forearms chosen for application with a fragrance-free liquid non-soap cleaner, rinse with clean water, spray forearms with diluted ethanol, and towel dry the arm prior to applications of the product (p. 22 in MRID 519127-01). Head nets were provided to protect head, face, and neck for times when subjects were outside of screened shelters. Gloves were fitted to subjects and bandaging was used to protect areas of the wrist and elbow joint at the outside margins of the treatment. Pre-determined volumetric amounts of product were dispensed onto each treated subject using a syringe to dispense the right amount of product unto the skin. The dispensed amount was rubbed evenly on the skin with a finger covered with a new, pre-weighed cot. Each finger cot was weighed prior to the repellent applications, placed in a labeled plastic bag, used only once to apply repellent to a subject, and then placed back into the bag that was sealed and weighed. The difference in finger cot weight before and after applications was used to quantify the weight of material lost from application (raw data on finger cots weights pre- and post-applications is provided on p. 272,

Appendix 5 in MRID 519127-01). The mean of material loss equaled 0.049 g ± 0.02 (± Standard Deviation (SD)), with representative values that ranged from of 2.82% to 23.1% of material loss. Loss of materials to finger cots result in a lower and consequently more conservative rate for repellency evaluation; therefore, loss of material resulting in a lower rate of application does not overestimate repellency or compromise efficacy. To ensure consistency in applications, three gloved researchers applied the product onto subjects at approximately the same time with subject application times differing by ≤ 20 minutes (timing of applications are described in notes to file on p. 274 for site 1 and p. 276 for site 2; see Table 3 below for individual times of applications). A separate researcher verified dosage and recorded time of application for each subject. Forearm surface area, volumetric dosage to achieve 0.00094 ml product/cm^2 of skin (see Formula 1 above), and application time for each subject, were provided in Appendix 5 in MRID 519127-01; summary statistics for average forearm surface area and average amount of product applied to subjects at each site are shown in Table 3.

Table 3. Mean and range of skin surface area and volumes of product applied to subjects

	Site 1		**Site 2**	
	Mean	Range	Mean	Range
Forearm surface area (cm^2)	543.45	416.88 – 670.63	530.59	401 – 671.25
Application amount (ml)	0.51	0.39 – 0.63	0.50	0.38 – 0.63

Note: Summary statistics calculated for this review from raw data in Appendix 5 [p. 263 (site 1) and p. 268 (site 2) in MRID 519127-01].

Field testing: Prior to the test day, subjects were reminded by a phone call to wear light, loose fitting long-sleeved shirt and pants for the study day. During this call, a request was also made to confirm that subjects avoided the use of repellents within the last 48 hours, and refrained from smoking, consuming alcohol, or using perfumed products since 9:00 pm the previous evening and to confirm that subjects can participate during the test day without smoking or consuming alcohol. At test days, product applications were made ~30 to 60 minutes prior to the first exposure period at Site 1, and ~ 2 to 2.5 hours prior to the first exposure period at Site 2 (data on time of applications on p. 264 for site 1 and p. 269 for site 2 in Appendix 5 in MRID 519127-01; see Table 4 in this review). Alternates were dismissed after the repellent was applied to treated subjects and by the end of the first two exposure periods. Control subjects were paired with a staff member, 12 treated members were paired together, and the remaining treated subject was paired with a staff member wearing protective clothing but no repellent. Treated subject pairs were "observer pairs" that were spaced close enough to effectively observe mosquito landings, whereas the staff members paired with control subjects were spaced close enough to effectively aspirate mosquitoes off control subjects. For each test day, the study director led subjects and attending researchers to an exposure area that he deemed to have optimal mosquito activity for each 5-minute exposure period. Three exposure areas were present in Site 1, whereas two exposure areas were present in Site 2. The exposure area maximum occupancy consisted of 20 subjects, two staff members accompanying control subjects, and typically two data-takers. The size of each exposure area was approximately 30 ft. by 100 ft. with paired subjects distributed within each exposure area at least 10 ft. (3 m) apart between pairs during exposure periods. Description is provided on p. 24 (MRID 519127-01). An exception to this 3 m spacing occurred during the first exposure period at Site 1, when some subject pairs were aggregated as close as 6-8 ft. due to a tree fall that reportedly limited accessible areas. This is reported as a protocol

deviation.

Landing pressure and exposure periods: Two control subjects (one male and one female) were used at each site to ensure adequate landing pressure immediately prior to the start of each treated subject exposure interval. Each control subject exposed their forearm for 5 minutes or until 5 mosquito landings were recorded, whichever occurred sooner. Total mosquito landings on each control subject were recorded for each exposure interval. Adequate landing pressure was established at five landings on each control subject per five minutes or less (a minimum rate of 1 mosquito per minute), except for three instances when 4 mosquitoes landed within 5 minutes. These exceptions occurred only once in one exposure interval at each test site. Treated subject exposures (5 minutes in 30-minute intervals) were conducted immediately after control subject exposures. During these periods, treated subjects exposed their forearms, reported mosquito landings on their skin, and if possible, aspirated landing mosquitoes. One or two staff members, typically one for control subjects and another for treated subjects, recorded landing events as landings were called out. Another staff member collected specimens from subjects that were later transported for identification and pathogen screening (p. 25 in MRID 519127-01). Data on mosquitoes aspirated from control and treated subjects at site 1 on Sept. 27, 2022, is summarized on Appendix 8 (p. 301). Data on mosquitoes aspirated from control and treated subjects at site 2 on October 3, 2022, are summarized on Appendix 8 (p. 303). Data are arranged by site, date of collection, pool number, species identification, number of species, number in pool, and test subject identification number (wherever applicable). Data on frequency and distribution of landings are provided in Appendix 7 in MRID 519127-01. Raw data on landings on control subjects are provided on pp. 289-291 for site 1 and on pp. 294-295 for site 2 (Appendix 7) and summarized in Table 5 on p. 26 (MRID 519127-01). Both exposure trials began around midday and ended in the evening.

Study results: Repellent efficacy was expressed as CPT for the duration of repellency. Summarized study outcomes at each site were based on CPT data analysis (pp. 27-28 of 380 in MRID 519127-01). CPT was measured for each subject as the duration of protection period from the time of product application to the time of FCL. A confirmed landing was defined in the study as a landing followed by a second landing within 30 to 60 minutes of the first landing (within the same exposure period or occurring during one of the next two exposure periods, within 30 to 60 minutes of the first landing). The confirmatory landing within 60 minutes is reported as a deviation.

Species identifications of mosquitoes aspirated from control and treated subjects are summarized in Table 4 below. Data in Table 4 is extracted from Table 5 on p. 26 in MRID 519127-01 and from Appendix 8 in MRID 519127-01 (p. 301) mosquitoes aspirated from control and treated subjects at site 1, and p. 303 mosquitoes aspirated form control and treated subjects at site 2, arranged by species). *Aedes melanimon* was the most captured species (Table 4). Figure 3 below illustrates the difference between sites in the number of *Culex* mosquitoes captured from control subjects at night versus day.

The mCPT for the spray repellent product was calculated with data from Site 2, with 12 out of 13 subjects receiving confirmed landings (See table 5). The mCPT at Site 1 could not be defined due to right-censored values provided by 11 out of 13 treated subjects but was estimated in the

final study report based on duration between product application and the termination of the exposure test. CPT data are reported on pp. 288-290 for site 1, and on pp. 292-293 for site 2 in Appendix 7 in MRID 519127-01 and summarized in Table 5 in this review. Subjects that received no landings and those that did not receive confirmed landings at the end of the test were considered right-censored data in the Kaplan Meier analysis with FCL listed as the last exposure period time for these subjects in the data summary tables. The more conservative estimate of mCPT was determined from data collected at Site 2, with mCPT calculated as 519 minutes (8.65 hours). No subjects were excluded, withdrawn, or removed on either test day. Tabulated CPT (landing) data for each treated subject are provided in Appendix 7, raw data sheets are provided on pp. 288 to 290 for site 1, pp. 292 to 293 for site 2 in Appendix 7 in MRID 519127-01. These data include the number of landings at each 5-minute exposure period, every 30-minute interval, application time, time of FCL, and time to FCL (or CPT). CPT measurements are summarized in Table 5 below.

Table 4. Mosquito species and number aspirated from control and treated subjects

Site		Treatment	Subject	Species	# Caught	Total Caught by Treatment	Total Landings by Treatment	% of Landings Caught
1		Control	25	*Aedes melanimon*	42	99	179	55.3%
				Aedes vexans	9			
			129	*Aedes melanimon*	36			
				Aedes sticticus	1			
				Aedes vexans	7			
				Anopheles freeborni	2			
				Culex tarsalis	2			
		Treated	30	*Aedes melanimon*	1	2	10	20%
				Aedes vexans	1			
2		Control	6	*Aedes melanimon*	37	102	158	64.5%
				Aedes nigromaculis	1			
				Aedes vexans	3			
				Anopheles freeborni	1			
				Culex tarsalis	11			
			101	*Aedes melanimon*	39			
				Aedes vexans	7			
				Culex tarsalis	3			
		Treated	7	*Aedes melanimon*	1	8	27	29.6%
				Aedes vexans	1			
			62	*Aedes melanimon*	1			
			69	*Aedes melanimon*	1			
			122	*Aedes melanimon*	1			
			132	*Aedes melanimon*	1			
			167	*Culex tarsalis*	1			
			178	*Aedes melanimon*	1			

Note: Data for species and numbers aspirated were taken from Table 5 for control subjects and Appendix 8 (p. 301 for site 1 and p. 303 for site 2) for control and treated subjects. Total aspirated by control or treated subject per site was calculated by the sum of numbers provided in the 'Number Aspirated' column. Total mosquito landings were determined by raw data sheets in Appendix 7 (CPT data on pp. 288 – 290 for site 1, and pp. 292 – 293 for site 2 in MRID 519127-01).

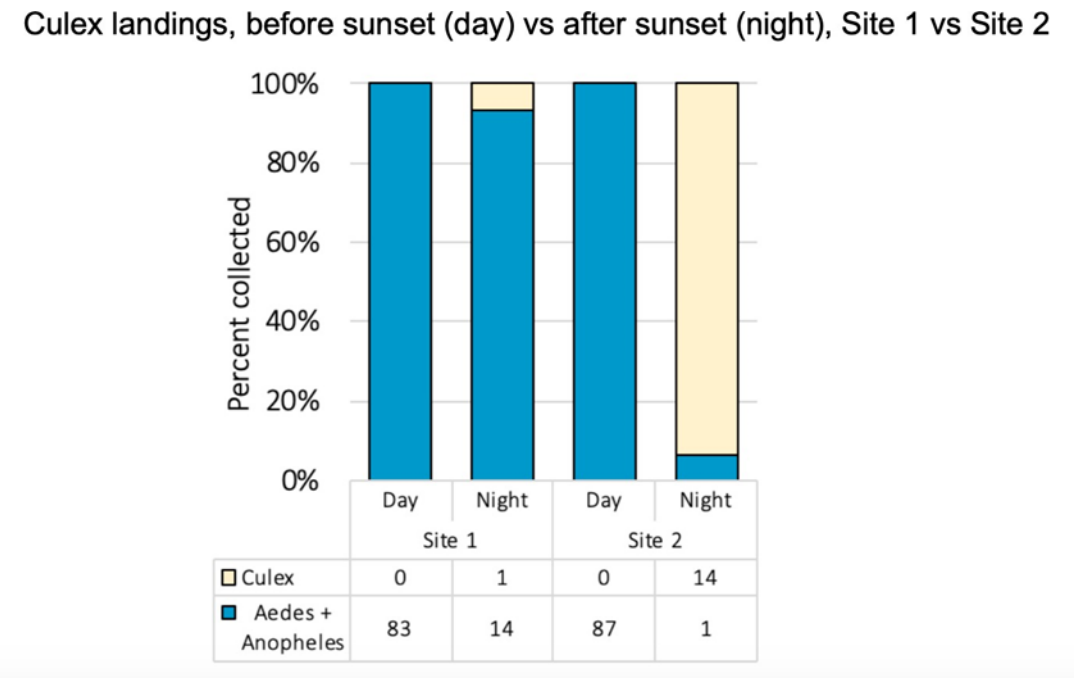


Figure 3. Comparison of day versus night (sunset and later) control subject landing counts of the three target mosquito genera between Site 1 and Site 2.Sunset was at 18:56 at Site 1 and 18:47 at Site 2 (source: https://sunrise-sunset.org/us/gridley-ca/2021/10).

Table 5. Summary of repellency test results for sites 1 and 2.

Site 1					
Subject	Amount applied (ml/cm2)	Application time (hrs:min)	Time to FCL (min)	Time to FCL (hrs:min)	CPT: Y or N (Yes or None)
104	0.39	10:53	361	6:01	Y
72	0.43	10:53	562	9:22	N
103	0.46	10:53	562	9:22	N
11	0.52	10:59	556	9:16	N
30	0.61	10:59	420	7:00	N
4	0.50	10:59	556	9:16	N
150	0.52	11:04	551	9:11	N
23	0.62	11:05	550	9:10	N
76	0.43	11:04	551	9:11	N
70	0.45	11:09	546	9:06	N
55	0.50	11:09	546	9:06	N
91	0.63	11:10	545	9:05	N
63	0.55	11:15	540	9:00	N
Site 2					
Subject	Amount applied (ml/cm2)	Application time (hrs:min)	Time to FCL (min)	Time to FCL (hrs:min)	CPT: Y or N (Yes or None)
7	0.42	9:06	584	9:44	N
33	0.44	9:07	527	8:47	Y
62	0.46	9:06	647	10:47	Y
69	0.54	9:11	491	8:11	Y
73	0.49	9:12	548	9:08	Y
98	0.38	9:15	519	8:39	Y
122	0.61	9:12	462	7:42	Y
123	0.40	9:16	458	7:38	Y
132	0.50	9:30	444	7:24	Y
167	0.56	9:24	595	9:55	Y
178	0.63	9:20	454	7:34	Y
181	0.57	9:26	448	7:28	Y
193	0.49	9:26	627	10:27	Y

Data from pp. 264, 268, 269, and pp. 285-286, 288, 292 and 293 in MRID 519127-01

Statistical analysis: A sample size of 13 subjects per treatment group was employed for testing efficacy as determined by the EPA power analysis for determination of sample size, "*Power Analysis/Sample Size of Field-Based Mosquito Studies*," dated July 2017, which is provided in Appendix 3 of the revised protocol. The protocol is included in Appendix 1 of MRID 519127-01. The mCPT at Site 1 could not be calculated due to right-censored values provided by more than half (11 out of 13) treated subjects. The mCPT and 95% confidence intervals (95% CI) were calculated using Kaplan-Meier Survival Analyses for landings data collected at Site 2, with 12 out of 13 subjects receiving confirmed landings. Statistical analyses used in the amended study report (MRID 519127-01) were performed in R and the analytical output provided in Appendix 11 (p. 317). The Agency analyzed the data using SAS via statistical methods recommended in the protocol, which provided a 95% CI (lower CI of 454 seconds, upper CI of 584 seconds) that differed from the 95% CI reported for Site 2, due to default transformation methods in the statistical programs used. The researcher used a log transformation and the Agency used a log-log transformation as recommended in the study protocol (Attachment 5). The results were

similar (Table 6). The mCPT at Site 2 was estimated as 519 minutes (8.65 hours) in both statistical methods (Table 6). EPA's statistical analysis report of the CPT data is provided in Attachment 5 of this review.

Table 6. Median CPT (mCPT) values determined via Kaplan-Meier Survival Analyses

Site	Time	EPA Analysis Results			MRID 519127-01Results			Precision K value (Lower 95%CI/mCPT)
		Est. mCPT	95% CI		Est. mCPT	95% CI		
1	minutes	**NA***	NA*	NA*	**NA***	NA*	NA*	NA*
2	minutes (hours)	**519** (8.7)	454 (7.6)	584 (9.7)	**519** (8.6)	458 (7.6)	NA*	0.87

*: NA = Not available, i.e., the value was greater than the length of exposure testing because 11 out of 13 subjects did not experience a confirmed landing by the end of the test day.

Statistical summary from EPA's analysis using SAS compared to the analysis in the study report (MRID 519127-01) using R, with medians and 95% confidence intervals (95 CI) provided as R output in Appendix 11 (p. 317 in MRID 519127-01).

The product label is provided in Attachment 7 of this review.

Deviations from Protocol (Listed in Table 6, pp. 31-33 in MRID 519127-01)**:**

- *Use of finger cot over glove for administration of product*: Gloves were replaced by single finger cot to facilitate dose application procedure and reduce weight of material lost to cots during application. This deviation has no ethical implications, and it is not expected to adversely affect the validity of results.

- *Measurements of lower legs were not performed*: Product was tested on non-dominant forearms only. Product was not applied on lower legs since scouting of mosquito activity at both sites indicated upper and not lower body, foraging. The study protocol indicated testing on lower legs to be conditional to mosquitoes' foraging behavior; therefore, skipping lower legs testing should not be considered a protocol deviation.

- *Use of different criteria defining a confirmed landing*: The criterion for determining first confirmed landing was applied to subjects numbered 30 and 104 for Site 1 (the first field test day) and for subject number 69 for Site 2 (the second field test day) as detailed in the Informed Consent Form (ICF) and not as described in the study protocol. As stated in the study protocol, a landing is confirmed by a second landing occurring within 30 minutes of the first; that is, a confirmatory landing may occur during the same exposure period as the first landing or during the next exposure period following the first landing. Exceptions only apply when there are skipped exposure periods or exposure periods of low landing pressure that occur just prior to, or immediately after, the period when the first landing took place, or the first landing occurs during the last exposure period of the field test day. The interpretation derived from language in the ICF implies that a confirmatory landing can occur during the same exposure period or in two

of three consecutive exposure periods following the period when the first landing occurred; that is within 30 or 60 minutes of the first landing. The metric followed a more conservative approach than the criteria described in the study protocol. Therefore, this deviation is not expected to compromise the validity of the data.

- *Subject pairs were positioned less than 10 feet apart*: The 10 ft. distance between pairs of subjects specified in the study protocol was altered to 6 and 12 ft. during for the first exposure at Site 1 due to a tree fall that was cleared before the second exposure and distance between pairs was restored to 10 ft. for the remining of the test day. This deviation is unlikely to adversely affect the validity of the data since the deviation occurred very early in the study, only during the first exposure period.

- *Total enrollment of subject increased from 40 (proposed) to 46*: On field test day at Site 1, 31 subjects were asked to come to the lab prior to transport to the field. Six subjects were randomly dismissed and 20 (13 treated, 2 controls and 5 alternates) retained for field testing. On field test day at Site 2, 24 subjects were summoned; 4 were randomly dismissed and 20 (13 treated, 2 controls and 5 alternates) were transported to the field. This deviation increased reliability for having enough numbers of alternate subjects to maintain the sample size and integrity of the study. Therefore, the increase in enrollment is not detrimental to the quality of the test.

- *Use of stratified randomization of treatment allocation within subject pools*: More female than male candidates responded to recruitment efforts in the time frame between EPA permission to conduct the study and the end of the mosquito field season, and a larger number of females than male subjects arrived for testing at Site 2. To account for these unexpected circumstances, treatment allocation was randomly stratified within the sexes to maintain a relatively balanced sex ratio as specified in the study protocol. All males were assigned to the treated group at Site 2, and females were randomized within their gender using the Excel-based randomization method described above. Randomization of subjects within sites does not invalidate the data since the separate statistical analyses were performed for data from each site and is unlikely to compromise the integrity of the study since a balanced sex ratio was maintained.

- *Use of supplementary weather station data*: Environmental data was not collected for the first five exposure periods at Site 1, so temperature data sourced from a nearby weather station (The Ranch – KCALIVEO2) was used to supplement data at these exposure periods. Conditions remained favorable to active mosquito foraging throughout the day. Collected environmental data records for both sites are provided in Appendix 10 (MRID 519127-01). Therefore, this deviation is unlikely to invalidate or adversely impact the integrity of the study.

COMMENTS, RECOMMENDATIONS and CONCLUSIONS

The registrant's response, dated May 12, 2022, provides complete answers to points of clarification requested in EPA's 90-Day Technical Screening Results of study MIM-006 (Attachment 2). The Registrant's rebuttal (dated June 22, 2022) to EPA's 75-Day deficiency letter regarding site independence is provided in Attachment 3 of this review.

Registrant's responses to points of clarification: Responses to Agency's comments are listed below in **bolded text.**

- **The registrant provided information describing the types of traps, frequency and times that surveillance traps were deployed at each site per week, how many traps were used and their locations within sites, the duration for which traps were deployed, and how frequently the traps were collected for specimen processing**. The response is acceptable. It is recommended for future studies that placement of traps during pre-test monitoring be depicted in the Site Maps provided in Appendix 16 (This information is CBI in CA in MRID 519127-01).

- **Procedures for attractiveness tests have been described. The researcher has clarified that individual subject exposures to mosquitoes in attractiveness tests were performed as a once-only procedure to allow non-mosquito-attractive subjects to withdraw before additional procedure are applied to the subject. All subjects qualified as attractive to mosquitoes**. **Raw data sheets are provided. No tests were stopped, therefore there is no report of stopped tests**. The registrant's response to details on how attractiveness test was conducted and whether tests were stopped is acceptable.

- **Procedures detailing aspirator training. The criteria used to determine "sufficient competence in aspirator use" is clearly stated. Each subject practiced aspirator training individually and as many times as needed. Proficiency was determined by a researcher, who also demonstrated how to use an aspirator for catching mosquitoes that land on subject's arm.** The response concerning how proficiency was determined in the use of aspirators is acceptable**.**

- **The formula(s) used to determine application dosage is reported** and the response is acceptable.

- **Arrangement of control and treated subjects in the field is explained. Each control subject was paired with a staff member, 12 treated members were paired together, and the remaining treated subject was paired with a staff member wearing protective clothing but no repellent** (as proposed in the protocol). The response is acceptable.

- The minimum number of two landings per minute was used for assessing subjects' attractiveness to mosquitoes in this study's lab-based attractiveness trials, departing from guideline recommendation of 5 landings/minute (according to OPPTS 810.3700

guidelines section (j)(12) "*five mosquito landings in five minutes or less*…"). However, the attractiveness test was conducted with a lower mosquito density (1 mosquito /4, 251 cm^3) than recommended in OPPTS 810.3700 guidelines section (1 mosquito/1160 cm^3) for testing repellency using the arm-in-cage method. **The lower mosquito density was provided by the registrant as a justification for the lower attractiveness threshold (Attachment 2)** and is acceptable. All subjects showed attractiveness by receiving 2 landings per minute or less. These guideline deviations are unlikely to overestimate attractiveness, and consequently, overestimate repellency. Therefore, these deviations do not compromise the validity of the data, but they should have been reported in the study report as deviations. **The registrant also noted that the OPPTS 810.3700 does not provide explicit guidance for mosquito thresholds and densities used in attractiveness cage assays (Attachment 2).** The Agency recommends that future studies refer to OPPTS 810.3700 guidelines section (j)(12) for guidance on mosquito cage assays, which includes those used for subject attractiveness tests.

- Clarification is provided on p. 23 (MRID 519127-01) for the protocol deviation concerning randomization of subjects within test days (refer to Recruitment and Randomization on p. 5 of this review for full details).

Recommendations for future studies:

The following recommendations should be considered for future studies. These specifications are not in the study protocol that was reviewed by the Agency and HSRB. Therefore, they are not reported in this study and not requested or required at this point:

- Details regarding how the study was conducted was not specified in the main body of the report and were only referred as indicated in the protocol. It is recommended that experimental details be incorporated in the main body of the study report sections preceding the appendices. This practice will facilitate and expedite the review process in the future.

- Neither *Aedes aegypti* nor *Aedes albopictus*, species that are important vectors of Zika, Dengue, and Chikungunya, and that were listed with probable sites in Table 1 of the protocol (Appendix 1, p. 34 of 362) were present at the selected study sites 1 and 2 (Table 2 above). Therefore, the data from test sites 1 and 2 do not support efficacy claim against these vectors of Zika, Dengue and Chikungunya on the product label. According to the rule *Pesticide Product Performance Data Requirements Claiming Efficacy Against Certain Invertebrate Pests*, effective June 14, 2022, data on these species will be required for general efficacy claims against mosquitoes of product labels, and consequently, for product registrations[1].

- While the two sites have been historically used to register repellent products and the PCA supports that species composition differs between sites (Attachment 3), there would be more confidence that the sites are distinct if there were separated by a

[1] 40 CFR 156, Subpart R

geographic distance greater than the flight distance of mosquito species present at the site(s). The Agency mapped the sites and determined that there is 15 km (~9 miles) between Site 1 and Site 2. From the total mosquitoes caught in both sites, the most trapped species found included *Aedes melanimon*, *Anopheles freeborni*, *Culex tarsalis*, and *Aedes vexans* (Table 1 above). The reported range of *Culex tarsalis*, *Aedes vexans* and *Anopheles freeborni* flight distances include those that are ≥ 15 km (Verdonschot and Besse-Lototskaya 2014).

- The study report indicates that exposure sites were moved to follow mosquito activity. Neither specific position of subjects nor specific position of monitoring traps in field sites are specified. The distribution of subjects amongst separate exposure areas within each site during each time interval, process of assigning subjects to each area, size of each area, positioning of subjects within areas, and the placement of pre-test monitoring traps in relation to these areas should be specified in future study reports.

Comments and recommendations made to study protocol and study report:

- Appendix 1 in MRID 519127-01, p. 61: "*To guard against inflating protection times, however, in the unlikely event that a treated subject receives a confirming landing in the first or second exposure after the exposure delay, that subject will be excluded from the study and replaced with an alternate*." This statement should have been removed from the protocol and it was not.

- The definition of CPT proposed in the protocol should also be included in the main study report to confirm that the same definition was used to assess the landings data collected in the study.

- The term *'biting pressure'* is used in the protocol regarding control subject exposures (Appendix 1 in MRID 519127-01, p. 62), and should have been replaced with *'landing pressure.'*

- Statements regarding a dermal absorption study should have been removed from the protocol on p. 117 in Appendix 1 (MRID 519127-01).

- A citation should be provided for the claim that DEET-based repellent may produce mild to serious side effects (Appendix 1 in MRID 519127-01, p. 35). A citation should also be included for the statement regarding how the CDC estimates that about 4 out of 5 people who are infected with WNV do not develop any type of illness made in the protocol (Appendix 1 in MRID 519127-01, p. 39) and the ICF (Appendix 1 in MRID 519127-01, p. 173).

- The term "bounce" in the CPT raw data sheets has been explained as not touching the skin and therefore has not been recorded as a landing for CPT determination. Regardless of the term used, this event did not impact the FCL. However, the EPA's guidelines do not include the term "bounce" and it will be considered as a landing in

future studies. Therefore, this terminology will not be accepted in the future.

Conformity with Protocol and Amendments:

The protocol was reviewed by EPA and the HSRB. The protocol was revised to address recommendations from both organizations and approved by the IRB on September 11, 2021. The original protocol was dated February 17, 2020, and was amended 3 times on the following dates: December 23, 2020; August 23, 2021; and September 5, 2021. The study was conducted according to EPA Guidelines OPPTS 810.3700 (with minor deviations concerning mosquito densities for assessing subjects attractiveness to mosquitoes) and study protocol MIM-006 (MRID 517071-02) as based on recommendations by EPA in the fourth protocol version dated September 5, 2021. Specific protocol amendments made prior to the start of efficacy testing are detailed under "Responsiveness to EPA and HSRB Science Comments on Draft protocol" tables in Attachment 6.

The following study details in the study report conformed with protocol provided in Appendix 1 of study report MRID 519127-01.

- Two distinct field sites with an abundance of mosquitoes were used. These sites had the presence of three mosquito taxa (*Aedes*, *Culex*, and *Anopheles* spp.) and the absence of mosquito-borne pathogens for a minimum of 1 month prior to testing.

- *Culex* spp. and *Aedes* spp. were tested for mosquito pathogens, whereas *Anopheles* spp. were not tested (Appendix 8, pp. 283-287). An exception to this was a mosquito sample for subject 225 that included *Anopheles freeborni* (Appendix 8, p. 287)

- Pre-test monitoring was performed weekly for a minimum of 1 month prior to the start of testing.

- Twenty subjects were selected to participate in efficacy trials, which includes a sample size of 13 treated subjects, 2 untreated control subjects (one male and one female), and 5 alternate subjects used at each site.

- Subjects used in efficacy field trials completed and passed attractiveness tests and aspirator training.

- Length and circumference of the non-dominant arm were measured and recorded.

- Multiple researchers (three) applied the standard application rate of 0.5 g of product per 600 cm^2 of skin to the non-dominant arm of treated subjects. A syringe was used to dispense the exact amount of product to each subject, adjusted to the skin surface area of each subject, to apply the standard dose across all subjects. Product weight loss from applications were accounted for.

- Control subjects were used in 5-minute exposure periods prior to each exposure period of treated subjects to assess landing pressure.

- Adequate landing pressure in the field was defined at a minimum rate of 5 mosquito landings within 5 minutes or less on each of the controls.

- Intermittent exposure periods of 5 minutes at 30-minute intervals were used.

- Tabulated data proposed in the protocol for control and treated subjects were included in the final report.

- Mosquitoes caught during pre-test site monitoring and aspirated from control and treated subjects were identified to species and screened for pathogens.

CONCLUSION

The methods used in this study are based on a protocol reviewed by the EPA in concordance with EPA's recommended revisions and the HSRB; the methods were partially amended to incorporate EPA and HSRB recommendations before testing began (Protocol version 4, dated September 5, 2021). Protocol deviations are reported in the study report. EPA's conclusion is based on review of the data and interpretation of test results, following standard recommendations from test guidelines OPPTS 810.3700, protocol procedures, and standard policy in the Agency's Repellency Awareness Guidance[2] for determination of CPT. This assessment concluded that the study results are acceptable to support a CPT of 8.0 hours against mosquitoes for the proposed spray repellent products containing 11% w/w of OLE and 7.75% w/w of MNK.

REFERENCES:

Verdonschot, P. F., & Besse-Lototskaya, A. A. (2014). Flight distance of mosquitoes (Culicidae): a metadata analysis to support the management of barrier zones around rewetted and newly constructed wetlands. *Limnologica, 45*, 69-79.

cc: Michelle Arling

- Attachment 1: Amended study report (MRID 519127-01) that replaces the original study report (MRID 517071-01) with study protocol therein (Appendix 1 in study report).
- Attachment 2: Registrant's updated response (dated May 12, 2022) to EPA's 90-Day Technical Screen

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

- Attachment 3: Registrant's technical report and rebuttal (dated June 22, 2022) to EPA's letter regarding site independence
- Attachment 4: EPA's review of the registrant's technical report and rebuttal June 22, 2022) regarding site independence (including EPA's statistical evaluation of the Principal Components Analysis (PCA) provided in the registrant's technical report and rebuttal)
- Attachment 5: EPA's statistical report on the Kaplan-Meier survival analyses provided in the study report
- Attachment 6: Responsiveness to EPA and HSRB Science Comments on the Draft Protocol
- Attachment 7: Product Label

Attachment 1

MRID 519127-01

(provided as a separate file)

Attachment 2

Registrant's updated response (dated May 12, 2022) to EPA's 90-Day Technical Screen

May 12, 2022

Via E-Mail

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

Re: Additional Responses to 90-day Preliminary Technical Screening Results of Field Test Efficacy Report, Mimikai, Inc., Study Number MIM-006, EPA File Symbol: 93616PA10, Action Case Number 00336661

Dear Ms. Hollis:

Bergeson & Campbell, P.C. (B&C®) is pleased to respond on behalf of Mimikai, Inc. (Mimikai) to the U.S. Environmental Protection Agency's (EPA) 90-day Technical Screen for Mimikai's "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray against Mosquitoes," Study ID MIM-006, MRID No. 517071-01. EPA has begun its in-depth review and concluded that the study report is "incomplete and that further information is needed". Mimikai also responded to the 90-Day Technical Screen with information, clarifications and proposed amendments on March 23, 2022, and also on April 26, 2022 with site specific geographical coordinates.

EPA requested explanations, discussions, and points of clarification for the science and ethical aspects of the study, many of which required amendment of the final report. Cumulative technical responses prepared by the testing facility, Carroll-Loye Biological Research (CLBR), are detailed in Appendix 1 of this letter. Submitted via CDX is the report, "Amended Final Report, Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes", Study Report MIM-006, MRID No. 519127-01 (replaces MRID No. 517071-01; remains a supplement to MRID Nos. 517071-02 and 517656-01).

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

Sincerely,

Dana S. Lateulere

Dana S. Lateulere

Attachments

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APPENDIX 1

EPA Comments *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
1) The study report should specify the exact locations of test sites. A note in Appendix 10 lists Site 1 in Glenn County and Site 2 in Butte County, California. These locations should be specified in the main body of the study report (not just an appendix).	CLBR proposes to amend Section 3, subsection 'Site Selection and qualification', of the final report as follows (added is bolded, underlined): "The Study Director chose two sites**, described as follows,** for efficacy testing based on their differences in their habitat structure and the presence of *Aedes, Culex* and *Anopheles* mosquitoes, along with the absence of sampled viruses in the trapped mosquitoes sampled within the month preceding efficacy testing at each Site**:** **Study Site 1:** **Flooded forest; Glenn County, California** **(approximate center-point: <*Cross-reference 1*>)** **Study Site 2:** **Open wetlands, irrigated pasture; Butte County, California** **(approximate center-point: <*Cross-reference 1*>)** **** Due to the confidential nature of the site locations, the specific coordinates have been redacted and moved to the Confidential Attachment of the amended final report (see Appendix 16).\"***
2) Explain why selected field sites did not include *Aedes aegypti* or *Aedes albopictus* required for testing vectors of Zika, Dengue and Chikungunya. The protocol listed potential field sites that included all main disease vectors within three genera of *Aedes*, *Culex*, and *Anopheles* but neither *Aedes aegypti* nor *Aedes albopictus* were found at the field sites selected for testing. The product label included in the study protocol claims efficacy against vectors that may transmit Zika and Dengue; therefore, either *Aedes aegypti* or *Ae. albopictus* must be tested to support these label efficacy claims. The Agency is currently requiring testing on the taxa listed in the	The collection of repellency data for the Zika, dengue and chikungunya vectors, *i.e., Aedes aegypti* and *Ae. albopictus,* were aspirational, as implied by the protocol, but not requisite for the execution of this study. In the conduct of this study, mosquito populations at the candidate field site that included those two targeted vector species were destroyed by Hurricane Ida in August 2021, leading us to adopt the next most suitable site for conducting the study within 2021.

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bullets below for a general mosquito claim to cover the most prevalent diseases transmitted by mosquitoes. The study protocol included a list of potential field sites where *Aedes aegypti* or *Aedes albopictus* are found. In addition, the Informed Consent Form explains to subjects the potential risks of contracting Zika and Dengue, which are transmitted by these specific vectors. No discussion has been provided in the study report that explains why the proposed field sites containing these vectors were not selected in support of this label claim. Guidance on mosquito species required for testing includes the species listed below, and can be found at: https://www.epa.gov/pesticide-registration/guidance-efficacy-testing-pesticides- targeting-certain-invertebrate-pests • *Anopheles* (*Anopheles quadrimaculatus* or *Anopheles freeborni* or *Anopheles punctipennis* or *Anopheles gambiae*), • *Aedes* (*Aedes albopictus* or *Aedes aegypti*), and • *Culex* (*Culex pipiens* or *Culex quinquefasciatus* or *Culex tarsalis*). Testing that includes the mosquito taxa specified above ensures that there are data supporting efficacy against the major disease vectors in these groups (e.g., *Aedes albopictus/Aedes aegypti* for Zika virus, etc.). Requiring data on major vectors is necessary to ensure that pesticide products are effective against species that may pose risks to public health.	Proposed label claims reflect the absence of those data (see master label dated January 14, 2022, submitted with registration application EPA No. 93616-R; appended as document number 353257; submitted on March 2, 2022 and March 23, 2022).
3) Specify in the study report whether finger cots were replaced after each dose applications.	CLBR proposes to amend Section 4 of the final report to specify that finger cots were single-use and replaced after each dose application and in response to

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	Comment #4 (see below), as follows (added text is bolded, underlined; deleted text is bolded and struck through): "**4) Dosage Determination** Limb measurements served as a basis for determining total volumetric dosage for each subject (see Appendix 5 for limb measurement raw data by subject). Surface area was determined from limb the average of four circumference values measured at the wrist joint, just below the elbow, and at two equally-spaced locations between, multiplied by the length from wrist to elbow. The resulting surface area calculations for each individual were recorded directly onto the repellent applications data sheet, then modified by the known specific gravity of the Test Material (0.8874 g/ml) to convert 0.5g/600cm^2 into ml for application of Test Material to subjects' forearms via tuberculin syringe (5th paragraph of Section 4.7, page 28 of the Study Protocol). **Each finger cot was pre-weighed prior to the beginning of repellent applications, placed in a labeled plastic bag, used once for application of repellent to one subject, then returned to the same bag and sealed in for subsequent weighing. It is unknown how much of each cot's weight change can be attributed to oils or sweat from the subject's skin receiving repellent application; this was presumed to be minimized by the limb washing and drying procedures that were completed shortly before applications.** Weights of finger cots measured before and after each Test Material application increased an average (±sd) of 0.049±0.02 grams (range 0.013-0.129 grams). Those values represent a loss of Test Material during applications ranging from 2.82 to 23.1%. How that loss might compare to loss on the skin of an ungloved hand used for personal application during consumer use is unknown. ~~**How that loss might compare to loss on the skin of an ungloved hand used for personal application during consumer use is unknown.**~~"

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4) Include in the study report an analysis of the correlation (or lack of it) between loss of material to cots and Complete Protection Time (CPT). CPT is defined in this study as the duration of time between application of the repellent at the standard application rate of 0.5 g/600 cm2 and time point of efficacy failure, signaled by the First Confirmed Landing (FCL).	Weighing the finger cots used to apply the test material was performed in response to a sound HSRB suggestion, CLBR found that substantial material sometimes remained on the finger cot after application. Importantly, from the practical perspective, our process of applying the test material, guided by the EPA approved protocol, emphasized covering uniformly the entire test area. That execution was guided by visual cues, friction against the skin surface, and subject perception, a method intended to minimize any possible 'gaps' in application that could have been associated with lower doses. The realized study outcome then, evident from the findings provided in the report, is that the calculated CPT was measured with a mean realized dosing rate of 0.451±0.02 g/600 cm^2 – a quantity that proved sufficient to provide full coverage and protect subjects for periods ranging from 361 to at least 647 minutes. It is noted anecdotally that the most briefly protected subject (~6 hrs) received one of the very highest estimated dosing rates of 0.48 grams per unit area, and the second most briefly protected subject (7 hrs) was likewise dosed at an above average rate (0.465 grams.) At the other end of the longevity spectrum, the longest (~10.75 hrs) and second longest (~10.5 hrs) protected subjects were below average in dosing (0.441 and 0.431 grams, respectively). While by themselves, these observations tell us very little, they may serve as a ready reminder that many factors in addition to variations in application dose likely impinge on product performance, and should be therefore taken into account when contemplating the design of a study to estimate a credible dose-response curve for protection against wild mosquitoes, which was not the objective of this study. CLBR therefore regards it to be unadvisable to conduct a post hoc regression analysis of CPT and net dose. That said, CLBR expects that any such analysis would identify no statistically discernable relationship between realized dose and CPT in this study.

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	CLBR proposes to amend the report to add the pertinent information regarding retention of test material on the finger cots (see proposed amendment to Section 4, *Dosage Determination*, in response to EPA Comment #3, above).
5) Specify whether subjects withdrew from the test and if withdrawn subjects were replaced during repellency testing.	CLBR proposes to amend Section 5 of the final report to include a subsection to specify that no treated or control subjects withdrew, were excluded, or were removed on either field test day, as follows (added text is bolded and underlined): ***"Withdrawal, Removal, and Exclusion of Subjects and Dismissal of Alternates*** **No treated or control subjects withdrew, were excluded, or were removed on either field test day. Alternates were dismissed after applications were completed on treated subjects and by the time the first two exposure periods were completed."**
6) Comparing efficacy of proposed products against DEET/ Picaridin products should be removed from the conclusion statement. The objective of the test is to characterize efficacy of the proposed product, not to compare efficacy with products containing different active ingredients.	CLBR proposes to amend Section 9 of the final report to remove the following statement (deleted text is bolded and struck through): ~~**These outcomes suggest that Lilly Pilly's performance is similar to other comparatively efficacious mosquito repellent formulations using conventional active ingredients such as deet and picaridin at similar, or higher concentrations.**~~

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7) Revise data entries for subjects with right-censored data as "NA" under the column heading "Time of FCL" in the treated subject summary tables in Appendix 7 of study report. If the first landing is at the last time interval, then there is no subsequent (confirmatory) landing available to determine FCL. If no landings occur, then neither event is present to mark "Time to FCL".	CLBR proposes to amend Appendix 7 of the final report to replace pages 272 and 277 with amended pages. See enclosed examples of the amended pages for Site 1 and 2 (document numbers 359350 and 359349, respectively; submitted on March 2, 2022 and March 23, 2022), wherein the data entries in question were annotated with "NA" (and "NA" defined in accompanying footnote) to reflect clearly the subjects with right-censored data.
8) Provide an updated proposed label to accompany the final study report.	A master label dated January 14, 2022, was submitted with registration application EPA No. 93616-R (appended as document number 353257; submitted on March 2, 2022 and March 23, 2022).
9) It is unclear what transformation was used to calculate 95% CI in the statistical methods. The transformation used should be specified, and the original R script files (.r file extension) and CSV input files should be provided.	The transformation was not explicitly specified in the script because it is the default condition for the function. CLBR proposes to amend Section 6, subsection *Landing data - treated subjects, Site 2, efficacy performance outcomes*, to include that information, as follows (added text is bolded, underlined): **"The 95% confidence intervals were calculated with data that were log transformed."** We are pleased to provide herewith the requested R script (as text and *.r files) and related data input files (*.csv) (submitted on March 2, 2022 and March 23, 2022): MIM-006_Analyses and Plots - Output.txt (document number 357625); MIM-006_Analyses and Plots.R (document number 357653); Control Landing Rate - Site 1 - Subject A.csv (document number 357629); Control Landing Rate - Site 1 - Subject B.csv (document number 357621); Control Landing Rate - Site 2 - Subject A.csv (document number 357622);

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	Control Landing Rate - Site 2 - Subject B.csv (document number 357623); Site_1_Treated_Duration.csv (document number 357626); and Site_2_Treated_Duration.csv (document number 357627).
10) Explain how the term "bounce," noted on the raw data sheet in Appendix 7 (p. 269 of 362), differs from a "landing." The term "bounce" is not defined and it should be.	Note to File on report page 271 clarifies the term "bounce" used in the context of one raw data annotation on page 269 of the final report. "Bounce" is CLBR vernacular for close approach of a flying mosquito that does not land but which may brush skin or arm hairs with the tips of the dangling legs followed instantaneously by a speedy retreat. No such notations are intended to function as data points for evaluating repellent failure. Rather, the notation only appears in an explanation for why a scored landing was corrected to a non-landing in one specific circumstance. Further clarification by report amendment is not considered necessary.
11) Describe in more detail the methodology for site monitoring prior to field testing. Specifically, report the following: • Frequency and time(s) in which surveillance traps were deployed at each site per week. • Number of traps used and their positions within the site, and how were they situated in relation to exposure areas (shown in Site Maps of Appendix 10) within each site. • How long were the traps deployed to catch mosquitoes at each sampling time? and • How frequently were traps collected for specimen processing?	CLBR proposes to amend Section 3 of the final report to include text detailing the methodology for site monitoring prior to field testing, as follows (added text is bolded and underlined; deleted text is bolded and struck through): *"Virus testing in trapped mosquitoes* **At each prospective field site, three mosquito traps were deployed, one in each of the three most humid and sheltered subsites that were also expansive and open enough at ground level to allow study subject pairs to distribute themselves safely at or beyond specified minimum distances. Samples from each trap were collected once in late June 2021, and then again once each week during the principal August-September 2021 sampling period at what would become the final two study sites. All three subsites were employed as exposure areas at different times of day at Site 1, depending on where mosquitoes were most abundant, whereas only two of the three subsites at site 2 were**

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	used as exposure areas (see site map sketches, Appendix 10). The third subsite at Site 2 was insufficiently sheltered from prevailing breezes on the day of the test. **Trapping was conducted for three hours each week, from approximately 6 pm to 9 pm. To bracket the early evening and crepuscular period when all three principal genera are simultaneously active, the start time was gradually shifted about 30 minutes earlier across the months sampled, from about 6:15 pm to about 5:45 pm. Trapping was not extended further into the evening because it would not enhance vector coverage and would instead risk depleting key scientific resource populations unnecessarily. The collection bag in each trap was removed and replaced hourly during the three-hour sampling period.** Trapped mosquitoes were **retained in trap bags and** transported to the laboratory in insulated boxes ~~**with dry ice**~~**. At the laboratory, mosquitoes were briefly cooled to below 0°C until quiescent for identification and counting.** They were then dispensed in aliquots of approximately 50 individuals for rapid identification to species by experienced taxonomically trained staff, referring where helpful to the keys of Darsie and Ward (2016). Mosquitoes were then placed in 50 ml centrifuge tubes by genus**and transferred to uniquely numbered vials that were further labeled with the abbreviation for our laboratory name, the field site name, and collection date. Each labeled vial was immediately transferred to an insulated box with dry ice (frozen carbon dioxide)**. ~~**Each tube was individually numbered with reference to an accompanying sample record sheet, and also labeled with the site and trapping date. Any specimens not immediately identifiable we returned to dry ice storage, and examined later, on a cold surface, with a stereomicroscope.**~~ All mosquitoes were identified to species. **Vial number along with the record of site, date, species identification and number of individuals per species were entered into a spreadsheet.**

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	Filled centrifuge tubes **on ~~were likewise quickly returned to~~** dry ice **~~storage prior to submission~~ were submitted** to the University of California, Davis Arbovirus Research and Training (DART) laboratory for RT-PCR analyses. Culex were assayed by DART for West Nile Virus, Western Equine Encephalitis, and St. Louis Encephalitis pathogens (collectively labeled WSW testing, Appendix 8), while *Aedes* were assayed for Chikungunya, Dengue and Zika pathogens (collectively, CDZ testing). One *Culex tarsalis* pool collected on 10 August at Site 1 was positive for West Nile Virus. No other samples were positive in our surveillance, (nor, ultimately, in our collections from study subjects, see below). Appendix 8 provides the records of species composition and viral screening results used to qualify the sites. The data were recorded directly into a spreadsheet compliant with DART Laboratory procedures for specimen submissions." *Further response:* a) Yes; three traps were deployed at each site for each sampling timepoint. This will be clarified in the amended final report. b) Yes; one trap was set up at each of the three subsites in Site 1. Amended sites maps will be included in the amended final report. c) There were three subsites at Site 2, each with one associated trap. The third subsite was not used on the day of the field efficacy trial because it proved insufficiently sheltered from breezes. The distribution of the traps amongst the relevant subsites will be clearly denoted on the amended site maps that will be included in the amended final report.
Your response states that "the traps were deployed in triplicate." **This response is incomplete. Further clarification is needed. See below questions:** a) Does your response mean that three traps were deployed at each site for each sampling timepoint? b) Was one trap set up at each of the three subsites in Site 1? You must amend the Site maps in Appendix 10 to show the placement and number of traps and submit the revised appendix. c) How were the traps distributed amongst the two subsites in Site 2? You must amend the Site maps in Appendix 10 to show the placement and number of traps and submit the revised appendix.	

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	See also the proposed amendment language above (highlighted text reflects changes from response submitted on March 23, 3022).
12) Describe the pre-test aspirator training in more detail. It is unknown what criterion was used for establishing subjects' proficiency in the use of aspirators if the test was performed as a once- only procedure (each subject placed their arms into mosquito cages only once). Describe how long subjects were trained to demonstrate proficiency, and how many attempts were permitted to demonstrate proficiency.	Here CLBR provides further context for, and descriptions of, the pre-test aspirator training. EPA will note that our accounts of how long subjects were trained to demonstrate proficiency, and how many attempts were permitted to demonstrate proficiency, are anecdotal rather than data-based. The pre-test aspirator training was conducted as described in the EPA and HSRB reviewed and IRB reviewed and approved aspirator training document (CLBR Training Manual §1.a. Observing mosquito landings and learning mechanical aspiration, version date 18 Dec 2020) as appears on page 183 of the final report. Neither that document nor any Carroll-Loye SOP document recommends or requires quantifying the time taken to complete the described procedures or the number of attempts a subject makes at effective aspirator use. For these reasons, CLBR did not record of how long subjects were trained or how many attempts were permitted to demonstrate proficiency. However, it is possible to estimate a range of likely durations of training and the likely range of the number of attempts permitted. These estimates are not provided in the final report because no data regarding duration of training or range of numbers of attempts was collected during the study. Based on queries of staff, researchers spent approximately ten minutes explaining and demonstrating the use of the aspirator and the process of aspirator training. Subjects then spent approximately 20 minutes practicing. Potential variation was anticipated, e.g., based on whether subjects had prior experience using aspirators, and therefore was addressed in the training document, where a time range of 15-30 minutes is offered. Since our recruitment outreach area included a university population that included entomology students and staff, some subjects already had aspirator experience. Each subject inserted

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Your response states that "HSRB reviewed, and IRB reviewed and approved aspirator training document (CLBR Training Manual §1.a. Observing mosquito landings and learning mechanical aspiration, version date 18 Dec 2020) as appears on page 183 of the final report." EPA requests that you update your response **by providing the additional information. See below questions:** a) What were the dimensions of the cage used for aspirator training? b) Were the same cages used for aspirator training and attractiveness tests?	his or her arms into the provided cage once, and practiced aspiration until they repeatedly and consistently captured landing mosquitoes before they took flight in response to the presence of the tip of the aspirator. The training manual document (specified above) indicates that each subject was to practice aspirator use as many times as they wished, with one and then two mosquitoes, before a determination was made at the trained researcher's discretion that the subject's use of the mosquito catcher was correct. Because CLBR's response to these EPA requests for additional details (see above) are based on anecdote rather than data, clarification by report amendment is not considered appropriate. *Further response:* a) As per study protocol, the dimensions of the cage used for aspirator training were 61 cm x 61 cm x 61 cm (24 in × 24 in × 24). This will be clarified in Section 2 of the amended final report. b) No; the same cages were not used for aspirator training and attractiveness tests. This will be clarified in Section 2 of the amended final report.

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13) Describe the mosquito attractiveness test in more detail. Please, confirm whether mosquito attractiveness tests were performed as a once-only procedure or whether subjects made more than one attempt to demonstrate their attractiveness to mosquitoes. If multiple attempts were permitted, how many attempts were permitted and how many subjects required more than one attempt in order to demonstrate attractiveness to mosquitoes? Please provide the raw data for all candidates tested for mosquito attractiveness. EPA requests that you update your response by **providing the additional information. See below question:** a) How long were mosquitoes deprived of food prior to their use in attractiveness tests?	Mosquito attractiveness tests were performed once only; all subjects passed the test. Please see the accompanying raw data for attractiveness assays for all subjects (document number 357624; submitted on March 2, 2022 and March 23, 2022). *Further response:* a) Mosquitoes were deprived of food during the 18 to 24 hours prior to their use in the attractiveness tests, in accordance with the OPPTS 810.3700 guideline. This methodological detail will be addressed in the amended final report; see further response to Comment #15 (below) for amendment language.
14) **The landing threshold for assessing subjects' attractiveness to mosquitoes deviated from guideline recommendation and is likely to compromise the validity of the data.** A threshold of less than two landings in two minutes (equivalent to less than one mosquito landing per minute) was specified in the ICF for assessing subject attractiveness (p. 161 of 362), which is ~5x lower than the landing rate of five landings per minute specified in OPPTS 810.3700 guidelines. We generally recommend following the Agency threshold of 5 landings per minute in laboratory attractiveness, provided in OPPTS 810.3700, specifically section *(j) Specific guidance for laboratory studies of mosquito or biting fly repellency*. **If a deviation from the recommended threshold is to be used, then a rationale for deviating from the guideline should be provided.**	Responses to EPA Comments #14 and #15 are combined; please refer to the response below to EPA Comment #15.

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15) **The mosquito density used for assessing subjects' attractiveness to mosquitoes deviated from guideline recommendation.** The mosquito density proposed for assessing attractiveness was 10 pathogen-free adult female *Aedes aegypti* mosquitoes in a 45.7 × 30.5 × 30.5 cm (18" × 12" × 12" cage), which is equivalent to a density of 1 mosquito for each 4, 251 cm^3 (Appendix 1, p. 37 of 362). This density is ~4x lower than the density recommended in the OPPTS 810.3700 guidelines, which is 1 mosquito for each 1,160 cm^3. We generally recommend following guidance provided in OPPTS 810.3700, specifically section *(j) Specific guidance for laboratory studies of mosquito or biting fly repellency*, should be followed in laboratory attractiveness tests. **If a deviation from the recommended density is to be used, then a rationale for deviating from the guideline should be provided.**	In response to the 75-day Deficiencies and Recommendations issued by the EPA (October 28, 2020)[1], CLBR expanded the study protocol description of its typical attractiveness assay to provide additional information in response to EPA's request and in accordance with OCSPP 810.3700 Guidelines for testing Insect Repellents to be Applied to Human Skin (July 7, 2010). The EPA-approved study protocol details procedures for confirming subject attractiveness to mosquitoes for the purpose of excluding subjects that fail to meet the specified criterion (§1.3.2, beginning with the second paragraph). OPPTS 810.3700 (Product Performance Test Guidelines: Insect Repellents to be Applied to Human Skin) specifies at several points that individual subject attractiveness should be established (e.g., §(c)(3)(i), p. 13.). Specific methods for assessing attractiveness, however, are not presented for field studies. In contrast, for laboratory arm-in-cage studies, a specific criterion of five mosquito landings on untreated forearms in one minute or less is given (§(j)(12), p. 26). While the density of mosquitoes for that attractiveness testing is not specified, one might assume the value given for efficacy testing in §(j)(7) on page 25 applies, i.e., one for each 1.16 liters. Note, however, that that rate might be more narrowly interpreted to apply strictly to challenging repellent treated arms, where subject safety and comfort might be much more readily protected than is the case for untreated arms under that condition. As noted in the technical responses to EPA's 75-day Deficiencies and Recommendations letter (October 28, 2020)[1], CLBR did not agree to adopt any arm-in-cage attractiveness assay involving more than a small number of mosquitoes. CLBR maintains that an attractiveness assay should either be a neutral model of expected biting pressure in the field or a conservative one. For

[1] See technical response letter addressing the 75-day Deficiencies and Recommendations issued by the U.S. Environmental Protection Agency on October 28, 2020, as submitted by Bergeson & Campbell, P.C. on January 11, 2021.

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	example, exposing subjects to 200 *Aedes aegypti* in starved condition is a much higher level of biting pressure than a subject would encounter in any field test we would conduct. A subject who passes such a test might prove unattractive to natural mosquito populations of mixed species, age class, and foraging avidity such as those in the conditions of the test, or that a typical end user might encounter during repellent use. Therefore, such an assay could result in the inclusion of subjects who might be unattractive to mosquitoes in the natural conditions of the test, thus inflating protection times estimated from study data. By conducting the assay using more than a few mosquitoes per subject, subjects would be exposed to a significant increase in bite-related risks and discomforts via a procedure likely to be counterproductive to the purpose, i.e., to ensure volunteers participating in the study are attractive enough to wild mosquitoes to adequately challenge the repellent's performance. Overall scientific and regulatory emphasis must remain on conservatism in estimates of repellent product performance. Use of an attractiveness assay that screens out only the most extremely non-mosquito attracting volunteers biases the entire study process towards overestimation of product performance, rather than underestimation, the preferred bias. More specifically, we note that the approved protocol specifies a minimum landing rate of two in one minute, which is double that ascribed by EPA. Given that CLBR conducted a field study, not a laboratory study, and so required more demanding attractiveness testing to avoid recruiting subjects insufficiently attractive to wild mosquitoes in a field study setting, our attractiveness bioassay was intended to align more closely with Guideline allowance for field landing rates on control subjects as low as 5 landings in 5 minutes. To provide adequately stringent attractiveness screening at densities more akin to those the subjects would likely encounter at the field sites, we employed 10 mosquitoes per 30.24 cm x 30.24 cm x 45.36 cm cage. The resulting density was

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	one for each 4.15 liters, or just 28% of the density recommended by the Guideline for arm-in-cage testing of repellent treated arms. We required two rather than five landings per minute, a rate that depended on landings by 20% of the mosquitoes present, rather than just 2.5% of the mosquitoes present per EPA's postulate regarding the Guideline. Note also that our minimum criterion of two landings per minute is equivalent to double EPA's recommended minimum for the untreated control subjects in these field studies. Lastly, and adding further confidence in the attractiveness of the subjects screened with our more rigorous assay, all subjects tested experienced two landings within 60 seconds of exposure, meaning that 20% of the mosquitoes present landed in one minute or less. In summary, OPPTS 810.3700 provides no explicit guidance for mosquito densities in attractiveness cage assays, but does specify a minimum control landing pressure at the field site. In this study, subject attractiveness was evaluated at cage densities more akin to field expectations in accordance with the protocol as reviewed and approved by EPA. This way, our subjects were shown to attract landings at a rate ≥2x that stipulated for field control subjects, by use of an appropriately demanding and biologically relevant bioassay. This assay provides greater confidence that we have measured efficacy rather than non-attraction, while protecting subjects from the inflated risks and irrelevant bites that higher density assays might engender. CLBR proposes to amend Section 2, *Training*, as follows, including reference to a new Note to File (see Appendix 3, page 210, of amended final report), all of which are based on the technical response provided above: **"<u>A field study requires more demanding attractiveness testing to avoid recruiting subjects insufficiently attractive to wild mosquitoes in a field study setting, where landing rates on control subjects may be as low as 5 landings in 5 minutes. Use of an attractiveness assay</u>**

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	that screens out only the most extremely non-mosquito attracting volunteers biases the entire study process towards overestimation of product performance, rather than underestimation, the preferred bias. To provide adequately stringent attractiveness screening at densities more akin to those the subjects would likely encounter at the field sites, 10 mosquitoes were used per 30.24 x 30.24 x 45.36cm cage, resulting in a density of one mosquito for each 4.15 liters (28% of the density recommended by the guideline for arm-in-cage testing of repellent treated arms). A rate of two landings per minute was employed in this study, which depended on landings by 20% of the mosquitoes present, rather than the guideline-recommended rate of five landings per minute (which is dependent upon landings by only 2.5% of the mosquitoes present). All subjects tested experienced two landings within 60 seconds of exposure, which corresponds to 20% of the caged mosquitoes present in each attractiveness assay landed in one minute or less (Appendix 3). Additional details regarding the rationale for the densities and landing threshold employed in the attractiveness assays in this study are provided in the Note to File in Appendix 3. In accordance with the EPA guideline OPPTS 810.3700, the mosquitoes used were starved for 18-24 hours prior to exposure to any given subject. **Attractiveness screening was conducted only once per subject. For each subject passing the test of attractiveness to mosquitoes, aspirator training was conducted immediately after that test using a cube-shaped cage 60.96 cm (24 inches) on a side, per the Study Protocol. Each subject was provided a scheduled time for completing both attractiveness screening and aspirator training such that each subject was trained individually in their own, separate training. Mosquitoes used for aspirator training were 5-12 days in age. See Appendix 3 for attractiveness screening raw data.**"

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At the end of your response to comment #15, you state "a note to file for appendix 3 was cited for additional details regarding this rationale." **This response is incomplete. Further clarification is needed. See below clarifications:** a) No such note to file exists in the submitted study report. The text to be used in the Note to File in Appendix 3 should be provided and should align with the rationale in the proposed amendment text to Section 2. This information is missing and should be provided and subject to review. You must a revised rationale to include an updated appendix. *[sic]*	*Further response:* CLBR reiterates that it proposes to amend Section 2, *Training*, to include the above text (text added the final report by amendment is bolded, underlined), which includes the reference to a newly proposed Note to File, that will be added to Appendix 3 in the final report by formal amendment (see page 210). The Note to File consists of the explanation in the response that immediately precedes the proposed text for amended Section 2, *Training*.

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16) Describe how landings were collected from subjects. Section 4.8.4 of the protocol states, *"Each subject will report the number of mosquitoes that land on their own treated skin during that five-minute period when asked by a researcher who will note it on a data sheet."* The study report should describe how landings were recorded. If landings were only recorded when asked, then the following should be specified: • The number of researcher(s) assigned to one or multiple subject pair(s), • The frequency and times at which researchers asked for landings for each subject at each time interval, and • The frequency and times the researchers recorded landings in the datasheets for each subject at each time interval. Alternatively, if landings were recorded more frequently than when asked, describe the procedures used for recording landings from all subjects exposed to mosquitoes during field trials.	CLBR proposes to amend Section 5, subsection *Test subject procedures for the test day, Exposures*, of the final report to provide the following text to address this request: **"Subjects called out landings to a technician as they occurred throughout all exposure periods. Typically, separate technicians recorded the times of each landing for control and treated subjects. The exception was late on Study Day 2, when only a few treated subjects remained in testing, and it was then more feasible for a single technician to record both treated and control outcomes. The technician recording landings on control subjects also recorded the number of seconds elapsed since the beginning of each exposure when landing was announced by each subject."**
17) Table 1 in Appendix A of this document summarizes data on species of mosquitoes collected by controls and treated subjects. The data in Table 1 is organized by subject type (control or treated) per site and total of mosquitoes collected by species. Data in Table 1 are summarized from Table 5 (§6, p. 20 of 362) and Appendix 8 (pp. 285, 287 of 362) of the study report. Total mosquito landings were determined by raw data sheets in Appendix 7 (pp. 269-270, 273-274, 276, 278 of 362). Percentage of landings caught were calculated by the formula: (total caught by treatment/total landings by treatment) ×100. Based on the data presented in the study report, it seems that only 20% to 65% of mosquitoes that landed on	The values presented by EPA below are correct. There are some complementary explanations that explain those values that might be regarded as 'low'. First, mosquito species studied appear to be more powerful fliers than many we encounter in, e.g., the more consistently densely vegetated mosquito habitats in the southeastern United States. They fly away more quickly. Mosquitoes are essentially always detected at the time of landing. Interventions with aspirators take place before skin penetration. More broadly, CLBR also perceives competing scientific objectives and ethical concerns in play. Landing mosquitoes that react quickly to the approach of an aspirator tip and are not captured are not identified, but also do not have the

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subjects in efficacy field trials were aspirated, identified to species, and screened for disease pathogens. These percentages are presented on Table 1 in Appendix A of this document. Please confirm whether the values represented in Table 1 are accurate. If the values are not accurate, indicate where can the information be found, and provide rationale for the low percentage of mosquito landings that were caught. *[Appendix A, as excerpted from the 90-day Preliminary Technical Screening Results[2], is included below].*	opportunity to bite subjects. The data set from controls provides a substantial sample of mosquitoes especially of species that approach subjects more quickly or that are active over a greater proportion of a study day. In early studies, CLBR used a landing criterion that included a mosquito extending its proboscis toward the skin, subjects (in practice only treated subjects) were compelled to look closely at any landing mosquito first, and that practice also improved capture success with little apparent cost in terms of allowing actual probing. Ideally, all mosquitoes would be captured. The perceived benefit of comprehensive mosquito capture might be greater for the science than the ethics. One reason, that just mentioned, is because lost mosquito events may be marginally safer for subjects than captures, which could risk the onset of probing. Another is that EPA's requirement that subject wear light, loose clothing, with no added protection other than gloves and head nets, means that they risk many more bites than otherwise. The agency has dismissed such concerns by emphasizing that testing is only conducted where preparatory surveillance has indicated very low pathogen presence. In comparison, the loss by individuals of mosquitoes that alight, but are frightened away before they probe, does not represent a lost chance to screen the source of one of their bites. It does represent a lost opportunity to identify the landing mosquito by species.

[2] 90-day Preliminary Technical Screening Results of Field Test Efficacy Report, Mimikai, Inc., Study ID MIM-06 issued by Biopesticides and Pollution Prevention Division, dated March 10, 2022.

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18) Describe the formula used for converting applied dose to volume using specific gravity of the product.	For a dose of 0.5 g/600 cm^2 skin area, by volume: Specific gravity of the product = 0.8874 g/ml 0.5g converted to volume: 0.5/0.8874 = 0.5634 ml Rate in ml: 0.5634 ml/600 cm^2 = 0.00094 ml/cm^2 From Page 17 of the final report: The resulting surface area calculations for each individual were recorded directly onto the repellent applications data sheet, then modified by the known specific gravity of the Test Material (0.8874 g/ml) to convert 0.5g/600cm^2 into ml for application of Test Material to subjects' forearms via tuberculin syringe (5th paragraph of Section 4.7, page 28 of the Study Protocol). Further clarification by report amendment is not considered necessary.
19) Specify whether the standard dose applied for testing repellency was applied to the dominant or non-dominant arm. The informed consent form notes "the researcher will measure the lengths of your dominant forearm and both of your lower legs" (p. 161) and also that for the measurements the subject would "roll up the sleeve of [their] non-dominant arm" (p. 167). The main report does not specify which arm was used (p. 18 of 362).	Application of the product was to the non-dominant forearm. CLBR did not take measurements of lower limbs because the procedure was determined to be unnecessary due to the fact that sampled mosquito populations at the sites had been coming to the upper body areas of researchers engaged in the four weekly samples collected by our researchers prior to field days at both sites. This is addressed in the final report as a protocol deviation on page 23 (section 8, first paragraph). Further clarification by report amendment is not considered necessary.
20) Describe the randomization procedure employed for assigning subjects to treated, untreated controls or alternate subjects. In addition, explain the randomization procedure employed for randomly selecting alternate subjects who were dismissed at the lab and those who accompanied subjects to the field.	For purposes of clarification, CLBR proposes to amend Section 5, subsection *Randomization of treatment condition*, of the final report as follows (added text is bolded, underlined; deleted text is bolded, struck through): "**<u>A single pool of candidates yielded two pools of subjects. Recruiting was conducted and continued to completion as if a single pool of</u>**

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	candidates would yield a single pool of subjects, as a working assumption in the Study Protocol. However, as the candidate pool was resolved into subjects, it became clear that several of the candidates were not available for both field test dates, precluding the intended randomization of subjects to their assigned test day. This meant the single candidate pool would resolve into two pools of subjects, without the possibility of randomization of subject assignments between the two field test dates (see Deviations 5, 6, and 7 in Table 6 for additional discussion). ~~**On both test days, we determined alternate subjects and assigned treatment status using lists of subject numbers generated randomly by Microsoft Excel. However, on the second test day, a minimum of male consented subjects arrived at the study site, so we had to account for gender bias by performing stratified randomization: all males were assigned to the treated group, and females were randomized within their gender, using the same randomization method as described above. This resulted in exclusively female alternate subjects.**~~ **On the morning of each study day, we collected the subject numbers of the subjects present at the laboratory. Those subject numbers were then entered into Microsoft Excel in two lists, one for male subjects and one for female subjects. Using the "RANDBETWEEN" and "CHOOSE" functions within Excel, subject numbers were chosen randomly, alternating by gender, until all 13 treated subjects were assigned either to the list of female subjects or the list of male subjects. The choice of the gender to initiate treated assignments was determined by simple randomization. Next, one subject per gender was randomly selected and assigned as untreated. Then, still alternating by gender, five additional subjects were assigned as alternates. These five alternates accompanied the subjects designated as treated and untreated to the**

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	field site on that day. All remaining subjects present in excess of the 20 previously selected subjects were then dismissed, compensated, and did not travel to the field site with the 20 designated subjects." See also page 193 in the final report for the resulting distribution of roles at each site. See also Deviation #5 and #6 on page 26 of the final report. This response also applies to the Ethics Comment below (see Pre-application preparations, Comment #1).

21) Please describe in detail how the aspirator training test was conducted and specify if the use of aspirators was demonstrated to subjects during the informed consent meeting. Please explain whether the attractiveness tests and pre-test aspirator were combined into one cage test set-up and conducted simultaneously or conducted as separate tests? If these tests were conducted simultaneously, explain if/how subjects were able to place both arms into the cage to assess both attractiveness and aspirator proficiency. Please include the age of the mosquitoes used in these test(s) in the main text of the report (preceding appendices). Your response referenced responses to comments 12 and 26. In comment #12 you indicate that you "conducted the aspirator training according to their CLBR training manual (part of the approved protocol)." Further, you state "we assessed attractiveness with the same cages that we used for aspirator training, so we changed to a larger cage to permit the subjects to have more freedom of movement when training with the aspirator." This response **is incomplete. Further clarification is needed. See below questions:** a.) Were attractiveness tests and aspirator training performed at the same time for each subject (conducted as the same test), or were they performed at different times for each subject (conducted as separate tests)? b.) What was the age of the mosquitoes used for aspirator training?	See responses to Comment #12 (above), including proposed amendment, and explanations regarding separate versus simultaneous tests and the age of mosquitoes used. In addition, see the response to Comment #26 (below) addressing demonstration of the use of aspirators during the consent meeting. *Further responses:* a) CLBR indicates its original response (March 23, 2022), above, includes a typographical error and confusing language. The same cages were not for attractiveness tests and aspirator training; rather, the two activities were completed in the same session with each individual subject. The attractiveness tests were performed first, followed immediately by the aspirator training if the subject passed the attractiveness test. The attractiveness assay was completed in one cage, sized 61 cm x 61 cm x 61 cm (24 in x 24 in x 24 in), per the approved protocol. The aspirator training was conducted in a different cage, sized 24" on a side, per the approved protocol, to allow both of the subject's arms – one for skin exposure to mosquitoes, the other for holding the aspirator – to be effectively inserted and moved within the cage volume. Since both activities were completed per the approved protocol, there were no deviations to report in regards to the size of cages used. The impression that we changed cage sizes given by original response (March 23, 2022), above, relates to the fact that we were using different cage sizes for the two activities, one following the other immediately in time, to complete all tasks for each subject.

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	Each subject had a unique time scheduled for attractiveness assay and training. Therefore, each subject was trained individually and at different times from those of other subjects, and thus as separate tests it that sense. b) The age of the mosquitoes used for aspirator training ranged from 5 to 12 days. These points will be clarified in section 2, 'Training', in the amended final report. See further response to Comment #15 (above) for amendment language.
22) The definition for confirmatory landing differed between the informed consent form and the study protocol. A confirmatory landing is defined in the protocol as a second landing occurring within 30 minutes of the first landing (that is, occurring during the same exposure period or on the next exposure period following the first landing). The definition in informed consent form, however, extends the confirmatory landing from 30 to 60 minutes (that is, to two exposures periods following the first landing). Although this deviation has been justified as being more conservative and unlikely to compromise integrity of results, it is unclear why the inconsistency between the informed consent form and the protocol occurred since it is expected that the informed consent form should have been revised concurrently with protocol revisions.	It appears that EPA means the difference in scoring confirmed landings via the timing of the confirmatory (confirming) landing. The protocol identifies a confirming landing as occurring within half an hour of the preceding landing, which becomes confirmed by the second landing occurring with that half hour period. The consent form specifies one hour rather than half an hour. See Deviation #3 (page 25 of the report) for a detailed explanation for CLBR's decision to follow the consent form specification. The difference is accidental, an editorial carry-over from previous studies that created a conflict between the study protocol and the ICF that went unnoticed by all editorial and regulatory reviewers. Further clarification by report amendment is not considered necessary.

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23) Subjects were recruited, then subjects were assigned to one test site, the other, or both, Appendix 3 (p. 193 of 362). Subjects who were alternates at Site 1 were also used as subjects in Site 2, shown in Appendix 3 (p. 193 of 362). Explain why there was an overlap in subjects who participated in both test sites when the protocol (Appendix 1, p. 51 of 362) indicates that subjects, including alternates, from separate pools will participate at each test site? The informed consent form also notes that "You will be asked to participate in one field test location but not the other." (p. 164 of 362).	In reference to Appendix 3, Note to File *Subjects used and role* of the final report, CLBR proposes to replace, by amendment to the final report, the current version of the table on page 193 with the attached, more detailed version (see document number 359786; submitted on March 2, 2022 and March 23, 2022) to provide a clearer accounting of the distribution of subjects within each test day. This revision also corrects the misassignment in the original table of five subjects as alternates for Site 1 (Test Day 1) who did not participate in any role on Test Day 1. As reflected in this amended accounting of subject roles in this study, no subjects went to the field on more than one study day. The apparent overlap in subject numbers that had suggested some went to the field on both test days was due to the original version of this table including the five non-participants in error, as well as pooling dismissed subjects and alternate subjects together in a single column labeled 'Alternates'.
24) Clarify the protocol deviation related to recruitment numbers. On p. 13, the study report says that 45 subjects consented. The deviations section (p. 26 of 362) in study report says that a total of 46 subjects were enrolled, and no explanation is provided for this discrepancy (Appendix 1, p.56 of 362). From 57 candidates that were contacted, 45 subjects consented (p. 13 of 362). From the 57 candidates who were contacted, explain how many subjects participated in a consent meeting and decided not to enroll and whether there were any candidates ineligible to enroll.	There is a typographic error in the final report. 46 subjects were enrolled, as supported by the dataset. CLBR proposes to correct this typographical error by report amendment (see response to EPA Comment #26, below). CLBR also proposes to amend section 2, 'Recruitment', in the final report as follows: Recruitment was completed primarily in the Davis and Sacramento, CA region. Using the approved advertising script (Appendix 2), initial outreach was completed via Craigslist, the UC Davis ECOSOCIAL list serve, theUC Davis Entomology Club email newsletter, and by word-of-mouth that resulted from all of the above. Fifty-seven candidates responded. After randomization (Study Protocol, pg. 18) and using the approved phone script (Appendix 1), follow-up calls were completed with the list of 57 candidates. **Regarding candidate attrition, as per phone script, Carroll-Loye left voice mails for candidates that did not pick up when we called. Six candidates never reached back out**

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	after voice mails were left. One candidate was not able to schedule a time for a consenting interview, indicated they would get back to Carroll-Love after reviewing their schedule, then did not contact Carroll-Love again. Three candidates were unavailable during either proposed field study day. Two candidates were ineligible to enroll when screened by phone for exclusion criteria.
25) Describe in detail how recruitment was conducted, including the rationale for recruiting from two different subject pools (p. 26 of 362) when the protocol called for recruiting from a single pool for geographically proximate sites within 2 hours driving distance (p. 56 of 362). Please provide more information and rationale about this protocol deviation. Your response states, "although deviation #7 on page 26 of the final report discusses recruitment as if two pools were used, we did not in fact recruit from two different pools of candidates. There was only one candidate pool." "…report amendment is not considered necessary." **EPA requests that you amend the report to avoid future confusion.**	Although Deviation #7 on page 26 of the final report discusses recruitment as if two pools were used, we did not in fact recruit from two different pools of candidates. There was only one candidate pool. Subjects from the single pool recruited, however, were not randomly distributed between sites because most subjects generally were available for one or the other field test days, not both. Some candidates who had completed consent interviews by phone were not available to sign the consent forms and participate in aspirator training or mosquito attractiveness assays until after the first field study date. Since all candidates were recruited into a single candidate pool, and their availability for only one of two field study dates would have constrained randomization regardless of whether they had completed consenting or not, we no longer consider there to be, in any scientifically meaningful way, two pools of subjects. ~~Further clarification by report amendment is not considered necessary.~~ *Further response:* Our single pool of candidates yielded two pools of subjects. Recruiting was conducted and continued to completion as if a single pool of candidates would yield a single pool of subjects as was the expectation of the Study Protocol. However, as the *candidate pool* was resolved into a *pool of subjects*, it became clear that few of the candidates becoming subjects were available for both field test dates. This meant the single *candidate pool* would resolve into two *pools of*

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	subjects, without the possibility of randomization of subject assignments between the two field test dates. CLBR proposes to amend Section 5, *Randomization of Treatment Condition*, in the amended final report, as indicated in the further response to Comment #20 (see above).
26) The study report did not include a description of what was discussed during the consent meeting. Please provide this information, including whether the meetings included a demonstration of the attractiveness testing, aspirator use, and product application such that the Agency can determine that candidates fully understood the process prior to giving informed consent to participate.	CLBR proposes to amend Section 2 of the final report to include a description of what was discussed during the consent meeting, as follows (bolded, underlined text is added; struck through text is deleted): *"Screening and Consenting* A total of ~~45~~ **46** subjects were consented (Appendix 3 & Section 8 of this report). In accordance with protocol procedures, PHRP-certified staff members read ICF documents to candidates, asked questions to ensure comprehension, **and as directed by the same ICF documents, performed demonstrations of repellent applications by mimicking the process on their own arm (but without applying any repellent to themselves), use of a mosquito cage (with an empty cage), and use of an aspirator by mimicking the action without mosquitoes present,** and provided **each subject with** copies of documents for review and reference (Protocol §3.4). Most such interviews were completed remotely via internet video and phone conferencing. In those cases, consent documents were initialed and signed at the beginning of the first in-person laboratory visit after the interviewing researcher asked the candidate if they still wished to participate and reminded the candidates that they are free to ask questions, request more time, or decline to consent. Staff exercised screening criteria during the reading of the ICF documents. Once consented, candidates, now subjects, were assigned a unique subject number from a list of random numbers previously generated in Microsoft Excel specifically for use in the study."

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27) Provide the number of subjects for whom the consent meeting (not the signing of the form) was held virtually and how many meetings were held entirely in person. Indicate how many virtual meetings held by phone, video conference (e.g., Zoom), or using another platform.	No subjects completed the consent process virtually, as the process requires an in-person signature, and the process is not complete without that signature. In-person consent procedures were completed out-of-doors. Of the 46 subjects consented, 14 completed the entire consenting interview process in person, and the remainder completed the process via an internet video call. No subjects participated in a consenting interview by voice only. See also the response to EPA Comment #26 (above). CLBR proposes to add the following text at end of the first paragraph of Section 2, subsection *Screening and Consenting,* in the final report as follows: **"In-person consent procedures were completed out-of-doors. Of the 46 subjects consented, 14 completed the entire consenting interview process in person, and the remainder completed the process as described above, via an internet video call. No subjects participated in a consenting interview by voice only. Subject ages ranged from 19 to 54; 20 were biologically male, 26 biologically female."**

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28) Confirm whether all female subjects took pregnancy tests prior to their participation in the attractiveness testing and aspirator training, as well as on each test day they participated in as a test subject, control, or alternate.	CLBR proposes to amend Section 2, subsection *Screening and Consenting*, of the final report with the addition of a final paragraph, as follows: **"All female subjects took pregnancy tests prior to their participation in the attractiveness testing and aspirator training, as well as on each test day they participated in as a test subject, control, or alternate (see also Note to File, Appendix 3)."** Confirmation for this statement appears in the first Note to File of Appendix 3 (first page of 'Notes to File on recruiting, screening, consenting, and training') in the final report.
29) Please provide the range of subjects' ages in the study report.	CLBR proposes to amend Section 2, subsection *Screening and Consenting,* of the final report to provide the range of subjects' ages. See response to EPA Comment #27 (above).

APPENDIX A: Mosquito Species and number aspirated from control and treated subjects

Site	Treatment	Subject	Species	# Caught	Total Caught by Treatment	Total Landings by Treatment	% of Landings Caught
1	Control	25	*Aedes melanimon*	42	99	179	55.3%
			Aedes vexans	9			
		129	*Aedes melanimon*	36			
			Aedes sticticus	1			
			Aedes vexans	7			
			Anopheles freeborni	2			
			Culex tarsalis	2			
	Treated	30	*Aedes melanimon*	1	2	10	20%
			Aedes vexans	1			
2	Control	6	*Aedes melanimon*	37	102	158	64.5%
			Aedes nigromaculis	1			
			Aedes vexans	3			
			Anopheles freeborni	1			
			Culex tarsalis	11			
		101	*Aedes melanimon*	39			
			Aedes vexans	7			
			Culex tarsalis	3			
	Treated	7	*Aedes melanimon*	1	8	27	29.6%
			Aedes vexans	1			
		62	*Aedes melanimon*	1			
		69	*Aedes melanimon*	1			
		122	*Aedes melanimon*	1			
		132	*Aedes melanimon*	1			
		167	*Culex tarsalis*	1			
		178	*Aedes melanimon*	1			

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
Pre-application preparations	
1) At what point were the subjects assigned to be a test subject, control, or alternate? The report notes that following consent, subjects were assigned a random number. What randomization was used to distinguish between alternate subjects who were dismissed at the laboratory and those who accompanied the test subjects to the field?	Please see CLBR responses to EPA Comment #20 (above).
Test day	
1) Please provide the information shared with subjects in the reminder call prior to the test day.	The information shared with subjects in the reminder call prior to the test day included the following: a reminder to wear light, loose clothing that covers the subject's arms and legs, but with sleeves and pant legs that can be rolled up; a request to confirm they have refrained from using repellents within the last 48 hours and from using perfumed products, smoking, or consuming alcoholic beverages since 9 p.m. the previous evening; and a request they confirm they can participate for the test day without smoking or consuming alcoholic beverages. ~~Further clarification by report amendment is not considered necessary.~~ Further response: CLBR proposes to amend the final report in the Section 5, 'Reminders', to clarify the information shared with subjects in the reminder call as follows: "Reminders to participating subjects were provided by phone call or email by the Director of Research on the Friday preceding each of the Sunday field tests. **The information shared with subjects in the reminder call prior to the test day included the following: a reminder to wear light,**

[3] Ethics-related comments were taken directly from *Appendix B* of 90-day Preliminary Technical Screening Results of Field Test Efficacy Report, Mimikai, Inc., Study ID MIM-06 issued by Biopesticides and Pollution Prevention Division, dated March 10, 2022.

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
	loose clothing that covers the subject's arms and legs, but with sleeves and pant legs that can be rolled up; a request to confirm they refrain from using repellents within the last 48 hours and from using perfumed products, smoking, or consuming alcoholic beverages since 9 p.m. the previous evening; and a request they confirm they can participate for the test day without smoking or consuming alcoholic beverages. At the field site and prior to repellent applications, the Study Director reminded subjects they were free to withdraw from the study at any time, privately and without penalty. The Study Director also reminded subjects of the exclusion criteria that applied to the 48-hour period preceding the morning of the field test day (Protocol §3.3.2, items 7 and 8 on pg. 19)."
2) Confirm that all subjects were contacted on the day of the study to determine whether they were experiencing COVID-19-related symptoms. Was any subject excluded from participation as a result of this screening?	All subjects were contacted on the day of the study to determine whether they were experiencing COVID-19-related symptoms (see pp 196-198). None were, so none were excluded for COVID-19 symptoms. Further clarification by report amendment is not considered necessary.
3) Where were the test sites and how far from the laboratory were they?	Test sites were approximately 1 hour 20 minute drive from the laboratory, located in wild areas near the Sutter Buttes land form. Further clarification by report amendment is not considered necessary.
4) Did any subjects transport themselves to the test site? If so, how many?	Yes. Though not recorded, the Director of Research confirms 4-5 subjects transported themselves each of the two field test days. The protocol allows for self-transport by subjects but does not require documentation thereof. Further clarification by report amendment is not considered necessary.

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
5) How many subjects showed up to the test site on each test day? How many were dismissed prior to the departure for the test site?	Twenty five subjects reported on the first test day; five were dismissed prior to the departure for the test site. On the second test day, 22 subjects reported; two were dismissed prior to the departure to the test site. CLBR proposes to amend Section 8, Protocol Deviations, Table 6, of the study report, the first paragraph of Deviation 5 (page 26) as follows: "Total enrollment was increased from the Study Protocol specification of 40 to a total of 46. Expanding the subject pool resulted in additional alternate subjects beyond the number specified in the Study Protocol. On field study test day 26 September 2021 (Site 1), ~~**31**~~ **25** subjects were asked to appear, **and did appear,** at the laboratory prior to departure to the field. ~~**Six**~~ **Five** were dismissed from the group gathering at random. The remaining 20 subjects went to the field site including the 5 alternates that would remain with the subject pool at least until all treated subjects received applications. Similarly, on field study test day 3 October 2021 (Site 2), 24 subjects were summoned, **22 appeared,** ~~**4**~~ **two** were randomly dismissed, and 20 went to the field." Please also see the response to EPA Comment #23 (above).
6) How long after arrival at the test site were alternates dismissed?	Alternates were dismissed after applications were completed on treated subjects and by the time the first two exposure periods were completed. No treated or control subjects withdrew, were excluded, or were removed on either field test day. Thus, Individual Stop Rule 1 was not invoked. Further clarification by report amendment is not considered necessary.

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
7) Who performed a check of the subjects' skin to determine continued eligibility to participate? Were any subjects dismissed as a result of this check? Was the skin of all subjects checked again at the end of the test day?	Inspection records are provided on pages 250 and 255 of the final report, both signed by Alex Duplantier (MPH, PA-C), who is listed on page 5 of the final report as contracted staff for the study. Further clarification by report amendment is not considered necessary.
8) Were any subjects withdrawn and/or removed at any point after the product was applied?	No. No treated or control subjects withdrew, were excluded, or were removed on either field test day. Further clarification by report amendment is not considered necessary.
9) Confirm that control subjects were paired with a staff member, 12 treated members were paired together, and the remaining treated subject was paired with a staff member wearing protective clothing but no repellent during field trials.	Confirmed. ~~Further clarification by report amendment is not considered necessary.~~ CLBR proposes to amend Section 5, subsection *Exposures* of the final report with the inclusion of a newly added sentence at the start of the first paragraph (which will become the 2nd paragraph in amended final report; see response to Comment #11 below), as follows: **"Control subjects were paired with a researcher wearing protective clothing but no repellent, twelve treated subjects were paired together, and the remaining treated subject was paired with a researcher wearing protective clothing but no repellent."**
10) What measures were taken to ensure that the integrity of the application to test subjects was maintained between the exposure periods?	Staff observed subjects and reminded them repeatedly to avoid contact of any treated skin with clothing or with surfaces in the screen shelter. The Study Director repeatedly praised subjects for their assiduous, compliant limb comportment.

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
	Further clarification by report amendment is not considered necessary.
11) How were the subjects distributed amongst the separate exposure areas within each site (areas shown in Site Maps of Appendix 10) during each time interval? What was the process of assigning subjects to each area? What was the size of each area? How were subjects positioned within each area?	See site maps on pages 314-315 of the amended final report. CLBR proposes to amend Section 5, subsection *Exposures* of the final report with the inclusion of a newly added first paragraph, as follows: **"Exposure areas were approximately 30 by 100' in area, and therefore large enough for 30 people at 10' spacings to occupy simultaneously. The total maximum occupancy consisted of 20 subjects, and two researchers attending control subjects, and typically two data-takers (one for the two untreated subjects and at least one for the treated subjects). Subjects and staff were distributed within an exposure area to create a minimum of 10 feet of distance between any two persons excepting observer pairs, who might briefly close that distance for a landing observation of a mosquito on one partner, and researcher-control subject pairs, when the attending researcher had to step closer to aspirate a landing mosquito (see also Deviation 4). In response to periodic drops in mosquito activity levels observed by the Study Director or research staff, before the following exposure, the Study Director (who was not wearing repellent but was wearing protective clothing), would visit each candidate exposure area to evaluate mosquito activity. The Study Director would then direct the entire group to the area he deemed optimal for the next exposure. Note that these micro-habitats were all considered to be part of a single site at each site, because any mosquitoes present anywhere in the total site area could easily distribute themselves by flying from one micro-habitat to another. Having multiple exposure areas on one site allowed the research team to respond to site conditions as they developed during the field study day."**

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
Subject safety	
1) The protocol indicated that CL would communicate with local public health agencies within a week of the field test about the presence of vectors at the potential field sites (p. 38 or 362), but that is not included in the study report. Please explain whether this occurred, and if so, provide a description of the process.	The statement refers to disease incidence, not vector presence. The California Department of Public Health Vector-borne Disease Section (WestNile.ca.gov) reported no pathogen records for the week preceding each field day. Further clarification by report amendment is not considered necessary.
2) Please confirm that females took pregnancy tests on both the test day and on the day of the attractiveness test/aspirator training and confirm that all female subjects were tested (how many, which instances).	Confirmation is provided in the first Note to File of Appendix 3 (first page of 'Notes to File on recruiting, screening, consenting, and training') of the final report). Further clarification by report amendment is not considered necessary.
3) Were any subjects replaced with alternates? If so, how many and at what point in the study?	No treated or control subjects withdrew, were excluded, or were removed on either field test day. Alternates were dismissed after applications were completed and two exposures had been completed. Further clarification by report amendment is not considered necessary.
4) Please describe the COVID-related precautions taken during the consent, attractiveness testing/aspirator training, and on the test day, especially during transportation to the test site.	See Note to File in Appendix 4 of the final report. Further clarification by report amendment is not considered necessary.
5) Confirm that subjects were provided with head nets to protect their head, face, and neck for times when they were outside of the screened shelter.	Subjects were provided with head nets to protect their head, face and neck for times when they were outside of the screened shelter. Further clarification by report amendment is not considered necessary.

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
Compensation	
1) Please explain the compensation provided to the subjects who went to the lab on the test day but who were not transported to the field.	The pay ($) per hour was logged from when the subject arrived until completion of study activities for that day, rounded up to the nearest hour. ~~Further clarification by report amendment is not considered necessary.~~ CLBR proposes to amend Section 5 of the final report to explain subject compensation, as follows (added text is bolded and underlined): **"Payment was to each subject regardless of role and for each hour of participation for every phase, rounding up to the nearest hour, and including the time spent in the consenting interview regardless of means (virtual or in-person), for which each subject was paid at the end of the first site visit. No candidates withdrew once they reached the consenting interview stage."**
2) Please explain the compensation provided to the alternate subjects who were transported to the test sites.	The pay ($) per hour was logged from when the subject arrived until completion of study activities for that day, rounded up to the nearest hour. ~~Further clarification by report amendment is not considered necessary.~~ CLBR proposes to amend Section 5 of the final report to explain subject compensation, as noted above (Compensation comment #1).

EPA Comments (from Appendix B in the 90-day Preliminary Technical Screening Results[3]) *(Additional comments dated April 29, 2022, are highlighted in yellow)*	***Registrant Responses*** *(Updates to responses dated March 23, 2022, are highlighted in yellow)*
3) Confirm the compensation paid for subjects attending the consent meeting, regardless of whether they consented.	Yes; compensation was paid for subjects attending the consent meeting. No candidates declined to consent if they proceeded to the consenting interview. ~~Further clarification by report amendment is not considered necessary.~~ CLBR proposes to amend Section 5 of the final report to explain subject compensation, as noted above (Compensation comment #1).

Attachment 3

Registrant's technical report and rebuttal (dated June 22, 2022) to EPA's letter regarding site independence

June 22, 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 00336661, EPA File Symbol Number 93616PA10

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) May 27, 2022, 75-Day Deficiencies letter regarding Mimikai's Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray against Mosquitoes, Master Record Identification (MRID) 517071-01, Carroll-Loye Biological Research (CLBR) Study Number MIM-006. EPA's letter (Attachment 1) and accompanying review were in response to Mimikai's rebuttal to EPA's letter of April 29, 2022, and the requested geographical site information provided to EPA on April 26, 2022.

The deficiencies noted in EPA's May 27, 2022, letter are summarized as follows:

- Two site locations were used that are not considered to be independent of each other (distinct sites), and the review and consultation with the Human Studies Review Board (HSRB) cannot move forward until data from an additional study is submitted to supplement MRID 517071-01;
- There are discrepancies in the total number of mosquitoes reported; and
- The explanation as to why the selected field sites did not include *Aedes aegypti* or *Aedes albopictus* is not acceptable.

Sites

In response to EPA's belief that the test sites in MIM-006 are not independent sites (Item 1, above), CLBR has prepared a technical response document to support Mimikai's firm

2200 Pennsylvania Avenue, NW, Suite 100W phone: 202.557.3800
Washington, DC 20037-1701 fax: 202.557.3836 www.lawbc.com

view that the sites are entirely distinct and MIM-006 therefore is sufficient as a stand-alone study to support any product label claims for registration purposes. *See* Attachment 2.

Number of Species

In response to Item 2 in EPA's letter, CLBR reevaluated the trap collection data reported in Table 4 and Appendix 8 of the amended final report for Study No. MIM-006 (issued on May 12, 2022, MRID No. 519127-01). Discrepancies in counts between Table 4 and the tabulated data in Appendix 8 were reviewed and found to be inadvertent transcription and spreadsheet computation errors. Corrections to these data will be reflected in a subsequent amendment to the final report and are summarized in Annex 1 of the Technical Response Document (Attachment 2).

***Aedes* Species**

During the June 8, 2022, meeting between EPA and Mimikai, EPA explained that a request had been sent to the Office of General Counsel to clarify if Mimikai's currently pending reports (and application) would be assessed against the species requirements of the new testing guidance, *Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests*, effective June 14, 2022, or against the standards in place at the time the study commenced. The new guidance requires that, in addition to specific *Culex* and *Anopheles* species, *Aedes aegypti* and/or *Aedes albopictus* must be present during field trials to support a general mosquito label claim. As reflected in the draft June 8, 2022, Meeting Minutes provided to EPA for comment on June 20, 2022 (Attachment 3), in a June 10, 2022, e-mail, Charles Smith stated, "we are not holding this current action to the requirements of the new product performance rule," but also, that any new testing (*i.e.*, a third site) should attempt to include the two *Aedes* spp. Mimikai thus considers the *Aedes* species deficiency resolved.

Conclusions

Based on the foregoing and attached, Mimikai maintains that Study No. MIM-006, pending amendment to address the data corrections (as indicated), is sufficient as a stand-alone study to support any mosquito label claims for registration purposes, including but not limited to the data used to derive the currently proposed median complete protection times (CPT) for mosquitoes. We are pleased to discuss any outstanding questions and the path forward for study acceptance and Office of Pesticide Programs (OPP) consultation with HSRB.

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

Sincerely,

Dana S. Lateulere

Attachments

Attachment 1

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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

May 27, 2022

****CONTAINS CONFIDENTIAL BUSINESS INFORMATION****

READ RECEIPT REQUESTED

Ms. Dana S. Lateulere
Bergeson & Campbell PC
Agent for Mimikai, Inc.
2200 Pennsylvania Ave.
Washington, DC 20037-1701

Subject: 75-day Deficiencies: Review of the response to the Agency's deficiency letter dated April 29, 2022, and submitted materials dated April 26, 2022.
Product Name: Field Test Efficacy Report, Mimikai, Inc, Study MIM-006
EPA File Symbol: 93616PA10
EPA Receipt Date: 08/26/2022
Action Case Number: 00336661
PRIA Due Date: 9/9/22

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 deficiencies and identifies your options under 40 CFR § 152.105.

At this time, the EPA has identified deficiencies in its review of the subject application that prevent the Agency from presenting at the Human Studies Review Board (HSRB) meeting scheduled for July 2022. Please refer to the attached review for details of the deficiencies. The attached review also includes previous deficiencies that have been satisfied, as well as additional considerations for future study planning. See below for a brief summary of the deficiencies.

Summary of Outstanding Deficiencies - Mimikai Mosquito - (Please refer to the attached review for specific details).

1) Two site locations were used that the Agency does not consider to be independent of each other (distinct sites). Due to the lack of independent test sites, MRID 517071-01 is not sufficient as a stand-alone study to support any product label claims for registration purposes. Therefore, the review of this study by BPPD and OPP's consultation with the HSRB cannot move forward until product performance data from an additional study is submitted to supplement MRID 517071-01. See attached review for details.

2) There are discrepancies in the total number of mosquitoes reported. See attached review for details.

3) The explanation that was provided as to why the selected field sites did not include *Aedes aegypti* or *Aedes albopictus* is not acceptable. See attached review for details.

Further review of your application and your response to the deficiencies may identify additional deficiencies and you will be so informed.

In accordance with 40 CFR § 152.105, you are allowed 75 days from the date of this letter to provide a response concerning the deficiencies listed in this letter. Please ensure that you consider each of the options below in determining how and when you respond to this letter. You have the following three options:

1. **Establish a New Due Date and Resolve the Issues.** You may resolve the issues by submitting the corrections through the Central Data Exchange (CDX) portal to complete the application by 08/08/2022, or you may submit an explanation of why it will take longer than 75 days to address the deficiencies. For the latter option, your explanation must include a written commitment and schedule for submitting the remaining information and/or data. When submitting information and/or data in response to this letter, a copy of this letter should accompany the submission to facilitate processing. Or you may work with us to establish a new Section 33/PRIA deadline that allows for an appropriate response to the 75-day letter.
2. **Withdraw the Application.** You may withdraw your application. If a fee was paid, the EPA will provide any applicable refund as soon as practicable.[1] A 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.
3. **Not Respond Properly.** If you do not respond to this letter by: 08/08/2022, 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 administratively withdraw your application. If a fee was paid, the EPA will provide any applicable refund as soon as practicable.[2] A 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.

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

Because this application requires the development of a new study and presentation to the HSRB, I highly recommend that we schedule a meeting with you to discuss how you wish to move forward. Upon receipt of this letter, please contact my staff, Menyon Adams, adams.menyon@epa.gov, Andrew Bryceland, Bryceland.andrew@epa.gov, or feel free to contact me directly, hollis.linda@epa.gov to schedule a meeting.

Sincerely,

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

Enclosure

Attachment 2

Carroll-Loye Biological Research

711 Oak Avenue Davis, California 95616 Tel (530) 902-8267 www.carroll-loye.com

Submission to Address Test Site Selection in a Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes (Carroll-Loye Biological Research Study No. MIM-006)[1]

June 22, 2022

Carroll-Loye Biological Research (CLBR) appreciates the care that the United States (US) Environmental Protection Agency (EPA) has taken in its evaluation of site habitat differences in Study No. MIM-006 and the continued engagement of EPA staff in these important considerations in support of registration of MIMIKAI's product, Lilly Pilly Repellent. Please note also that the responses herein are based on verified trap count tabulations, which reflect data corrections (see Annex I) in response to outstanding deficiencies noted in EPA's 75-day deficiency letter dated May 27, 2022[2].

Study Sites 1 and 2, used in this study, have been central to our work in the mosquito rich habitats of Northern California for 30 years. We agree with EPA's observation that, in the broad overview, the mosquito populations at Sites 1 and 2 show similar composition for several important species. But we also emphasize that this observation should not be taken as indicating habitat equality, nor, as we will show, evidence of reliable similarities in species composition, diversity or relative abundance.

Instead, major ecological differences in the biotic and seasonal attributes of larval mosquito habitats create important differences between Sites 1 and 2 in species composition, and in the availability of key, complementary species for safely conducting conclusive human subject repellant efficacy trials. Indeed, the efficacy data underlying the successful and enduring registrations of the approximately 80 Picaridin, IR3535, and Oil of Lemon Eucalyptus (OLE) products in the US are from testing conducted at Sites 1 and 2 conducted by CLBR.

The following paragraphs address how ecological differences between Sites 1 and 2 help inform the best ways to evaluate their degree of overlap in mosquito community structure. CLBR will also show how functional complementarity of these sites renders them particularly ideal for the evaluation of insect repellent efficacy on a national level.

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[1] MRID Number 519127-01; supplement to MRID Numbers 517071-02 and 517656-01, and replacement of MRID Number 517071-01.

[2] Letter from U.S. Environmental Protection Agency, Office Of Chemical Safety And Pollution Prevention (OCSPP), dated May 27, 2022, regarding "75-day Deficiencies: Review of the response to the Agency's deficiency letter dated April 29, 2022, and submitted materials dated April 26, 2022", EPA File Symbol: 93616PA10.

The major conclusions and emergent points CLBR will support are these:

1. Principle Components Analysis (PCA) of the trap data shows Sites 1 and 2 had largely distinct mosquito communities, a strategic priority of the Study Director in choosing sites.
2. The two site-specific mosquito communities are the ecological result of habitat differences, influenced by when and where species-specific larval environs are available.
3. Pre-test trapping data made available to CLBR by LivFul (accepted by EPA, MRID No. 507791-01) show that while their adjacent Florida sites were comparatively more speciose, with less species overlap than CLBR's sites, if the Florida sites are examined in terms of landings data for *Aedes, Culex* or *Anopheles* on control subjects, the Florida sites appear no more and perhaps somewhat less diverse and distinct than CLBR's Sites 1 and 2.
4. Hence, it is important not to over-rely upon trap assays to characterize diversity relevant to efficacy studies, and in judging habitat differences from species composition to inform the study of human-mosquito interactions.
5. Together, Sites 1 and 2 produce enormous, subject-safe populations of some of North America's most dangerous and important mosquito genera, *Culex, Aedes* and *Anopheles.*
6. In contrast to mega-diverse southeastern mosquito communities, which parse their habitat uses more finely, all Site 1 and Site 2 mosquitoes are highly anthropophilic, rendering all, rather than just a small fraction, of the trapped species relevant to our MIMIKAI repellency evaluation.
7. US-based studies of long-acting repellents rely on large *Aedes* populations for control landing pressure. The pairing of Sites 1 and 2 in autumn combines that requisite day pressure with complementary high nocturnal pressure from *anthropophilic Culex* – our most important vector mosquito – at a season when West Nile Virus (WNV) is not detected in trap samples of said populations.

Statistical Analyses of Diversity and the Distinction Between the Two Sites

Principle Components Analysis (PCA) is useful to visualize and compare the spread of data within, and distinctions between, large and complex data sets. Here CLBR uses PCA to characterize presence and abundance patterns for trapped mosquito counts, combining all of the 4 weekly samples preceding efficacy testing days within each site, and with the scheduling of both qualifying trapping and efficacy testing intentionally offset by one week between sites. Figure 1 illustrates the results of such an analysis:

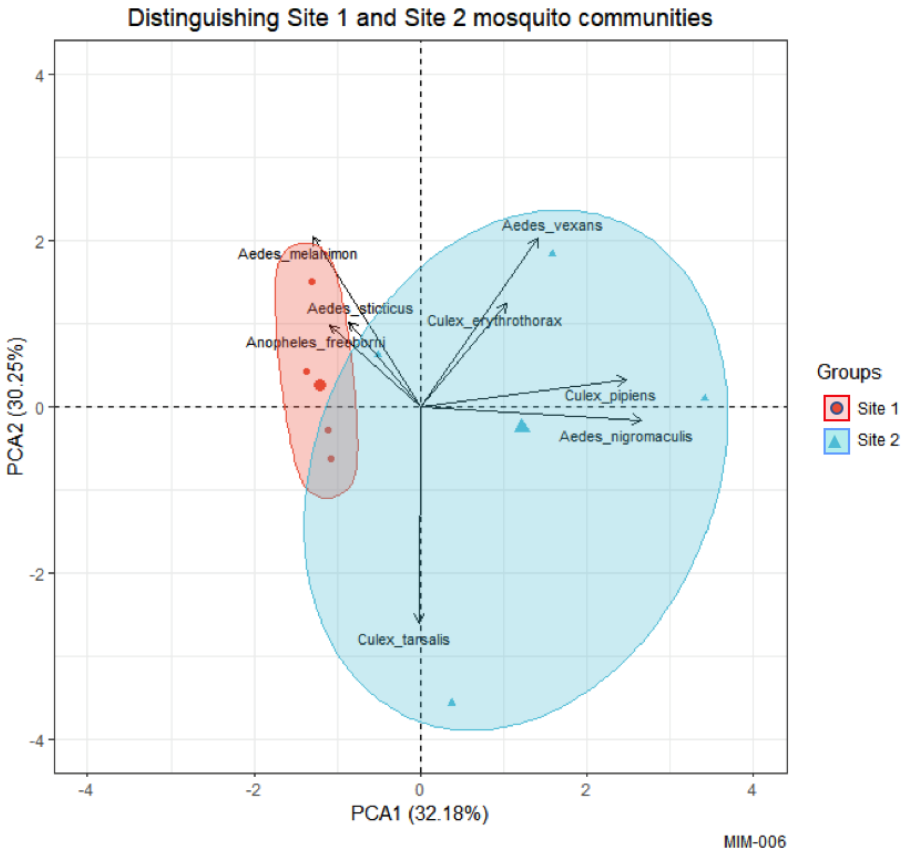


Figure 1: Principal components analysis (PCA) using mosquito species presence and abundance data from four weekly-interval trapping collections completed prior to human subject efficacy testing at Sites 1 and 2 for Study No. MIM-006. The X and Y axes are percentages that represent how much each axis explains the variation in the data. Arrows are representations of the principal components explaining the dissimilarity between the two sites. The length of an arrow (principal component) correlates with a specific variable's explanatory impact on observed variation in the data. The cumulative interaction of the principal components influences the location of each Site's centroid (the large red circle for Site 1, and the large blue triangle for Site 2).

The high relative abundance of *C. tarsalis* and *A. nigromaculis,* plus the presence *C. pipiens* and *C. erythrothorax* at Site 2, and presence of *A. sticticus* at Site 1, drive substantial overall distances between site centroids, with only minor overlap, and thus demonstrating dissimilarity between sites. Note that even a single species in common between any two sites will create some overlap in the PCA. Sites and their respective four predecessor weekly trap counts were intentionally offset by one week while waiting for the rise of *Culex* spp. populations at Site 2, a condition that would usefully enhance the distinction between sites for the purposes of the study. By analyzing the weekly trap collection data used to qualify each site together, the divergent nature of the mosquito communities supports the qualification of Site 2 as sufficient on its own and as sufficiently different from Site 1 to merit classification as distinct

for study purposes. Whereas EPA's assessment emphasized comparing near-simultaneous collections between sites (which excluded the fourth trapping date at Site 2), CLBR maintains including the four collection dates for each of the respective sites, as prescribed by the approved amended Study Protocol[3] and Office of Chemical Safety and Pollution Prevention (OCSPP) Guideline 810.3700[4], is essential for qualifying the site within the week prior to the efficacy test day.

In addition to following the requirements of the Study Protocol[3] and the imperatives for analysis and final reporting, CLBR's approach also retains an informative analytical opportunity not available in the approach taken by EPA. CLBR also used PCA to compare EPA's approach to CLBR's approach (Figures 2a and 2b, respectively).

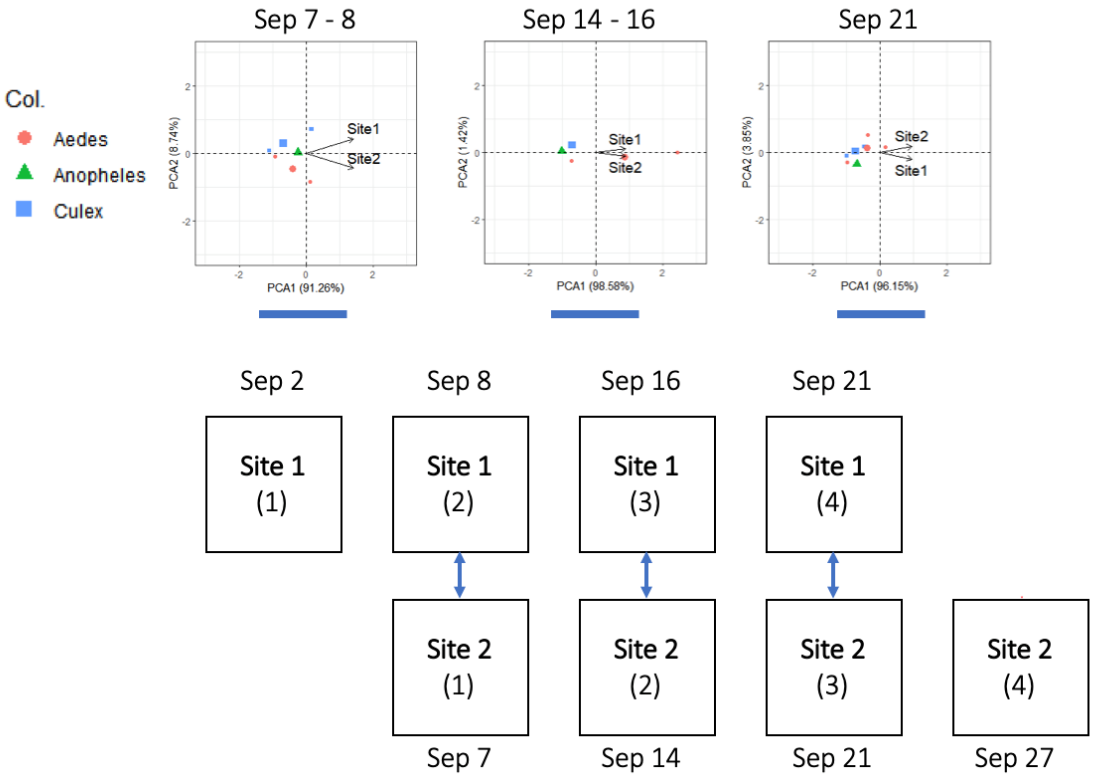


Figure 2a: Comparing Site 1 with Site 2 trapping data emphasizing coincidence in time (per EPA). Separate PCAs for Sep 7-8, Sep 14-16, and Sep 21 show little difference between sites. Individually, each uses much less data to draw comparisons than PCA of the overall trap data (Figure 1).

[3] Amended Protocol (No. 2) for study entitled, "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes", Carroll-Loye Biological Research Study Number MIM-006.
[4] U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention (OCSPP). (2010) Product Performance Test Guidelines: OCSPP 810.3700: Insect Repellents to be Applied to Human Skin, July 7, 2010.

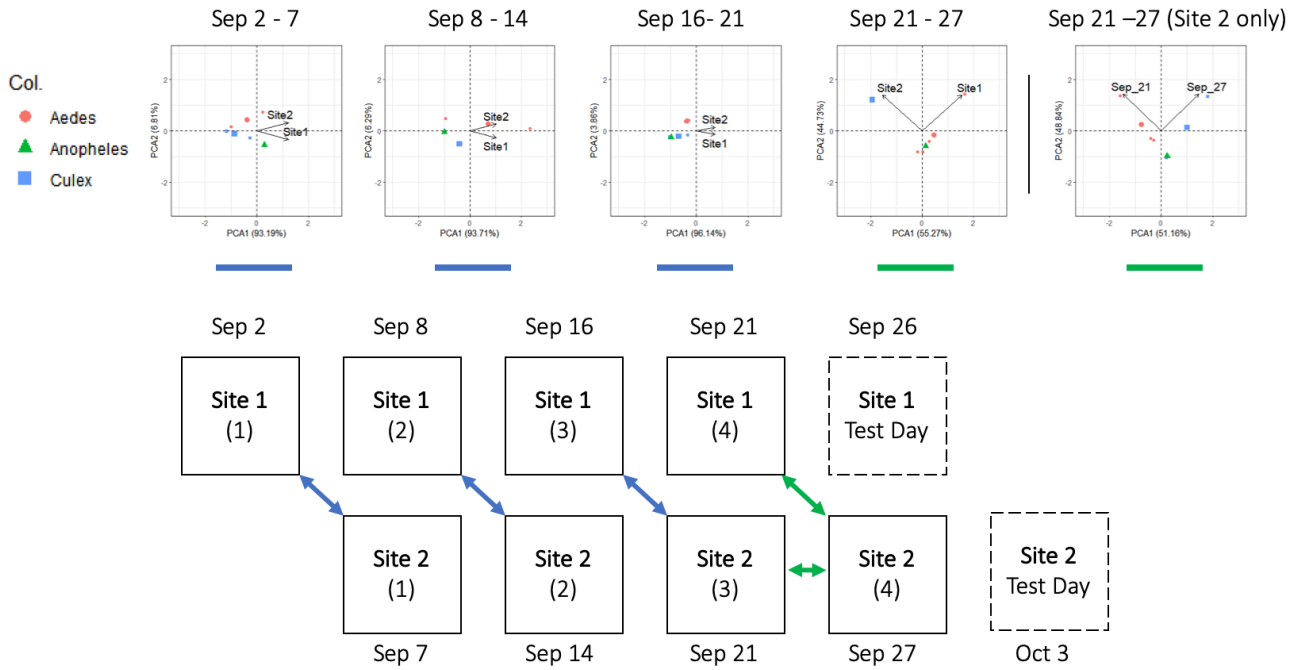


Figure 2b: Comparing development of differences in mosquito species along the planned sequence of 1st, 2nd, 3rd, or 4th trap collection over four weeks immediately preceding testing at each site. The separate PCAs for September 21-27, 2021, between sites, and September 21-27, 2021, within Site 2, demonstrate divergence.

The ultimate, striking divergence of Site 2 from its prior state, and from Site 1, was anticipated based on annual wildlife habit flooding at Site 2 that causes marked larval mosquito habitat divergence between the two sites, and resulting shifts in adult mosquito populations. Site differentiation in terms of mosquito ecology was known in advance to be a function of both location (as driver of each site's physical habitat characteristics) and time, thus CLBR's strategy of separating the test dates in time. Note that separation of sites in time would also necessarily arise in nearly any effective field efficacy study, as sites free of mosquito vectored human diseases for a full month prior to efficacy test dates and of appropriate and useful diversity rarely coincide in time within the US, regardless of geographic separation.

Thus to deepen perspective on the distinctions between the mosquito ecology of the two sites, CLBR provides the comparative analysis by proximate calendar date per EPA's approach (Figure 2a) in contrast with CLBR's approach (Figure 2b) of comparing trap counts in the weekly sequence for each site (1st, 2nd, 3rd, or 4th sample in the sequence over the four weeks before the efficacy test day for each site). Note that CLBR's process to qualify distinct sites, per the Study Protocol, was to use the four weekly trap samples for each prospective site that preceded the actual test day as a confirmation (A) that all three genera were present as anthropophilic species; and (B) that each site at the time of efficacy testing would be divergent in mosquito species composition, as expected due to known differences in larval habitat and in response to real-time changes in influential ecological factors between the two sites. CLBR therefore was

not making qualifying choices in site selection by comparing site trapping data coincident in time, but rather comparing development of differences along the sequence of weekly trap collections at each site for the month prior to each test day, as required per the OCSPP Guideline 810.3700. This is the same approach that would be used to assess and qualify any other test site on a future test date, for which a proximate date-to-date comparison would neither be possible nor appropriate. Trapping at Site 1 was not undertaken at Site 1 after efficacy testing was concluded because it is not a productive habitat for *Culex* in autumn and those data would have had no bearing on the testing completed at Site 1 on September 26, 2022. Also, trapping at a site after efficacy testing was not part of the study design in the approved Study Protocol.

Thirdly, CLBR regards the strong contrast in *Culex* abundance between the two sites as what may be the most regulatorily important aspect of the differences between Sites 1 and 2. Figure 3, illustrating the difference between Sites 1 and 2 in terms of *Culex* landings on control subjects on the respective test days, shows the striking shift in the dominant species landing on control subjects from *Aedes* spp. to *Culex* spp. during exposures after sunset, including a recorded role in repellent failure at Site 2. *Culex* spp. landings are rarely successfully represented in repellant efficacy studies. The ability to assess so effectively the repellency of this product against *Culex* spp. is attributed to the divergence in mosquito diversity at Site 2 as demonstrated by trap collections leading up to and landings on control subjects during Test Day 2.

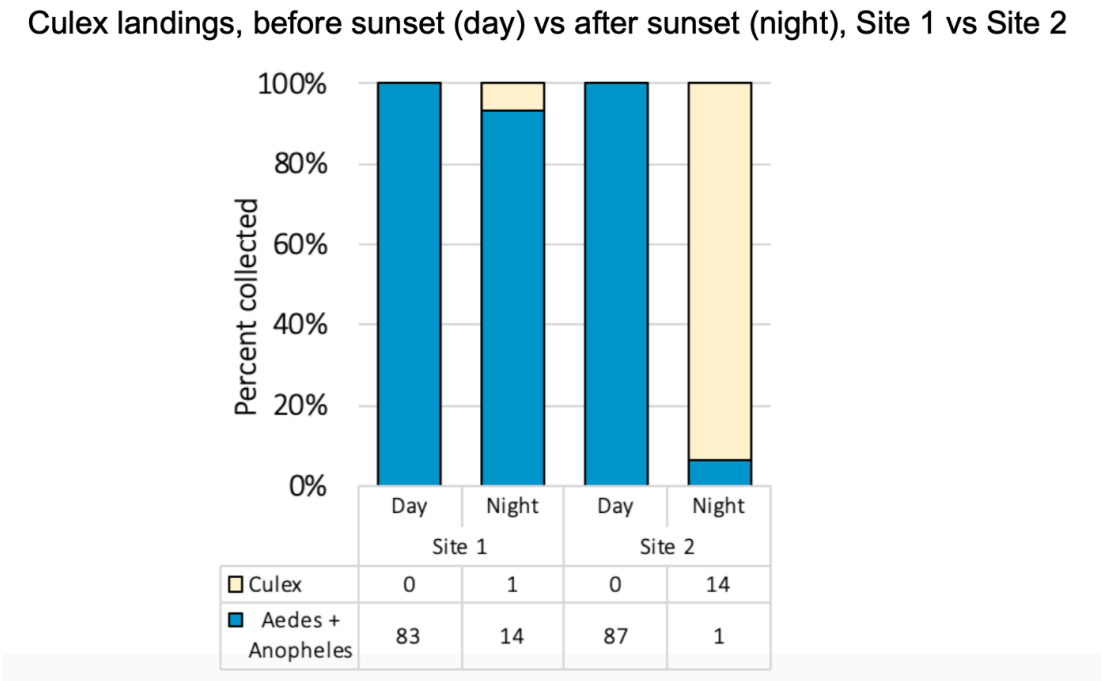


Figure 3: Comparison of day versus night (sunset and later) control subject landing counts of the three target mosquito genera between Site 1 and Site 2. Sunset was at 18:56 at Site 1 and 18:47 at Site 2 (source: https://sunrise-sunset.org/us/gridley-ca/2021/10).

Discussion: How Carroll-Loye Biological Research's two study sites address efficacy testing against genera and species of regulatory concern

In regards to the three mosquito genera of concern and important member species, CLBR's autumnal testing is organized around habitats providing reliably large, dense and subject-safe populations of otherwise WNV-vectoring *Culex*, while simultaneously targeting large numbers of *Aedes* and *Anopheles.* While these California field sites are representative of some of North America's most important public health and nuisance mosquitos, major ecological differences in the biotic and seasonal attributes of larval mosquito habits lead to important differences in species composition and in the timing of availability of key species for safely conducting conclusive human subject efficacy trials.

- ***Culex* spp.**

  A reliably large WNV-free population at Site 2
  The most important difference between the Sites 1 and 2 is the preponderance of the dominant autumnal species, *Culex tarsalis*. Repellent claims against WNV vectors should include rigorous tests against *Culex* mosquitoes. The avian WNV pathogen is the leading cause of human mosquito-borne diseases in the continental United States. It is most commonly spread to people by the bite of an infected *Culex* mosquito. *Culex tarsalis* is the most important vector of WNV in the western US and, given this species' expansive geographic range, perhaps nationally. Understanding the factors that determine the intensity of spillover of this zoonotic pathogen from birds to humans via mosquitoes is a prerequisite for predicting suitable study areas for testing mosquito repellents relevant to public health. While birds are known as the preferred hosts of WNV-vectoring *Culex* mosquito species, autumnal shifts in their preferences from birds to mammals, including humans, are key to geographic and seasonal patterns of WNV epidemic dynamics in the US[5].

  Culex are nocturnal feeders, and their discrete evening feeding period is limited to no more than an hour after dusk in many habitats (*pers. obs*.). A single-day test of a long-lasting mosquito repellent for EPA registration requires pairing large populations of avid, mainly day-active *Aedes* species, with very large populations of *Culex,* which while seasonally aggressive, are never as anthropophilic as most *Aedes* species.

  From the regulatory testing standpoint, Site 2 is the best place to safely test against *Culex tarsalis* at an acceptable biting pressure throughout the exposure period. Due to localized flooding schedules for duck hunting, Site 2 is unique in supporting very large

[5] Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol. **4**:e82 doi:10.1371/journal.pbio.0040082

autumnal, rather than just early summer populations of *Culex*. Only in autumn does WNV disappear from the large *Culex* surveillance sampling pools. Substantial autumnal regeneration of *Cx. tarsalis* have not been observed at Site 1, where the flooding schedule and habitat structure is not conducive to their reproduction. CLBR knows of no other study area than Site 2 that supports the subject-safe, massive 'second wind' of *Cx. tarsalis* in the US. Reduced and retarded wildlife habitat flooding schedules resulting from drought management in 2022, led to a delayed autumnal *Cx. tarsalis* emergence than we anticipated. Upon detection of this later than anticipated seasonal shift at Site 2, CLBR prepared promptly for testing.

- ***Aedes* spp.**

  SITE 2: Irrigated pasture mosquitoes important to agriculture and rural communities

  Aedes nigromaculis is a crepuscular and day-active nuisance species that breeds primarily in irrigated pastures and fields. Ecologically, irrigated pastures and fields are a widespread, highly specific habitat for mosquito larvae characterized by ephemeral surface water. This habitat is rare or absent from Site 1. *Ae. nigromaculis e*ggs left dormant on the ground through winter only hatch with warming temperatures, and populations increase during the late spring and summer months, impacting agricultural workers and operations. Adult mosquitoes emerge wherever water stands in irrigated fields for at least four days. Often, they emerge in large numbers and invade rural communities.

  Site 2 irrigated pasture permit testing against a species relevant to important, widespread but underserved agricultural economies and communities. A regulatory handicap to delivering this service under strict conformance to OCSPP guidelines is that our carbon dioxide traps often catch few *Ae. nigromaculis* even when they are frequently observed landing on surveillance staff. Moreover, in the presence of fast flying, aggressive congeners such as *Ae. melanimon* and *Ae. vexans,* they are rarely captured on control subjects, who normally finish their exposures quickly under the conditions required to ensure EPA minimum landing pressures even through the mid-to-late afternoon hours when dayflying mosquito species are normally less active. Nonetheless, *Ae. nigromaculis* are observed both flying around and landing on the clothing of subjects and research staff during the five-minute treated exposures. EPA has asked that we refrain from hand-netting from subjects during or between exposures; CLBR therefore lacks those supporting data in this study. However, scientifically, we regard the absence of *Ae. nigromaculis* landings on treated subjects' arms as a meaningful outcome of study MIM-006.

  SITE 2: Autumnal *Aedes vexans* to pair with subject-safe *Culex tarsalis*

  Aedes vexans is a floodwater species that occurs throughout the county and has perennially been the number one nuisance mosquito species, as well as a chief

encephalitis vector, confronting US vector control agencies. As with *Cx. tarsalis*, late-season controlled flooding in support of wildlife adjacent to Site 2 creates strong fall *Ae. vexans* populations there, whereas at Site 1 they are typically present in large numbers only until mid-summer, when *Ae. melanimon* increases and appears to outcompete them in the forest-dominated habitat. This year, for unknown reasons, *Ae. melanimon* remained more numerous than *Ae. vexans* at Site 2, although the trapped count ratio of the former to the latter was a much lower at Site 2 (2.6 to 1) compared with Site 1 (9.6 to 1). CLBR cannot rule out the possibility that the unanticipated abundance of late-season *Ae. melanimon* at Site 2 may have been due to supplementation from populations at nearby forested National Wildlife Refuges. However, the value of the Site 2 data in providing a more robust test against *Ae. vexans* than was possible with the much lower numbers of *Ae. vexans* at Site 1 remains clear. In addition, Site 2 featured presence and activity of the key taxa *Cx. tarsalis* and *Ae. nigromaculis,* neither of which appeared impacted by *Ae. melanimon* at the site.

SITE 1: Huge Autumnal *Aedes* populations of flooded forest species

Like its nearly identical and similarly important Eastern US sister species, *Ae. dorsalis, Ae. melanimon* is an aggressive nuisance mosquito species and a major vector of encephalitis viruses. Site 1 annually supports a very large fall-active, non-hurricane-dependent *Aedes* mosquito population. *Ae. melanimon* is a floodwater species that also takes advantage of forest pools, which hold water late into the year. At Site 2, where *Ae. vexans* prevails in autumn, *Ae. melanimon* populations generally drop off substantially earlier and more steeply than those at Site 1, again due to habitat differences in larval habitat structure and water availability for breeding. *Ae. sticticus*, an aggressive river bottom species in Site 1, and has a range that extends broadly across the Eastern US, is not known from Site 2.

- ***Anopheles* spp.**

SITES 1 and 2: Differences in *Anopheles* malaria mosquitoes from adjacent rice cultivation and uplands

The highly anthropophilic *An. freeborni* is an historically leading malaria vector in North America. By adopting irrigated rice agriculture habitats, this species has remained extremely common in our study region, peaking annually between mid-July and mid-September. Site 1 sustains large *Anopheles* populations longer into the autumn than is the case at Site 2. Site 1 is immediately adjacent to extensive areas of flooded rice fields, which geographically almost surround the nearly contiguous woodlands *Anopheles* shelter within Site 1. Site 2 has less sheltering habitat and is bordered more by private and public areas managed for waterfowl, with reduced *Anopheles* populations. Site 2 also harbors the malaria vector species, *An. punctipennis*, in greater numbers, likely due to adjacent foothills landscapes immediately to the south. This ecologically different species was not abundant in this study, however, again likely due to lingering drought.

In regards to the useful distinctions between mosquito species present and active at the two sites as demonstrated by mosquitoes captured landing on subjects, Site 2 featured 15 *Culex* spp. captured landings versus only two for Site 1, a more than seven-fold difference. Subdominant species differed between sites, with *Ae. sticticus* landing on a subject at Site 1 and *Ae. nigromaculis* landing on a subject at Site 2. Five species representing the three genera of concern landed on controls at Site 1 and at Site 2 such that all three genera were represented at both sites. *Aedes* spp. were captured only on treated subjects at Site 1, and *Aedes* spp. + *Culex tarsalis* for treated subjects at Site 2. At both sites, *Anopheles* spp. were captured making landings on control subjects, but not on treated subjects.

The presentation of data in Table 4 of the final report was unfortunate in that it obscured important information about species diversity at the sites as indicated by trapping. A clearer and more complete version is presented as follows (see also Annex 1):

Table 1: Species counts for each trapping date at each site. Red text indicates values that are corrected relative to Table 4 in the amended final report for Study No. MIM-006 (see Annex 1 for detailed summary of data corrections). Blue text indicates species totals by site for all trapping dates at each site. Bolded text indicates the data used in the Principle Components Analysis (PCA) addressed in Figures 1-3 (above). Note: Site 2 data from 27 September 2021 are subsamples, not the full count of all trapped mosquitoes from that collection sample, which is estimated to include 8,000 to 9,000 mosquitoes (see explanation in text below).

	Aedes melanimon	*Aedes vexans*	*Aedes nigromaculis*	*Aedes sticticus*	*Anopheles franciscianus*	*Anopheles freeborni*	*Anopheles punctipennis*	*Culex erythrothorax*	*Culex pipiens*	*Culex tarsalis*	Grand total
Site 1											
27-Jun-21	33					578				57	668
4-Aug-21	444					363				10	817
10-Aug-21	383	50				423				391	1247
17-Aug-21	150	17				560				21	748
25-Aug-21	250	39				497				19	805
2-Sep-21	**650**	**100**				**312**				**182**	1244
8-Sep-21	**650**	**50**				**128**				**300**	1128
16-Sep-21	**800**	**15**				**13**				**128**	956
21-Sep-21	**750**	**158**		**36**		**97**				**47**	1088
Total per spp.	4110	429		36		2971				1155	8701
Site 2											
31-Aug-21	400	200			11	916	2	7		172	1708
7-Sep-21	**550**	**281**	**50**			**123**		**5**	**4**	**85**	1098
14-Sep-21	**700**	**182**				**76**				**100**	1058
21-Sep-21	**500**	**178**	**158**			**25**			**19**	**108**	988
27-Sep-21	**124**	**32**	**40**			**20**				**800**	1016
Total per spp.	2274	873	248		11	1160	2	12	23	1265	5868

Finally, CLBR notes that totals for one site versus the other may appear skewed because there were nine trapping dates total at Site 1 and five trapping dates at Site 2. CLRB SOPs call for weekly trapping at our most utilized sites during summer months to support all field research activities, *i.e.,* not limited to Study No. MIM-006. Although all trapping counts are included in the above Table 1 and Amended Report Table 4 (see Annex I), only the four weekly trapping dates preceding each efficacy test day at each of the two sites were used for site qualification in Study No. MIM-006, as indicated by bolded values in Table 1 below and reflected in the PCA (Figures 1-3). Also, per the approved Study Protocol, CLBR did not necessarily count, separate, and identify more than approximately 1000 mosquitoes per trapping day. In one case (31 August 2021; Site 2) 1708 were counted; all other trap count totals were lower. CLBR sorted and counted all trapped mosquitoes with one exception -- on the final trapping day at Site 2, where most of the very large trap sample (estimated to include up to 9,000 mosquitoes) were not separated and counted. Rather, proportional sub-samples of each species present were taken until approximately 1000 mosquitoes were separated out, of which an estimated 90 to 95 percent were *Culex* spp. Each trap's total sample was placed in individual piles on a work surface. From each, a subsample was taken of an estimated appropriate size for a total of 1000 mosquitoes combined. After this subsampling, the trap samples remaining were combined into one pile as shown in the photo. The subsamples were combined then separated into three piles by species, as shown in the photo. The photograph below (Figure 5) shows the activity of this particular sorting, with the densest of the three smaller piles of mosquitoes being *Culex* (rightmost of the three). The largest among the four sort piles is 90 to 95% *Culex* and was visually estimated to contain between 5,000 and 8,000 mosquitoes. Although CLBR's documentation of the distinction between sites would have been more robust if all of the final trap sample mosquitoes for Site 2 had been counted, the provided visual documentation supports our contention that the two Sites were distinct.

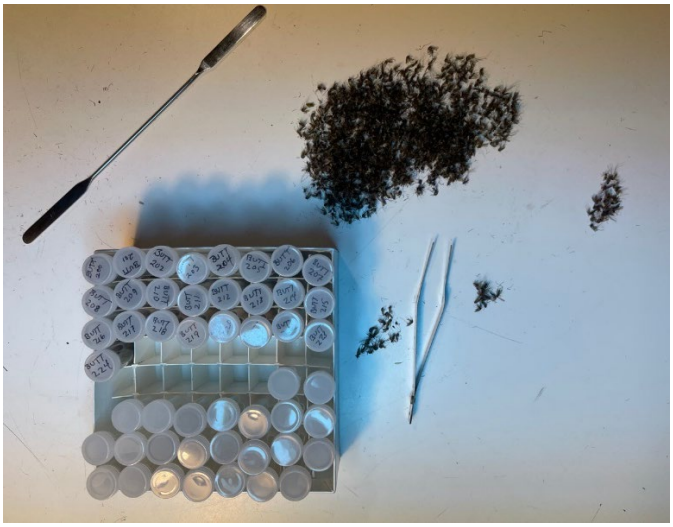


Figure 5: Trap sample collected 27 Sep 2021 for Site 2. The largest sort pile (upper center) was visually estimated to contain 5,000 to 8,000 mosquitoes, consisting of approximately 90 to 95% *Culex* spp.

Proven utility of the sites in question and CLBR's approach to evaluating efficacy of skin-applied repellents.

EPA has accepted field efficacy study data, generated in accordance with established testing guidance and Good Laboratory Practice Standards, from the same pair of sites used in MIM-006 (Site 1 and Site 2) for validation of all the key DEET-alternative products developed for the US market in this millennium, including for all Picaridin, IR3535, and OLE products, as well as para-menthane-3,8-diol (PMD). These products account for approximately 80 EPA-registered products as of 2016, including: 54 Picaridin products, at least 14 IR3535 products, and 12 OLE products.[6] Subsequent to that work, CLBR has gained additional insights into the differences between the two habitats represented by Sites 1 and 2 in larval ecology, allowing us to better time our work so as to increase the breadth and complementarity of the Sites for robust, subject-safe repellent evaluations. The success of those products for long-lasting protection against mosquitoes of public health significance testifies to the enduring utility of the data collected from those sites. Table 2 (below) summarizes the seminal registration data generated by CLBR utilizing the test sites under discussion in this document.

Table 2: Summary of insect repellent efficacy data sets accepted by EPA and generated by CLBR at the two field sites discussed herein.

	SPONSOR	TEST YEAR	ACTIVE INGREDIENT	%, DELIVERY	MRID
1	EMD	2006	IR3535	10%, lotion	46979003
2	EMD	2006	IR3535	20%, pump	46979004
3	Spectrum	2007	Oil of Lemon Eucalyptus (OLE)	30%, pump	47217601
4	LANXESS	2009	Picaridin	20%, spray 20%, cream	47506401
5	Del Cielo	2010	para-menthane-3,8-diol (PMD)	16%, lotion	48577201

Conclusions

CLBR believes it is important to distinguish data evaluation for qualification of a test site from evaluation of data from study execution for regulatory utility. Intentions and procedures for each of those study activities are distinct in the Study Protocol and in practice. Data quality evaluation can only be applied to the data sets themselves after study execution and in the

[6] Tally based on data from 2016, as provided by https://coastalhealthdistrict.org/wp-content/uploads/2016/02/EPA-Registered-Insect-Repellents.pdf; more recent developments not reflected here.

context of the field conditions imposed by nature during a given study day. As noted above, although trapping is used to get an idea of species presence and activity for the purposes of site qualification, it does not necessarily or directly correlate with which species will land on subjects, or directly confirm that the foraging activity will be both useful and adequate for evaluating the repellent.

Because the aims of both CLBR as a scientific team and EPA as a regulatory team is to clearly understand and effectively evaluate the quality of the data set in terms of utility for product registration purposes, it is reasonable and appropriate to examine the nature of what constitutes quality and utility to be certain we are not rejecting study data, gained at high cost to the Sponsor and meaningful risk to test subjects, based upon an *a priori* criterion that may or may not be significant in terms of the ultimate regulatory objective of the study. On principle, if the scientific merit of the data set and the degree to which it was ethically obtained are both judged to be sound, and the regulatory utility of the data is understood to be adequate or better on the merits of the clearly demonstrated efficacy of the product in two distinct habitats with differing species distributions, then it behooves EPA to accept and use that data set, rather than requiring additional Sponsor expense and subject risks on the chance that a singular, different or desired species might be present in another time or place. Herein we have made the case, scientifically, that the two test sites utilized for Study No. MIM-006 are indeed sufficiently different to qualify as ecologically distinct, complimentary mosquito habitats, which produced datasets of high evaluative quality for the purpose of regulatory decision-making and to inform the ultimate product label to ensure safe and effective use by consumers.

ANNEX I

The trap collection data reported in Table 4 and the tabulated data in Appendix 8 of the amended final report for Study No. MIM-006, issued on May 12, 2022[1], were re-evaluated in response to Item 2 of the outstanding deficiencies noted in EPA's 75-day deficiency letter, dated May 27, 2022[2], quoted as follows:

> *'2) A total of 8,701 mosquitoes were collected from Site 1 (MRID 517071-01, Appendix 8), but a total of 8,112 mosquitoes were reported to be collected and identified (Table 1 above). A total of 5,868 mosquitoes were collected from Site 2 (MRID 517071-01, Appendix 8), but a total of 5,645 were reported to be collected and identified (Table 1 above). An explanation should be provided regarding these discrepancies in reported numbers.'*

Discrepancies in counts between Table 4 and the tabulated data in Appendix 8 were confirmed as inadvertent transcription and spreadsheet computation errors. Corrections to these data will be reflected in a subsequent amendment to the final report, and are summarized as follows (red, struck-through data are replaced by bolded, underlined data); these corrected data were used in the principle components analyses discussed in the technical response above:

Amended Table 4. Species counts of mosquitoes trapped at the two field sites during the four weeks preceding each test day.

Genera	Species	Site 1	Site 2
Aedes	*Aedes melanimon*	~~3,671~~ **4,110**	~~2,224~~ **2,274**
	Aedes vexans	~~329~~ **429**	~~857~~ **873**
	Aedes nigromaculis	0	~~90~~ **248**
	Aedes sticticus	36	0
Anopheles	*Anopheles freeborni*	2,971	1,160
	Anopheles franciscanus	0	11
	Anopheles punctipennis	0	2
Culex	*Culex tarsalis*	~~1,105~~ **1,155**	~~1,266~~ **1,265**
	Culex pipiens	0	23
	Culex erythrothorax	0	12
Total numbers:		~~8,112~~ **8,701**	~~5,645~~ **5,868**

Amended Trap Collection Data by Site, Date and Species [from Appendix 8, pages 299-303 of the Amended Final Report, issued May 12, 2022]. Errors in tallies and data transcription arose where multiple species were entered into a single cell in the 'Species' column. The enclosed version shows multiple-species entries converted to one species per row such that the trapping count (Number in pool) for each species at each site on each date is unambiguous. The column identifying County is removed.

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
Site 1	1	27 June 2021	*Aedes melanimon*	Female	33	Yes			Neg.	668
Site 1	2	27 June 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	3	27 June 2021	*Culex tarsalis*	Female	7			Neg.		
Site 1	4	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	5	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	6	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	7	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	8	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	9	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	10	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	11	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	12	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	13	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	14	27 June 2021	*Anopheles freeborni*	Female	50			Neg.		
Site 1	15	27 June 2021	*Anopheles freeborni*	Female	28			Neg.		
Site 1	NA	4 August 2021	*Aedes melanimon*	Female	444					817
Site 1	NA	4 August 2021	*Culex tarsalis*	Female	10					
Site 1	NA	4 August 2021	*Anopheles freeborni*	Female	363					
Site 1	16	10 August 2021	*Culex tarsalis*	Female	41			Neg.		1247
Site 1	17	10 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	18	10 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	19	10 August 2021	*Culex tarsalis*	Female	50			WNV + Ct 20.1		
Site 1	20	10 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	21	10 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	22	10 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	23	10 August 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 1	24	10 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	25	10 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
Site 1	26	10 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	27	10 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	28	10 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	29	10 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	30	10 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	31	10 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	32	10 August 2021	*Aedes melanimon*	Female	33	Yes			Neg.	
Site 1	NA	10 August 2021	*Anopheles freeborni*	Female	423					
Site 1	33	17 August	*Culex tarsalis*	Female	21			Neg.		748
Site 1	34	17 August	*Aedes vexans*	Female	17	Yes			Neg.	
Site 1	35	17 August	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	36	17 August	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	37	17 August	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	NA	17 August	*Anopheles freeborni*	Female	560					
Site 1	38	25 August 2021	*Culex tarsalis*	Female	19			Neg.		805
Site 1	39	25 August 2021	*Aedes vexans*	Female	39	Yes			Neg.	
Site 1	40	25 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	41	25 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	42	25 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	43	25 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	44	25 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	NA	25 August 2021	*Anopheles freeborni*	Female	497					
Site 1	45	2 September 2021	*Culex tarsalis*	Female	50			Neg.		1244
Site 1	46	2 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	47	2 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	48	2 September 2021	*Culex tarsalis*	Female	32			Neg.		
Site 1	49	2 September 2021	*Aedes vexans*	Female	50				Neg.	
Site 1	50	2 September 2021	*Aedes vexans*	Female	50				Neg.	
Site 1	51	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	52	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	53	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	54	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	55	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	56	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	57	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	58	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	59	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	60	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	61	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	62	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	63	2 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	NA	2 September 2021	*Anopheles freeborni*	Female	312					
Site 1	64	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	1128
Site 1	65	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	66	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
Site 1	67	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	68	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	69	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	70	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	71	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	72	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	73	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	74	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	75	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	76	8 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	77	8 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 1	78	8 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	79	8 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	80	8 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	81	8 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	82	8 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	83	8 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	NA	8 September 2021	*Anopheles freeborni*	Female	128					
Site 1	84	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	956
Site 1	85	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	86	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	87	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	88	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	89	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	90	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	91	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	92	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	93	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	94	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	95	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	96	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	97	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	98	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	99	16 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	100	16 September 2021	*Aedes vexans*	Female	15	Yes			Neg.	
Site 1	101	16 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	102	16 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 1	103	16 September 2021	*Culex tarsalis*	Female	28			Neg.		
Site 1	NA	16 September 2021	*Anopheles freeborni*	Female	13					
Site 1	104	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	1088
Site 1	105	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
Site 1	106	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	107	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	108	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	109	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	110	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	111	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	112	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	113	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	114	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	115	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	116	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	117	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	118	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 1	119	21 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 1	120	21 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 1	121	21 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
~~Site 1~~	~~122~~	~~21 September 2021~~	~~*Aedes vexans, sticticus (36)*~~	~~Female~~	~~44~~	~~Yes~~			~~Neg.~~	
Site 1	**122**	**21 September 2021**	***Aedes vexans***	**Female**	**8**	**Yes**			Neg.	
Site 1	**122**	**21 September 2021**	***Aedes sticticus***	**Female**	**36**	**Yes**			Neg.	
Site 1	123	21 September 2021	*Culex tarsalis*	Female	47			Neg.		
Site 1	NA	21 September 2021	*Anopheles freeborni*	Female	97					
~~Site 1~~	~~200~~	~~27 September 2021~~	~~*Aedes melanimon (42), vexans (9)*~~	~~Female~~	~~52~~	~~Yes~~	~~Control Subject 25~~		~~Neg.~~	
Site 1	**200**	**27 September 2021**	***Aedes melanimon***	**Female**	**42**	**Yes**	**Control Subject 25**		**Neg.**	
Site 1	**200**	**27 September 2021**	***Aedes vexans***	**Female**	**9**	**Yes**	**Control Subject 25**		**Neg.**	
~~Site 1~~	~~201~~	~~27 September 2021~~	~~*Aedes melanimon (36), vexans (7), sticticus (1)*~~	~~Female~~	~~43~~	~~Yes~~	~~Control Subject 129~~		~~Neg.~~	
Site 1	**201**	**27 September 2021**	***Aedes melanimon***	**Female**	**36**	**Yes**	**Control Subject 129**		**Neg.**	
Site 1	**201**	**27 September 2021**	***Aedes vexans***	**Female**	**7**	**Yes**	**Control Subject 129**		**Neg.**	
Site 1	**201**	**27 September 2021**	***Aedes sticticus***	**Female**	**1**	**Yes**	**Control Subject 129**		**Neg.**	
Site 1	202	27 September 2021	*Anopheles freeborni*	Female	2	Yes	Control Subject 129		Neg.	
Site 1	203	27 September 2021	*Culex tarsalis*	Female	2		Control Subject 129	Neg.		
~~Site 1~~	~~204~~	~~27 September 2021~~	~~*Aedes melanimon (1), vexans (1)*~~	~~Female~~	~~2~~	~~Yes~~	~~Treated Subject 30~~		~~Neg.~~	
Site 1	**204**	**27 September 2021**	***Aedes melanimon***	**Female**	**1**	**Yes**	**Treated Subject 30**		**Neg.**	
Site 1	**204**	**27 September 2021**	***Aedes vexans***	**Female**	**1**	**Yes**	**Treated Subject 30**		**Neg.**	

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
Site 2	124	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	1708
Site 2	125	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	126	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	127	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	128	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	129	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	130	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	131	31 August 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	132	31 August 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	133	31 August 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	134	31 August 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	135	31 August 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	136	31 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	137	31 August 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	138	31 August 2021	*Culex tarsalis*	Female	50			Neg.		
~~Site 2~~	~~139~~	~~31 August 2021~~	~~*Culex tarsalis, erythrothorax (7)*~~	~~Female~~	~~29~~			~~Neg.~~		
Site 2	**139**	**31 August 2021**	***Culex tarsalis***	**Female**	**22**			**Neg.**		
Site 2	**139**	**31 August 2021**	***Culex erythrothorax***	**Female**	**7**			**Neg.**		
Site 2	NA	31 August 2021	*Anopheles freeborni*	Female	916					
Site 2	NA	31 August 2021	*Anopheles franciscianus*	Female	11					
Site 2	NA	31 August 2021	*Anopheles punctipenis*	Female	2					
Site 2	140	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	1098
Site 2	141	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	142	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	143	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	144	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	145	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	146	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	147	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	148	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	149	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	150	7 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	151	7 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	152	7 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	153	7 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	154	7 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	155	7 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
~~Site 2~~	~~156~~	~~7 September 2021~~	~~*Aedes vexans, nigromaculis (13)*~~	~~Female~~	~~44~~	~~Yes~~			~~Neg.~~	
Site 2	**156**	**7 September 2021**	***Aedes vexans***	**Female**	**31**	**Yes**			Neg.	
Site 2	**156**	**7 September 2021**	***Aedes nigromaculis***	**Female**	**13**	**Yes**			Neg.	
Site 2	157	7 September 2021	*Aedes nigromaculis*	Female	37	Yes			Neg.	
Site 2	158	7 September 2021	*Culex tarsalis*	Female	50			Neg.		
~~Site 2~~	~~159~~	~~7 September 2021~~	~~*Culex tarsalis, erythrothorax (5), pipens (4)*~~	~~Female~~	~~44~~			~~Neg.~~		
Site 2	**159**	**7 September 2021**	***Culex tarsalis***		**35**			**Neg.**		
Site 2	**159**	**7 September 2021**	***Culex erythrothorax***		**5**			**Neg.**		
Site 2	**159**	**7 September 2021**	***Culex pipiens***		**4**			**Neg.**		
Site 2	NA	7 September 2021	*Anopheles freeborni*	Female	123					
Site 2	160	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	1058
Site 2	161	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	162	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	163	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	164	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	165	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	166	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	167	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	168	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	169	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	170	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	171	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	172	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	173	14 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	174	14 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	175	14 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	176	14 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	177	14 September 2021	*Aedes vexans*	Female	32	Yes			Neg.	
Site 2	178	14 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	179	14 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	NA	14 September 2021	*Anopheles freeborni*	Female	76					
Site 2	180	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	988
Site 2	181	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	182	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	183	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	184	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	185	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	186	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	187	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
Site 2	188	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	189	21 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
Site 2	190	21 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	191	21 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
Site 2	192	21 September 2021	*Aedes vexans*	Female	50	Yes			Neg.	
~~Site 2~~	~~193~~	~~21 September 2021~~	~~*Aedes vexans, nigromaculis (22)*~~	~~Female~~	~~50~~	~~Yes~~			~~Neg.~~	
Site 2	**193**	**21 September 2021**	***Aedes vexans***	**Female**	**28**	**Yes**			**Neg.**	
Site 2	**193**	**21 September 2021**	***Aedes nigromaculis***	**Female**	**22**	**Yes**			**Neg.**	
Site 2	194	21 September 2021	*Aedes nigromaculis*	Female	50	Yes			Neg.	
Site 2	195	21 September 2021	*Aedes nigromaculis*	Female	50	Yes			Neg.	
Site 2	196	21 September 2021	*Aedes nigromaculis*	Female	36	Yes			Neg.	
Site 2	197	21 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	198	21 September 2021	*Culex tarsalis*	Female	50			Neg.		
~~Site 2~~	~~199~~	~~21 September 2021~~	~~*Culex tarsalis, pipiens (19)*~~	~~Female~~	~~27~~			~~Neg.~~		
Site 2	**199**	**21 September 2021**	***Culex tarsalis***	**Female**	**8**			**Neg.**		
Site 2	**199**	**21 September 2021**	***Culex pipiens***	**Female**	**19**			**Neg.**		
Site 2	NA	21 September 2021	*Anopheles freeborni*	Female	25					
Site 2	205	27 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	1016
Site 2	206	27 September 2021	*Aedes melanimon*	Female	50	Yes			Neg.	
~~Site 2~~	~~207~~	~~27 September 2021~~	~~*Aedes melanimon (24), vexans (26)*~~	~~Female~~	~~50~~	~~Yes~~			~~Neg.~~	
Site 2	**206**	**27 September 2021**	***Aedes melanimon***	**Female**	**24**	**Yes**			**Neg.**	
Site 2	**207**	**27 September 2021**	***Aedes vexans***	**Female**	**26**	**Yes**			**Neg.**	
~~Site 2~~	~~208~~	~~27 September 2021~~	~~*Aedes vexans (6), nigromaculis (40)*~~	~~Female~~	~~46~~	~~Yes~~			~~Neg.~~	
Site 2	**208**	**27 September 2021**	***Aedes vexans***	**Female**	**6**				**Neg.**	
Site 2	**208**	**27 September 2021**	***Aedes nigromaculis***	**Female**	**40**				**Neg.**	
Site 2	209	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	210	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	211	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	212	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	213	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	214	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	215	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	216	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	217	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	218	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	219	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	220	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	221	27 September 2021	*Culex tarsalis*	Female	50			Neg.		

Trial Site	Pool number	Date of collection	Species	Sex	Number in pool	CDZ testing	Study Subject No. (no entry=NA)	WSW	CDZ	Total trapped and submitted [a]
Site 2	222	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	223	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	224	27 September 2021	*Culex tarsalis*	Female	50			Neg.		
Site 2	NA	27 September 2021	*Anopheles freeborni*	Female	20					
~~Site 2~~	~~225~~	~~3 October 2021~~	~~*Aedes melanimon (37), vexans (3), nigromaculis (1), Anopheles freeborni (1)*~~	~~Female~~	~~42~~		~~Subject 6~~		~~Neg.~~	
Site 2	**225**	**3 October 2021**	***Aedes melanimon***	**Female**	**37**		**Subject 6**		**Neg.**	
Site 2	**225**	**3 October 2021**	***Aedes vexans***	**Female**	**3**		**Subject 6**		**Neg.**	
Site 2	**225**	**3 October 2021**	***Aedes nigromaculis***	**Female**	**1**		**Subject 6**		**Neg.**	
Site 2	**225**	**3 October 2021**	***Anopheles freeborni***	**Female**	**1**		**Subject 6**		**Neg.**	
Site 2	226	3 October 2021	*Culex tarsalis*	Female	11		Subject 6	Neg.		
~~Site 2~~	~~227~~	~~3 October 2021~~	~~*Aedes melanimon (39), vexans (7)*~~	~~Female~~	~~46~~		~~Subject 101~~		Neg.	
Site 2	**227**	**3 October 2021**	***Aedes melanimon***	Female	**39**		**Subject 101**		**Neg.**	
Site 2	**227**	**3 October 2021**	***Aedes vexans***	Female	**7**		**Subject 101**		**Neg.**	
Site 2	228	3 October 2021	*Culex tarsalis*	Female	3		Subject 101	Neg.		
~~Site 2~~	~~229~~	~~3 October 2021~~	~~*Aedes melanimon, vexans*~~	~~Female~~	~~2~~		~~Treated Subject 7~~		~~Neg.~~	
Site 2	**229**	**3 October 2021**	***Aedes melanimon***	**Female**	**1**		**Treated Subject 7**		**Neg.**	
Site 2	**229**	**3 October 2021**	***Aedes vexans***	**Female**	**1**		**Treated Subject 7**		**Neg.**	
Site 2	230	3 October 2021	*Aedes melanimon*	Female	1		Treated Subject 62		Neg.	
Site 2	231	3 October 2021	*Aedes melanimon*	Female	1		Treated Subject 69		Neg.	
Site 2	232	3 October 2021	*Aedes melanimon*	Female	1		Treated Subject 122		Neg.	
Site 2	233	3 October 2021	*Aedes melanimon*	Female	1		Treated Subject 132		Neg.	
Site 2	234	3 October 2021	*Culex tarsalis*	Female	1		Treated Subject 167	Neg.		
Site 2	235	3 October 2021	*Aedes melanimon*	Female	1		Treated Subject 178		Neg.	

[a] Summed totals include all trapped mosquitoes, but none that were caught from subject landings; "mean trap counts" were removed from this data presentation.

DRAFT

Meeting Minutes

Mimikai, Inc.

U.S. Environmental Protection Agency, Office of Pesticide Programs

June 8, 2022

3:00 p.m. (EDT)

On June 8, 2022, Mimikai, Inc. (Mimikai) met with the U.S. Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP) and its Biopesticides and Pollution Prevention Division (BPPD) to discuss Mimikai's Mosquito Field Test Efficacy Report, MIM-006, MRID No. 517071-01, conducted by Carroll-Loye Biological Research (CLBR). A copy of the agenda is appended.

1.0 **Introductions** -- The meeting attendee list is attached. Attendees from OPP, Mimikai, and Mimikai's consultant, Bergeson & Campbell, P.C. (B&C®), introduced themselves.

2.0 **Meeting Objective** -- Mimikai requested the meeting to discuss the deficiencies noted in EPA's May 27, 2022, 75-Day letter.

2.1 EPA did not consider the two study site locations to be independent of each other and requested that data for an additional site be submitted to supplement MRID 519127-01 (which replaced MRID 517071-01).

2.2 EPA noted discrepancies in the total number of mosquitoes reported.

2.3 EPA did not find acceptable CLBR's explanation why the field sites did not include *Aedes aegypti* or *Aedes albopictus.*

3.0 **Discussion** -- Mimikai and OPP discussed the following.

3.1 Site Locations

3.1.1 Mimikai noted that CLBR was preparing a technical rebuttal to EPA's conclusion that the two sites are not distinctive sites, and Mimikai may request that EPA consider the rebuttal *in lieu* of requiring testing at a third site.

2200 Pennsylvania Avenue, NW, Suite 100W phone: 202.557.3800
Washington, DC 20037-1701 fax: 202.557.3836 www.lawbc.com

3.1.2 Mimikai shared an overview of what CLBR will address in its rebuttal of why EPA should consider the two sites as independent and distinct.

3.1.3 EPA explained that the basis for its conclusion that the two sites are not independent is a lack of species diversity that the predominant species did not differ sufficiently between the two tested sites. EPA indicated it had previously reviewed studies that utilized two sites that were relatively close geographically but that represented vastly different habitats with different predominant species.

3.1.4 EPA noted that if testing at a third test site is conducted, an (sub)urban environment, where *Aedes aegypti* and *Aedes albopictus* are more prevalent, should be considered to address EPA's current recommendations for species diversity and habitat distinction.

3.2 Status of Amended MIM-006 Report

3.2.1 The deficiencies identified in EPA's March 10, 2022, 90-Day Technical Screen of MIM-006 required amendment to the final report. Mimikai submitted the fully amended MIM-006 report, MRID 519127-01, on May 12, 2022. Mimikai requested OPP's comments on the process and format used for the amended mosquito study, as it moves forward with the required amendment of the tick report, MIM-007. OPP confirmed that it had not reviewed the amended MIM-006 report and could not provide comment on the process or format.

3.3 Mosquito Species

3.3.1 EPA discussed the new testing guidance, *Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests*, effective June 14, 2022. The new guidance requires that, in addition to specific *Culex* and *Anopheles* species, *Aedes aegypti* and/or *Aedes albopictus* must be present during field trials to support a general mosquito label claim. Although at least three *Aedes* spp. were present at each field site, neither of the specified *Ae*. spp. were present in Study No. MIM-006. EPA explained that a request had been sent to the Office of General Counsel to clarify if Mimikai's currently pending reports (and application) would be held to the species requirements of the new guidance.

[DONE -- In a June 10, 2022, follow-up e-mail, Charles Smith stated, "we are not holding this current action to the requirements of the new product performance rule," but also, that any new testing (*i.e.*, a third site) should attempt to include the two *Ae*. spp.]

3.4 Third Site Testing

3.4.1 Mimikai and EPA agreed that if an additional third site were tested and submitted as a supplement to MRID 517071-01, the following actions would apply.

3.4.1.1 The current Pesticide Registration Improvement Extension Act (PRIA 4) due date (**September 9, 2022**) must be renegotiated.

3.4.1.2 Study No. MIM-006 would need to be reopened and the current protocol would need to be amended to include the new site and subjects. Protocol amendment and further testing would require Institutional Review Board (IRB) approval and oversight, but not Human Studies Review Board (HSRB) approval.

3.4.1.3 Upon completion of additional testing, the final study report would need to be amended formally (again) in accordance with Good Laboratory Practice (GLP) Standards.

3.4.1.4 EPA confirmed that the third site would need only to focus on *Ae. aegypti* and/or *Ae. albopictus,* and not the *Culex* or *Anopheles* spp., as the latter were adequately represented in the current study.

4.0 **Next Steps**

4.1 EPA stated that presenting MIM-006 at the **October 2022** HSRB meeting was not achievable. Mimikai countered that if the current study were reclassified as acceptable (*i.e.*, that the two sites were considered distinct), an **October 2022** HSRB appearance could be achievable.

4.2 Mimikai will provide CLBR's technical response.

The meeting adjourned at approximately 4:00 p.m. (EDT).

Attachments:
Meeting Attendee List
Agenda

Meeting Attendee List

EPA, OPP, BPPD

Charles Smith
Linda Hollis
Menyon Adams
Clara Fuentes
Richard Fehir
Brandall Ingle
Robert Mitchell
Kathryn Korthauer
Angela Myer
Shannon Borges

EPA, OPP

Michelle Arling

Mimikai

Stephanie Watson
Martin Mulvihill (SaferMade)
Adrian Horotan (SaferMade)

Lynn L. Bergeson (B&C)
Lara A. Hall (B&C)
Dana S. Lateulere (B&C)

FINAL AGENDA

Virtual Meeting
MIMIKAI, INC. and
U.S. Environmental Protection Agency, Office of Pesticide Programs,
Biopesticides and Pollution Prevention Division

June 8, 2022
3:00 p.m. (EDT)

Zoom/Teams Link: Click here to join the meeting
Call in (audio only) 1 571-429-6099
Passcode: 664582204#

1.0 Welcome and Introductions

2.0 Objective: Mimikai, Inc. (Mimikai) wishes to discuss the path forward to address the deficiencies identified by EPA in its May 27, 2022, review of Mimikai's Mosquito Field Test Efficacy Report, MIM-006; Action Case Number 00336661. Mimikai also requests a status update of the review on the Tick Laboratory Efficacy Report, MIM-007, although understands this may not be available at this time.

3.0 Discussion

- 3.1 Key deficiencies identified by EPA that are the basis for Study No. MIM-006 being considered SUPPLEMENTAL and INCOMPLETE;
- 3.2 Status of EPA's review of the amended final report for Study No. MIM-006 (MRID 519127-01) and proposed product label;
- 3.3 Options for addressing deficiencies:
  - 3.3.1 Practical considerations;
  - 3.3.2 Scientific considerations; and
  - 3.3.3 Ethics considerations;

4.0 Other Issues;

5.0 Summary of Action Items; and

6.0 Adjourn

Attachment 4

EPA's review of the registrant's technical report and rebuttal June 22, 2022) regarding site independence (including EPA's statistical evaluation of the Principal Components Analysis (PCA) provided in the registrant's technical report and rebuttal)

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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Response to Rebuttal for Registration of MIMIKAI Lilly Pilly Repellent, a bag-on-valve spray formulation, containing 11% w/w Oil of Lemon Eucalyptus (OLE) (Citriodiol) and 7.75% w/w Methyl Nonyl Ketone (MNK) (2-undecanone)

FROM: Clara Fuentes, Ph.D. Entomologist
Risk Assessment Branch
Biopesticides & Pollution Prevention Division
Office of Pesticide Programs

THROUGH: Shannon Borges, Chief
Risk Assessment Branch
Biopesticides & Pollution Prevention Division
Office of Pesticides Programs

TO: Linda Hollis, Chief
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division
Office of Pesticide Programs

REFERENCE: Response to Rebuttal: Action Case Number 00336661, EPA File Symbol Number 3616PA10

I. ACTION REQUESTED

Agency's response to Technical Response and Rebuttal document prepared by Carroll-Loye Biological Research (CLBR), and submitted on June 22, 2022, by Bergeson & Campbell, P.C. (B&C) on behalf of Mimikai, Inc. (MIMIKAI). This Technical Response and Rebuttal document addresses the following deficiency items, identified in a 75-Day Agency's deficiency letter, dated May 27, 2022 (in Attachment 1 of Technical Response and Rebuttal Document):

Item 1. Agency's assessment of geographical site information provided to EPA on April 26, 2022.

Item 2. Discrepancy between reported and tabulated counts of collected mosquitoes recorded in Table 4 and in raw data sheets of study report MRID 517071-01, and

Item 3. Justification for not including sites where *Aedes aegypti* and *Aedes albopictus* are present.

II. EXCUTIVE SUMMARY AND CONCLUSIVE REMARKS

The Agency has reviewed the registrant's rebuttal concerning sites assessment, and scientific information provided in the Technical Response document. Based on the scientific validity of the provided information, the Agency concludes that field data generated by study report, MIM-006, conducted per approved protocol and in accordance with OCSPP Guidelines 810.3700, supports a general repellency claim against mosquitoes, and against vectors of West Nile virus (WNV), malaria virus and mosquito-borne viruses that transmit Western encephalitis, Western Equine encephalitis, and St. Louis encephalitis on the product label. However, efficacy claims against vectors that may transmit Zika virus, Chikungunya virus, and Dengue virus are not supported by the data submitted, because those specific vectors have not been tested. Consequently, field data on *Aedes albopictus*, or *Aedes aegypti* are needed to support label repellency claims against mosquitoes that may transmit Zika virus, Chikungunya virus, and Dengue virus.

This conclusion is based on the following considerations:

- Results of the Principal Component Analysis (PCA), conducted by the registrant and verified by the Agency, showed temporal dissimilarity between sites in terms of species composition and abundance at the time of repellency testing on sites 1 and 2. Although the Agency recommends against the use of the "proportional sampling" method for estimating abundance of mosquito species, and asks for further clarification concerning numbers of mosquito species reported in Table 1 for site 2 on September 27, 2021, following the "proportional sampling" method, the estimated value of *Culex* mosquitoes used in the CLBR's PCA should be acceptable because the estimate is based on the large amount of *Culex* mosquitoes collected on trap day September 27, 2021, at site 2. Therefore, the Agency accepts the estimated abundance of mosquito species on the last trap day, September 27, 2021, at site 2, because it is based on the large amount of *Culex* mosquitoes collected from that trapping date, and consequently, it should not compromise the reliability of the PCA analysis, which includes those estimated values.

- Historical records of registered skin-applied repellents tested at the same California sites 1 and 2.

- Site monitoring trapping data a month prior to field testing, and mosquito landing data on control and treated subjects at sites 1 and 2, showed difference between sites regarding prevalence of *Culex* mosquitoes at time of repellency testing at site 2 (Refer to Figure 3).

- The Agency acknowledges that:

    - The study was conducted according to a previously approved protocol.

    - Physical distance between sites has not been scrutinized before as a requirement for site selection, and that the study conforms to the OCSPP 810.3700 Guidelines

regarding testing sites that do not need to be geographically distant to have different mosquito habitats where predominant species differ. The information provided in the Technical Report substantiates CLBR's argument that the difference between sites 1 and 2 is seasonal with the rise of *Culex* species in the fall.

- The study was conducted prior to June 14, 2022, when the *Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests*, became effective; therefore, the current study, MIM-006, is not subject to the three representative species requirement per the rule to support a general mosquito repellency claim on the label. Additional field data on *Aedes albopictus*, or *Aedes aegypti* is required to support label repellency claims against mosquitoes that may transmit Zika virus, Chikungunya virus, and Dengue virus.

III. SUMMARY OF SUBMITTED INFORMATION

Item #1. <u>Field sites assessment</u>:

Carroll-Loye Biological Research (CLBR) has provided a technical response addressing Agency's assessment of similarities in composition of mosquito species encountered at field sites 1 and 2 (Attachment 2 in Technical Response and Rebuttal document).

CLBR conducted a Principal Components Analysis (PCA), using trap collection data from four weekly samples, collected during site monitoring prior to efficacy testing. Results from the PCA showed composition of mosquito populations diverging between site 1 and site 2. This dissimilarity between sites is attributed to physical, biological and ecological characteristics and over time difference in availability of species-specific habitats within and between sites. Results from the PCA are illustrated in Fig. 1 (pg. 3 Attachment 2 in Technical Response and Rebuttal document).

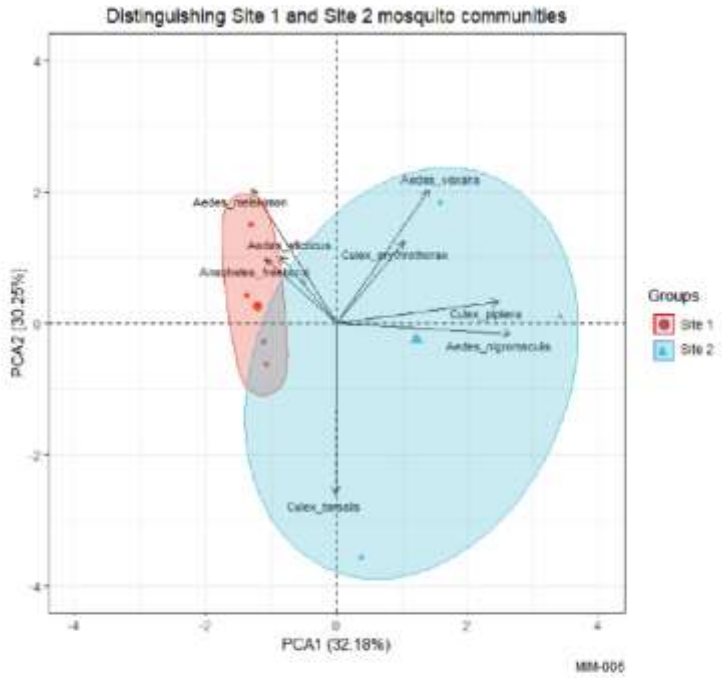


Figure 1: Principal components analysis (PCA) using mosquito species presence and abundance data from four weekly-interval trapping collections completed prior to human subject efficacy testing at Sites 1 and 2 for Study No. MIM-006. The X and Y axes are percentages that represent how much each axis explains the variation in the data. Arrows are representations of the principal components explaining the dissimilarity between the two sites. The length of an arrow (principal component) correlates with a specific variable's explanatory impact on observed variation in the data. The cumulative interaction of the principal components influences the location of each Site's centroid (the large red circle for Site 1, and the large blue triangle for Site 2).

According to the PCA, the distances in sites' centroids is driven by high relative abundance of different mosquito species at either site. The following mosquito species are present at site 2: *C. tarsalis* and *Ae. nigromaculis, C. pipiens* and *C. erythrothorax* while, *Ae. sticticus* is predominantly present at Site 1. Overall distance between sites' centroids indicates differences in distribution and composition of mosquito species between sites, likely attributed to ecological dissimilarities between sites.

In contrast, EPA's site analysis compares sites' data from simultaneous collection days, not including the last (4th) collection date (September 27, 2021) at site 2. CLBR includes data from the 4th collection date at both sites, September 21 at site 1 and September 27 at site 2. PCA comparison of September 21 and September 27 for sites 1 and 2, and September 27 for site 2, show differences in species composition between sites 1 and 2, and differences within species composition at site 2. Difference in species composition between sites is attributed to physical differences in habitat structure between sites. Shifts in species composition, diversity and abundance within site 2 is attributed to seasonal changes in ecological / environmental conditions within site 2. In summary the main difference between EPA and CLBR analysis for site comparison is that EPA analysis compares coinciding sampling dates and excludes the last 4th sampling date at site 2, while CLBR analysis includes all four sampling dates prior to repellency

testing day. Consequently, CLBR analysis captures the sequential change in species composition and abundance occurring over time between and within sites during the entire site monitoring period prior to efficacy testing. These results are illustrated in Figs. 2a and 2b (pp. 4 and 5 of Technical Report document).

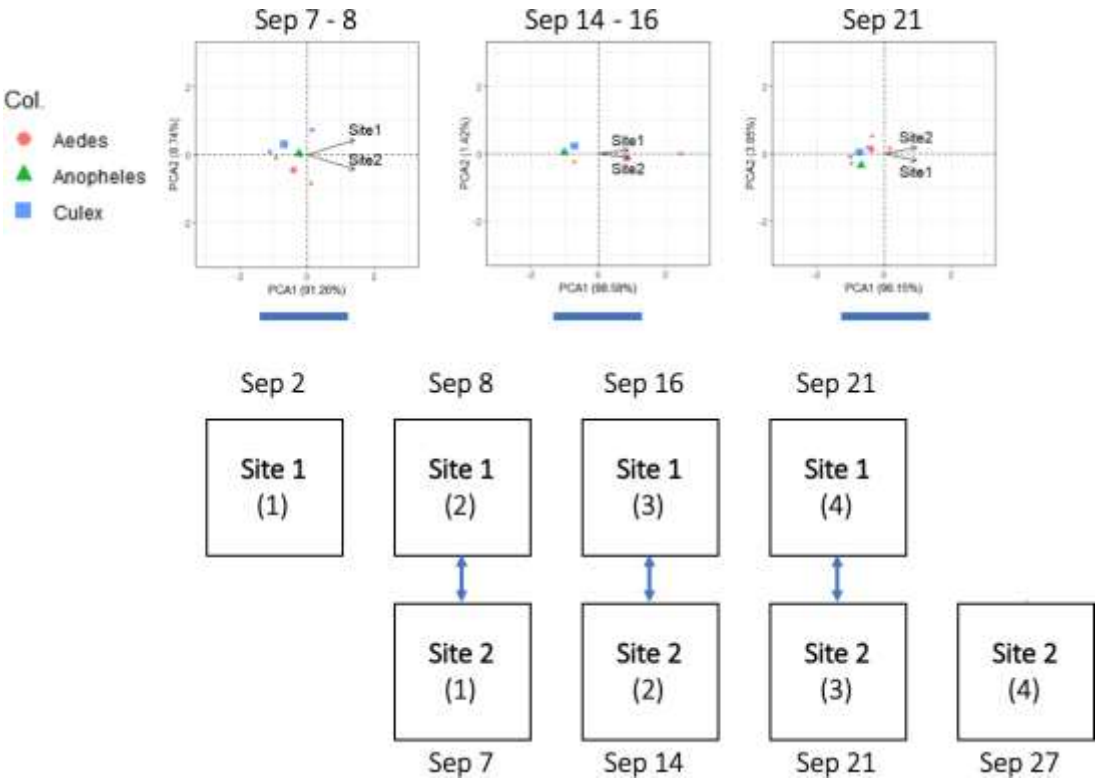


Figure 2a**:** EPA analysis of site monitoring sampling data using coinciding dates.

Comparing Site 1 with Site 2 trapping data emphasizing coincidence in time (per EPA). Separate PCAs for Sep 7-8, Sep 14-16, and Sep 21 show little difference between sites. Individually, each uses much less data to draw comparisons than PCA of the overall trap data (Figure 1).

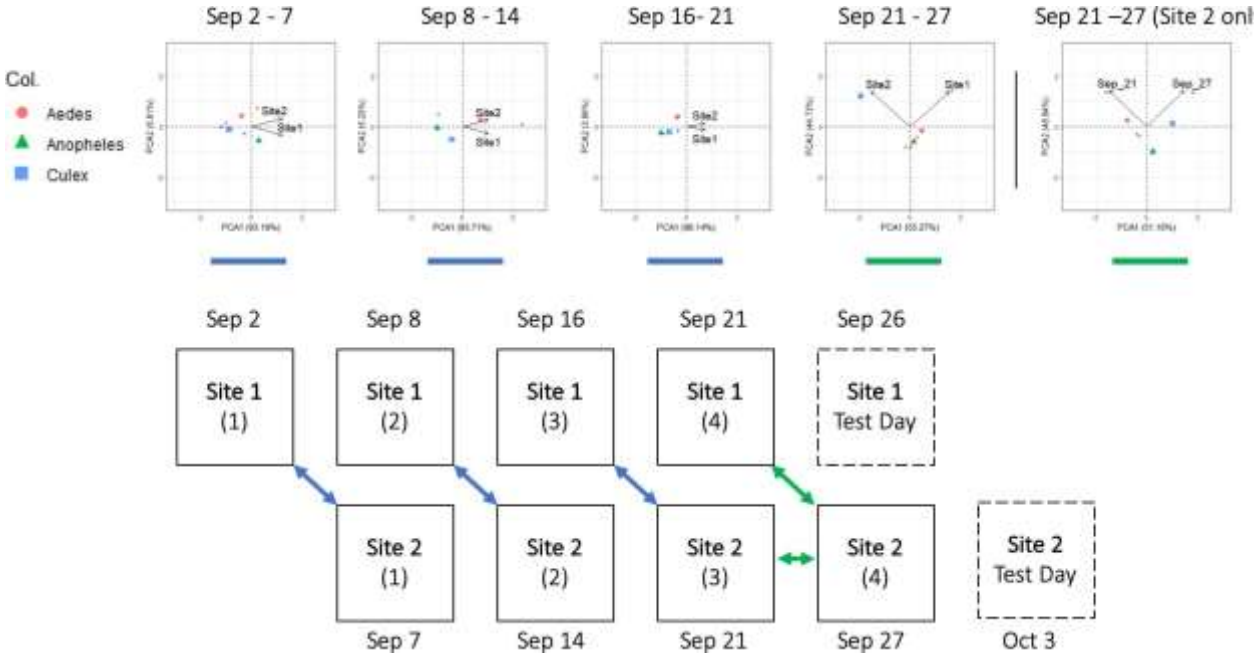


Figure 2b: Comparing developmental differences in composition of mosquito species along the planned sequence of 1st, 2nd, 3rd, or 4th trap collection over four weeks immediately preceding testing at each site. The separate PCAs for September 21-27, 2021, between sites, and September 21-27, 2021, within Site 2, demonstrate divergence.

The main difference in species composition between sites is the abundance of *Culex* species at site 2 prior to testing efficacy. In addition, difference in landings of *Culex* mosquitoes on control subjects at site 1 and site 2 is illustrated in Fig. 3 on pg. 6 of the Technical Report document. These data show more landings of *Culex* species at site 2 than at site 1 during efficacy testing.

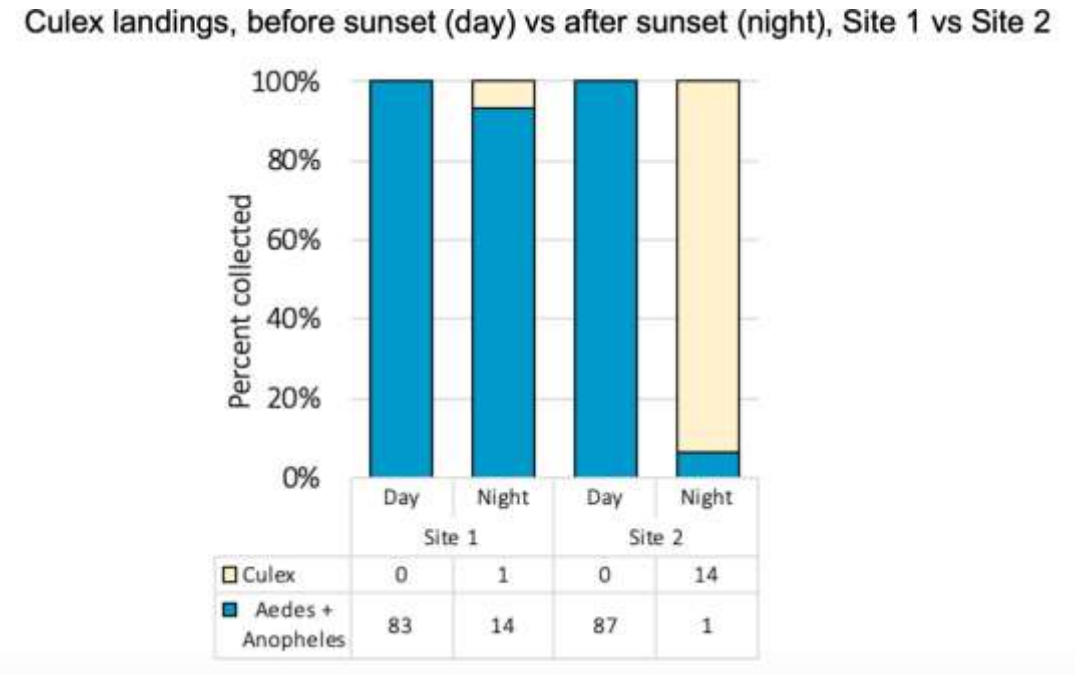


Figure 3**:** Comparison of day versus night (sunset and later) control subject landing counts of the three target mosquito genera between Site 1 and Site 2. Sunset was at 18:56 at Site 1 and 18:47 at Site 2 (source: https://sunrise- sunset.org/us/gridley-ca/2021/10).

Item # 2. <u>Number of collected mosquito species</u>:

The discrepancy between reported and tabulated counts of collected mosquitoes in study report MRID 517071-01 were addressed in supplemental MRID 519127-01 and in the Technical Report document. Counts were revised and amended as indicated in Table 1 below and on pg. 10 of the Technical Report document and amended final report MRID 519127-01. Raw data sheets are corrected accordingly. Amended tables and raw data sheets are provided in subsequent amendment to the final report, MRID 519127-01, summarized in Annex 1 (Attachment 2; pp. 15 - 22, of the Technical Report and Rebuttal document).

Table 1**.** Mosquito abundance and species distribution at two field sites (pre-test monitoring data). Table 1 in amended report (MRID 519127-01) replaces data from Table 4 of study report MRID 517071-01 (§3, p. 15 of 362). Amended Table 4 is presented below as provided in amended report MRID 519127-01. Corresponding corrected raw data are provided and presented in Appendix A of this document.

	Aedes melanimon	*Aedes vexans*	*Aedes nigromaculis*	*Aedes sticticus*	*Anopheles franciscianus*	*Anopheles freeborni*	*Anopheles punctipennis*	*Culex erythrothorax*	*Culex pipiens*	*Culex tarsalis*	Grand total
Site 1											
27-Jun-21	33					578				57	668
4-Aug-21	444					363				10	817
10-Aug-21	383	50				423				391	1247
17-Aug-21	150	17				560				21	748
25-Aug-21	250	39				497				19	805
2-Sep-21	**650**	**100**				**312**				**182**	1244
8-Sep-21	**650**	**50**				**128**				**300**	1128
16-Sep-21	**800**	**15**				**13**				**128**	956
21-Sep-21	**750**	**158**		**36**		**97**				**47**	1088
Total per spp.	4110	429		36		2971				1155	8701
Site 2											
31-Aug-21	400	200			11	916	2	7		172	1708
7-Sep-21	**550**	**281**	**50**			**123**		**5**	**4**	**85**	1098
14-Sep-21	**700**	**182**				**76**				**100**	1058
21-Sep-21	**500**	**178**	**158**			**25**			**19**	**108**	988
27-Sep-21	**124**	**32**	**40**			**20**				**800**	1016
Total per spp.	2274	873	248		11	1160	2	12	23	1265	5868

Data from Table 1 in Technical Report and Rebuttal document. Species counts for each trapping date at each site. Red text indicates values that are corrected relative to Table 4 in the amended final report for Study No. MIM-006 (see Annex 1 for detailed summary of data corrections). Blue text indicates species totals by site for all trapping dates at each site. Bolded text indicates the data used in the-Principal Component Analysis (PCA) addressed in Figures ; 2a and 2b (above). Although all trapping counts are included in Table 1 and revised Table 4 in Amended Study Report , MRID 519127-01, only the four weekly trapping dates preceding each efficacy test day at each of the two sites were used for site qualification in Study No. MIM-006, and used in the PCA (Figures 1, 2a and 2b). Note: Site 2 data from 27 September 2021 are subsamples, not the full count of all trapped mosquitoes from that collection sample, which is estimated to include 8,000 to 9,000 mosquitoes.

Mosquito Counts procedure: CLBR explained that per the approved protocol, no more than 1,000 mosquitoes were counted, separated, and identified per trapping day. The highest count was reported on August 31 at site 2. *"In one case (31 August 2021; Site 2) 1708 were counted; all other trap count totals were lower."* (See Table 1). September 27 had the largest trapping sample at site 2, with an estimated sample of 9,000 mosquitoes, of which 5,000 to 8,000 mosquitoes in the sample were visually estimated to be *Culex* species. Mosquitoes from trap collections on September 27 were not counted. Rather, the number of trapped species was visually estimated via "proportional subsampling." The procedure is explained as follows: Collections from each trap were placed in individual piles and subsamples of approximately 1,000 mosquitoes were taken out from each pile. After subsampling from each pile was done, the remaining individual piles per trap were combined into one single pile. All subsamples of approximately 1,000 mosquitoes from individual piles were combined in one single pile of all subsamples combined, and species were sorted out to form three separate piles per species as shown in Fig. 4 below (corresponding to Fig. 5 in the Technical Report document).

Figure 5: Trap sample collected 27 Sep 2021 for Site 2. The largest sort pile (upper center) was visually estimated to contain 5,000 to 8,000 mosquitoes, consisting of approximately 90 to 95% *Culex* spp.

CLBR claims that the photograph in Fig. 5 *"shows the activity of this particular sorting, with the densest of the three smaller piles of mosquitoes being Culex (rightmost of the three). The largest among the four sort piles is 90 to 95% Culex and was visually estimated to contain between 5,000 and 8,000 mosquitoes. Although CLBR's documentation of the distinction between sites would have been more robust if all of the final trap sample mosquitoes for Site 2 had been counted, the provided visual documentation supports our contention that the two Sites were distinct."*

Item # 3. <u>Absence of *Aedes aegypti* or *Ae. albopictus* at field sites</u>:

Agency's communications from June 8th meeting, e-mail message from Charles Smith on June 10th, and meeting minutes, dated June 20, 2022, are provided in Attachment 3 of Technical Report and Rebuttal document. The Agency acknowledges that this study was conducted according to protocol, reviewed and approved prior to the representative mosquito species requirement as specified in *Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests*, was effective on June 14, 2022.

In summary, the rationale provided in support of selected field sites and mosquito species encountered at those sites is based on the ecology and public health significance of mosquito species encountered at both sites, absence of mosquito-borne pathogens prior to field testing, and history of products registered in the USA that have been tested at those same sites. Each of these points are summarized below:

- Dense populations of *Aedes*, *Anopheles* and *Culex* species, vectors of West Nile virus (WNV) and other encephalitis viruses, are encountered at the California sites selected for testing, while no mosquito-borne pathogens were detected prior to field testing.
    - *Culex* species, in particular *Culex tarsalis*, one of the main vectors of WNV, becomes predominant at site 2 during the fall. "*While birds are known as the preferred hosts of WNV-vectoring Culex mosquito species, autumnal shifts in their preferences from birds to mammals, including humans, are key to*

geographic and seasonal patterns of WNV epidemic dynamics in the US (Kilpatrick et al. 2006)*."* While peak biting activity of *Culex* mosquitoes is limited to a brief feeding period after dawn, the presence of complementary and more anthropophilic *Aedes* species with daylong biting activity, maintain adequate landing pressure throughout exposure periods at site 2. Thus, the combination of *Aedes* and *Culex* mosquito species present at site 2, together with absence of mosquito-borne pathogens prior to field testing, allows for ethically acceptable human testing against *Culex* mosquitoes at an adequate landing pressure throughout exposures.

Aedes vexans mosquitoes, vectors of St. Louis encephalitis virus, Western and Eastern equine encephalitis viruses, are abundant at site 1 until mid-summer when the population of *Aedes melanimon* becomes more predominant. In contrast, *Ae. vexans* populations are more abundant at site 2 than at site 1 during fall, with the exception of 2021, when *Ae. melanimon,* another major vector of Western Equine and California Group Encephalitis, became more abundant than *Ae. vexans* at site 2.

Site 1 sustains large populations of *Anopheles freeborni*, a malaria vector. *An. freeborni* is less predominant at site 2, where larger populations of *An. punctipennis,* another malaria vector, are found, except in 2021 due to drought conditions.

- *"Proven utility of the sites in question and CLBR's approach to evaluating efficacy of skin-applied repellents"*

    - "*EPA has accepted field efficacy study data, generated in accordance with established testing guidance and Good Laboratory Practice Standards, from the same pair of sites used in MIM-006 (Site 1 and Site 2) for validation of all the key DEET-alternative products developed for the US market in this millennium, including for all Picaridin, IR3535, and OLE products, as well as para-menthane-3,8-diol (PMD). These products account for approximately 80 EPA-registered products as of 2016, including: 54 Picaridin products, at least 14 IR3535 products, and 12 OLE products. Subsequent to that work, CLBR has gained additional insights into the differences between the two habitats represented by Sites 1 and 2 in larval ecology, allowing us to better time our work so as to increase the breadth and complementarity of the Sites for robust, subject-safe repellent evaluations. The success of those products for long-lasting protection against mosquitoes of public health significance testifies to the enduring utility of the data collected from those sites. Table 2 (below) summarizes the seminal registration data generated by CLBR utilizing the test sites under discussion in this document*."

Table 2: Summary of currently registered products tested at site 1 and site 2.

	SPONSOR	TEST YEAR	ACTIVE INGREDIENT	%, DELIVERY	MRID
1	EMD	2006	IR3535	10%, lotion	46979003
2	EMD	2006	IR3535	20%, pump	46979004

3	Spectrum	2007	Oil of Lemon Eucalyptus (OLE)	30%, pump	47217601
4	LANXESS	2009	Picaridin	20%, spray 20%, cream	47506401
5	Del Cielo	2010	para-menthane-3,8-diol (PMD)	16%, lotion	48577201

DISCUSION, COMMENTS AND RECOMMENDATIONS

Item 1: <u>Field sites assessment</u>

The Agency conducted a PCA analysis using amended mosquito count data as shown in Table 1. Results from the Agency's PCA analysis (presented in Fig. 5 below) coincide with those provided by CLBR. Therefore, the Agency has no questions/concerns about the results from CLBR's PCA analysis.

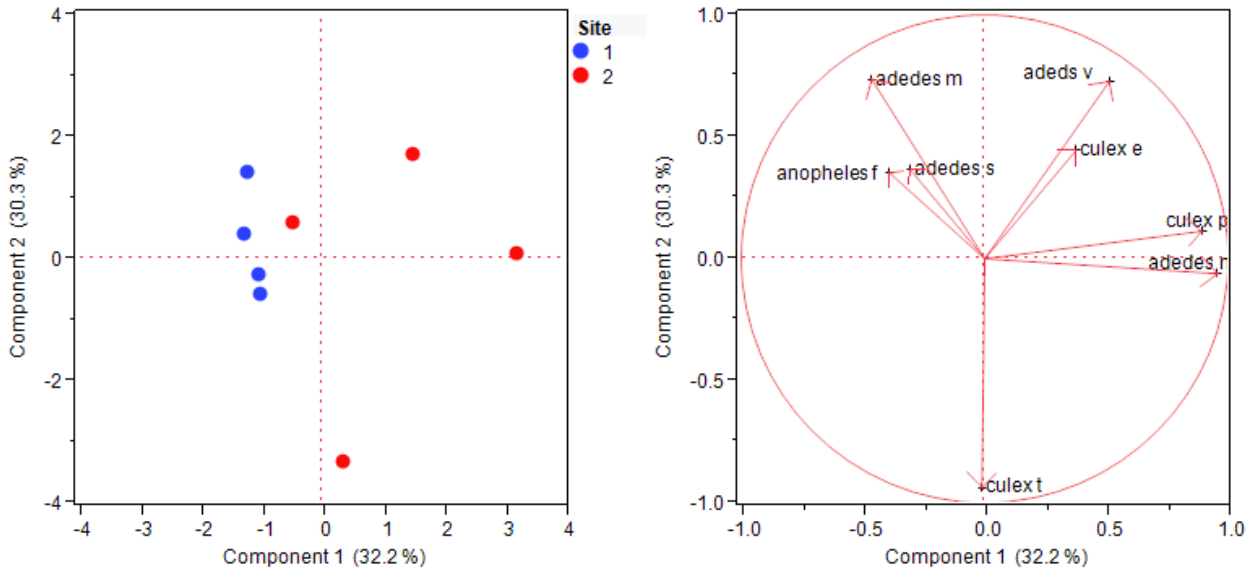


Figure 5. Results from Agency's PCA. From the plot of scores, all datapoints of site 1 (blue) were relatively far left and all datapoints of site 2 (red) were far right on the scale of component 1. This indicates that the data of sites 1 and 2 were not similar. Therefore, although the two sites are close in proximity, the PCA indicates that the two sites are two distinct habitats, leading to differences in species composition.

<u>Structural differences between sites</u>: site 1 is described as a seasonally flooded ,riparian forest habitat dominated by heavy tree cover as well as nearby marshes and cultivated rice fields that provided mosquito breeding habitats. In contrast, site 2 is described as a native moist grassland habitat around a small lake with permanent body of water that shelter wildlife such as birds and mammals. Mosquitoes that breed in the permanent standing water as well as in adjacent irrigated pastures and marshes at site 2, also feed on birds and mammals inhabiting site 2.

Item # 2. <u>Number of collected mosquito species per site</u>

The Agency recommends against the "proportional subsampling" method employed by the researcher for estimating abundance of mosquito species because it is based on subjective visual evaluation and consequently, less accurate than actual count or weight of sample. In addition, the three small piles per species shown in Fig. 4 of this document and Fig. 5 of Technical Report and Rebuttal document, do not correspond to number of species reported on Table 1 for site 2 on September 27, 2021. There are five mosquito species, not three, reported for site 2 on September 27, 2021 (Table 1). These species are: 124 *Aedes melanimon*; 32 *Ae. vexans;* 40 *Ae. nigromaculis*; 20 *Anopheles freeborni*, and 800 *Culex tarsalis* for a total of 1,016 mosquitoes. Therefore, further clarification should be provided concerning the reported values.
Estimated numbers, rather than actual counts, from the last trap day, September 27, 2021, at site 2 were used in CLBR's PCA analysis that contrasts with EPA analysis by including data from September 27, 2021, from site 2. At this time, the Agency accepts the estimated abundance of mosquito species on the last trap day, September 27, 2021, at site 2, based on the large amount of *Culex* mosquitoes collected from that trapping date, and consequently, the reliability of the PCA analysis, which includes those estimated values.

In addition, the Agency also takes into consideration landing data on control and treated subjects for assessing difference in species composition between sites during efficacy testing. Differences in landings of *Culex* mosquitoes on control subjects at sites 1 and site 2 is illustrated in Fig. 3 in this document and on pg. 6 of Technical Report and Rebuttal document. Mosquito landings' data on control subjects is summarized in Table 5 of MRID 517071-01 (pg. 20 of 362) and mosquito landing data on controls and treated subjects per site are summarized in Table 2 in this document. Fifteen *Culex* mosquitoes were recorded landing on controls at site 2 and only two *Culex* mosquitoes were recorded landing of controls at site 1. One *Culex* mosquito was recorded landing on treated subject at site 2. No *Culex* mosquitoes were recorded landing on treated subject at site 1 (Table 3).

"In regard to the useful distinctions between mosquito species present and active at the two sites as demonstrated by mosquitoes captured landing on subjects, Site 2 featured 15 Culex spp. captured landings versus only two for Site 1, a more than seven-fold difference. Subdominant species differed between sites, with <u>Ae. sticticus</u> landing on a subject at Site 1 and <u>Ae. nigromaculis</u> landing on a subject at Site 2. Five species representing the three genera of concern landed on controls at Site 1 and at Site 2 such that all three genera were represented at both sites. Aedes spp. were captured only on treated subjects at Site 1, and Aedes spp. + <u>Culex tarsalis</u> for treated subjects at Site 2. At both sites, Anopheles spp. were captured making landings on control subjects, but not on treated subjects." (See Table 3).

Table 3**.** Mosquito species and number aspirated from control and treated subjects per site.

Site	Treatment	Subject	Species	No. Caught	Total Caught by Treatment	Total Landings by Treatment	% of Landings Caught
1	Control	25	*Aedes melanimon*	42	99	179	55.3%
			Aedes vexans	9			
		129	*Aedes melanimon*	36			
			Aedes sticticus	1			
			Aedes vexans	7			
			Anopheles freeborni	2			
			Culex tarsalis	**2**			
	Treated	30	*Aedes melanimon*	1	2	10	20%
			Aedes vexans	1			
2	Control	6	*Aedes melanimon*	37	102	158	64.5%
			Aedes nigromaculis	1			
			Aedes vexans	3			
			Anopheles freeborni	1			
			Culex tarsalis	**11**			
		101	*Aedes melanimon*	39			
			Aedes vexans	7			
			Culex tarsalis	**3**			
	Treated	7	*Aedes melanimon*	1	8	27	29.6%
			Aedes vexans	1			
		62	*Aedes melanimon*	1			
		69	*Aedes melanimon*	1			
		122	*Aedes melanimon*	1			
		132	*Aedes melanimon*	1			
		167	***Culex tarsalis***	**1**			
		178	*Aedes melanimon*	1			

Note: Data for species and numbers aspirated were taken from Table 5 (§6, p. 20 of 362) for control subjects and Appendix 8 (pp. 285, 287 of 362) for treated subjects. Total aspirated by subject type per site was calculated by the sum of numbers provided in the 'Number Aspirated' column. Total mosquito landings were determined by raw data sheets in Appendix 7 (pp. 269-270, 273-274, 276, 278 of 362).

As CLBR explains in the Technical Report and Rebuttal document, efficacy testing days at each site were scheduled according to these temporal differences in species composition between site 1 and site 2. Efficacy testing at site 1 was conducted on September 26 and efficacy testing at site 2 was conducted on October 3, 2021, to capture the temporal rise in number of *Culex* species at site 2. Therefore, the Agency agrees that regardless of physical proximity and consequent species overlap, the study was conducted according to guideline recommendations for testing efficacy at two sites that do not need to be geographically distant, but provide distinctly different mosquito habitats where predominant mosquito species differ. The difference in species composition between these two sites is a function of seasonal variation in habitat availability, causing a seasonal increase in *Culex* species at time of repellency testing at site 2. Since efficacy testing days were scheduled to capture these temporal differences between site, it is possible to conclude that the product was tested at two ecologically different sites where species composition differed at time of efficacy testing. **Data on Figure 3 supports this conclusion**. The difference between sites is based on seasonal ecological changes and habitat availability within site 2.

"CLBR's process to qualify distinct sites, per the Study Protocol, was to use the four weekly trap samples for each prospective site that preceded the actual test day as a confirmation (A) that all three genera were present as anthropophilic species; and (B) ***that each site at the time of efficacy testing would be divergent in mosquito species composition, as expected due to known differences in larval habitat and in response to real-time changes in influential ecological factors between the two sites****. CLBR therefore was not making qualifying choices in site selection by comparing site trapping data coincident in time, but rather comparing development of differences along the sequence of weekly trap collections at each site for the month prior to each test day."* Difference in species composition between sites is illustrated in Fig. 1, pg. 16 of 362 in original report MRID 517071-01.

At present, the Agency acknowledges the historical record of registered products (listed in Table 2) that have been tested at the same California sites. However, **future studies will require data on specific vectors according to *Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests*, effective June 14, 2022, to support a general mosquito claim on product labels. Sites should be selected on the basis of predominant required species that differ between field sites as well as the absence of vector-borne pathogen a month prior to efficacy testing. Based on this requirement, the current California sites will qualify as one field site and a second ecologically different site where *Aedes albopictus* or *Aedes aegypti* are predominant must be selected in support of product registration.**

Item # 3. <u>Absence of *Aedes aegypti* or *Ae. albopictus* at field sites</u>

Although the protocol listed potential field sites that included all main disease vectors within three genera of *Aedes, Culex*, and *Anopheles*, neither *Aedes aegypti* nor *Aedes albopictus* were found at the field sites selected for testing. In addition, the Informed Consent Form explains to subjects the potential risks of contracting Zika, Chikungunya, and Dengue viruses, which may be transmitted by these specific vectors. This information, in addition to proposed label claim against vectors of Zika, Chikungunya, and Dengue viruses, implies the intention of choosing sites where these vectors are encountered. Consequently, the protocol was approved and the study was conducted according to

protocol prior to the representative mosquito species requirement as specified in *Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests*, effective June 14, 2022. Future studies, however, must be conducted at field sites where required mosquito species (listed below), including *Aedes aegypti* or *Aedes albopictus*, are present. As of June 14, 2022, the following mosquito species are required for a general mosquito claim to cover most prevalent diseases transmitted by mosquitoes:

- *Anopheles* (*Anopheles quadrimaculatus* or *Anopheles freeborni* or *Anopheles punctipennis* or *Anopheles gambiae* or *Anopheles hermsi* or *Anopheles albimanus* or *Anopheles stephensi*);
- *Aedes* (*Aedes albopictus* or *Aedes aegypti*), and
- *Culex* (*Culex pipiens* or *Culex quinquefasciatus* or *Culex tarsalis*).

Guidance on mosquito species required for testing can be found at: https://www.epa.gov/pesticide-registration/guidance-efficacy-testing-pesticides-targeting-certain-invertebrate-pests

The Agency acknowledges that the study was conducted prior to June 14, 2022, and for this study at this time, field data on mosquito species representing the three major genera, *Aedes*, *Culex*, and *Anopheles* support a general repellency claim against mosquitoes on the label, and against vectors of WNV, malaria, Western encephaliti, St. Lous encephalitis and Western equine encephalitis viruses, specifically. However, efficacy claims against vectors that may transmit Zika, Chikungunya, and Dengue viruses are not supported by data on product labels. Consequently, field data on those specific vectors are needed to support efficacy claims against those specific vectors on product label.

Future studies will require data on specific vectors according to *Pesticide Product Performance Data Requirements for Products Claiming Efficacy Against Certain Invertebrate Pests*, effective June 14, 2022, to support a general mosquito claim on product labels. Sites should be selected on the basis of predominant required species that differ between field sites as well as the absence of vector-borne pathogen a month prior to efficacy testing. Based on this requirement, the current California sites will qualify as one field site and a second ecologically different site where *Aedes albopictus* or *Aedes aegypti* are predominant must be selected in support of product registration.

REFERENCE

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol. 4:e82 doi:10.1371/journal.pbio.0040082.

Attachment 5

EPA's statistical report on the Kaplan-Meier survival analyses provided in the study report

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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: September 27, 2022

SUBJECT: **Data Analysis of "*FIELD EFFICACY TEST OF AN OIL LEMON EUCALYPTUS AND METHYL NONYL KETONE-BASED REPELLENT SPRAY AGAINST MOSQUITOES*" Study**

PC Code: 040522, 044102
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: N/A
MRID No.: 51707101

DP Barcode: N/A
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: 1245629-80-4, 112-12-9
40 CFR: N/A

FROM: James Nguyen, Mathematical Statistician
Chemistry and Exposure Branch (CEB)
Health Effects Division (7509T)

THRU: David J. Miller, Branch Chief
Chemistry and Exposure Branch (CEB)
Health Effects Division (7509T)

TO: Angela Myer, Biologist
Risk Assessment Branch VIII
Health Effects Division (7509T)

Background

CEB has been asked to review in advance of a planned April 2022 Human Studies Review Board (HSRB) a study report (MIM-006) of the completed field efficacy testing of a mosquito repellent product (an OIL OF LEMON EUCALYPTUS AND METHYL NONYL KETONE-BASED REPELLENT SPRAY AGAINST MOSQUITOES). The study was conducted per a protocol which was reviewed and approved by the HSRB at a HSRB meeting on 20-21 April 2021. That protocol had originally been reviewed by CEB and found to be acceptable. CEB was asked to review the data analysis and statistical methods used by the registrant in study report.

Study Design

The study objective was to determine the Complete Protection Time (CPT) of applying a typical consumer dose of the product to skin of people against mosquito landings (on treated skin areas). The study included the 13 treated subjects and 2 controls for each of two testing sites in Butte County, California (Site 1, a "seasonally flooded riparian forest dominated by heavy canopy and sub-canopy tree cover area" and Site 2, a "native moist grassland habitat around a small lake, large cottonwood trees, large shrubby willows, and irrigated pasture"). Each treated subject would be exposed in the field for 5-minute intervals immediately following product application and then for 5 minutes every 30 minutes until a "first confirmed mosquito landing" occurred or until the end of the testing day if no confirmed landing. The CPT of each treated subject would be the duration from the product application time to a confirmed landing time or the end of testing day (right censored CPT data), rounding down to the nearest half hour.

Statistical Methods and Results of Data Analysis

To analyze the CPT data, the registrant used a Kaplan-Meier survival data analysis to create a Kaplan-Meier survival curve and estimate the median CPT (and 95% CLs) of the product at each of the two testing sites. CEB reviewed the statistical methods used by the registrant and considers them to be appropriate; the results of data analysis and the Kaplan-Meier survival curves in the study report are statistically reasonable.

CEB attempted to replicate the statistical results submitted in the reports using SAS's Kaplan-Meier estimator to create Kaplan-Meier survival curve and estimate the median (and 95% CI) of CPT of the product for each testing site. Table 1 below presents the results of estimated median CPT from CEB data analysis and registrant data analysis. Figures 1 and 2 present the Kaplan-Meier survival curves of CPT data at sites 1 and 2 from CEB's data analysis. (CEB's SAS code and associated SAS outputs are presented in the Appendix to this memorandum.)

Table 1: Results of CEB data analysis and registrant's data analysis

Site	Time	CEB Analysis Results			Registrant's Results			Precision K value[b]
		Est. Median CPT	95% CI		Est. Median CPT	95% CI		
1	minutes	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]
2	minutes (hours)	519 (8.7)	454 (7.6)	584 (9.7)	519 (8.6)	458 (7.6)	NA[a]	0.87

[a] NA = Not available, i.e., the value was greater than the length of testing session because many subjects had not experienced a confirmed landing at the end of the testing day.
[b] equal to Lower 95%CI/mCPT

Figure 1: CEB's Kaplan-Meier survival curve of CPT at site 1

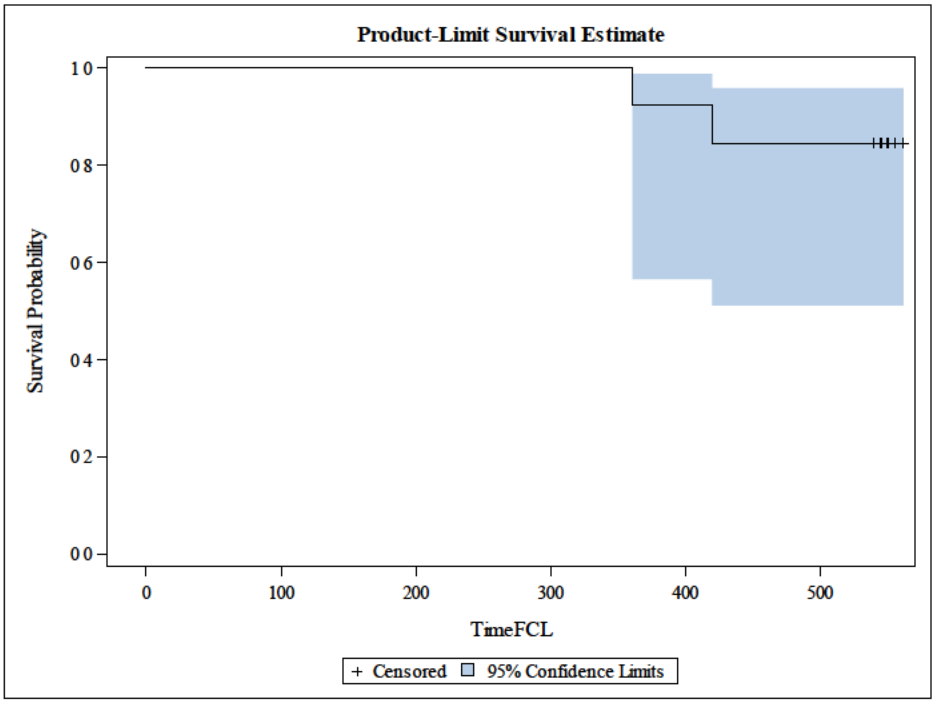
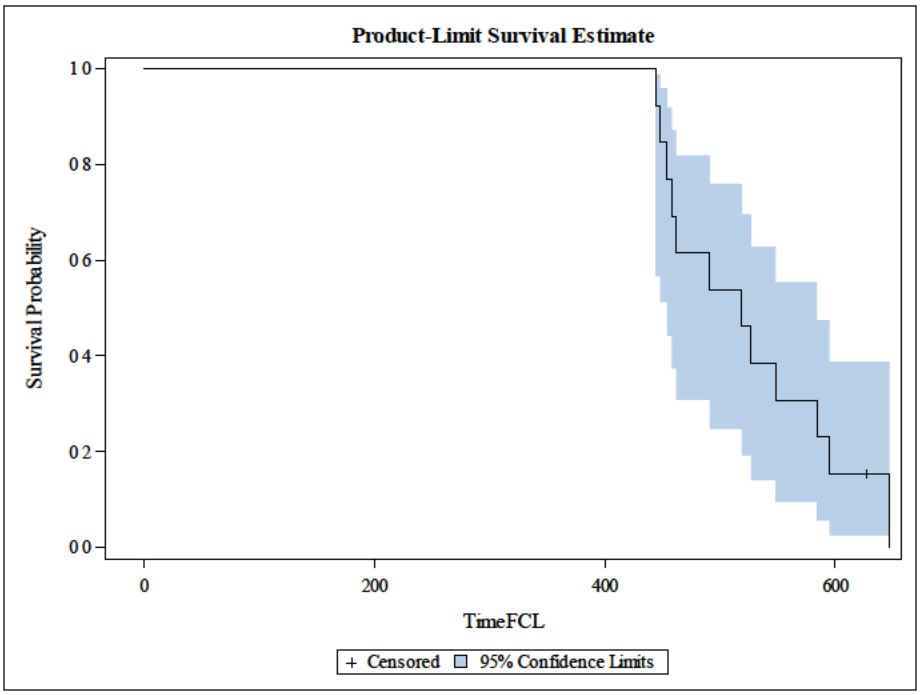


Figure 2: CEB's Kaplan-Meier survival curve of CPT at site 2



CEB comments

From the results of CEB analysis and registrant's data analysis, we note that the *confidence interval* of the estimated median of CPT at site 2 from CEB data analysis did not match those from the registrant results (but the *point estimate* did match).[1] CEB determined that the discrepancy was due to the use of different methods by SAS (used by CEB) and R (used by the registrant in the submission) as each software's default techniques for computing the confidence interval of the estimated median differed. The default statistical technique to compute the confidence intervals of estimated median CPT was *loglog* transformation for SAS statistical software (used by CEB statisticians) while the default technique to compute the confidence intervals in the R statistical software (as used by the registrant) was *log* transformation. While the point estimate and confidence bound results from the registrant and CEB data analyses were similar and did not affect the overall conclusion, CEB statisticians believe it is more appropriate to report the 95% CI that used what we understand to be a more standard *loglog* transformation technique since it was used by CEB to conduct the simulations of sample size vs. power analysis for the study[2,3] and to thus support the 13 subject sample size used here.

Based on the results, the precision K value[4] of the estimated median CPT at site 2 was 0.87. This means that the lower 95% confidence bound of the mCPT is 87% of the mCPT and thus the estimate of the mCPT is reasonably precise[5]. Note that as determined by CEB in a prior simulation, a study with a sample size of 13 subjects would have sufficient power to obtain a precision of the estimated median CPT of $K \geq 0.6$ if the P5MR ≥ 0.5 or sufficient power to obtain a precision of the estimated median CPT of $K \geq 0.7$ if the P5MR ≥ 0.6, where P5MR = the 5th percentile/median of the CPT distribution (P5MR indicates how spread of CPT distribution: a P5MR value close to zero indicates that the CPT distribution is widely spread; a P5MR value of or close to 1 indicates that the CPT distribution is narrow). Risk managers might consider the level of precision of the estimated median CPT and the variation of the CPT distribution in their

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[1] The estimated median CPT was not able to be calculated for Testing Site 1 since more than half the participants did not fail prior the termination of testing. Thus, the estimated median CPT is longer than the testing period.

[2] The Power Analysis was included on pages 86-101 of the submission.

[3] Note: when reviewing the registrant-submitted study protocol prior the April-2021 HSRB meeting, CEB statisticians recommended to delete some lengthy and statistically nonsensical text about statistical methods and replace it with a simple sentence provided by CEB statisticians (i.e., "*The CPT data will be analyzed using Kaplan-Meier Estimator for survival data analysis, and the median CPT (mCPT) and its 95% CI (using log-log transformation is applied to survival function to obtain the confidence interval) will be estimated and presented along with a figure of Kaplan-Meier survival curves*"); however, it appears that the original text (that CEB statisticians recommended to be removed) was deleted (page 94 of the study report indicated the deletion), but the sentence provided by CEB statisticians was not added to the final protocol.

[4] K = the lower bound of 95% CI/estimated median CPT.

[5] A low precision K value would mean that the mCPT would not be known to any great deal of precision and thus may be substantially inaccurate. The sample size simulations performed by CEB as part of the determination that a sample size of 13 is sufficient (see Power Analysis on pages 86-101 of the submission) incorporated the need for sufficient precision/accuracy by using the P5MR as a benchmark which is reflective of the shape of the distribution. Low P5MRs reflect a low ratio between the 5th percentile of the distribution and the mCPT and thus are indicative of large differences (more specifically, high variation) in population protection times across the population for the repellent product; conversely, large P5MRs reflect large ratios between the 5th percentile and mCPT and thus small variation across the population. CEB's power simulations that were used to determine the recommended sample size of 13 used P5MRs of 0.5 and 0.6 which means that the 5th percentile of the population would have a CPT of 50% or 60%, respectively, of the mCPT.

decision-making process since a low P5MR suggests 1) a substantial degree of variation in the protection time across the population and 2) that the mCPT may be quite inaccurate for a substantial fraction of users.

Conclusions

Due to the default computational method in the statistical software R used by the registrant, the 95% confidence interval of the estimated median of Complete Protection Time (CPT) at site 2 from the registrant analysis was slightly larger than that from CEB analysis using the statistical method recommended in the protocol. However, the estimated median of CPT was consistent between the statistical methods, and the overall conclusion was also consistent.

The estimated median (95% CI) of the CPT of site 2 from CEB reanalysis was 519 (454, 584) minutes. The data show that the median of CPT at site 1 was longer than the time duration conducted in the study and was thus not estimable.

APPENDIX

SAS outputs of Quartile Estimates of CPT data

---------- Site=1 ----------

The LIFETEST Procedure

Quartile Estimates

Percent	Point Estimate	95% Confidence Interval Transform	[Lower	Upper)
75	.	LOGLOG	.	.
50	.	LOGLOG	.	.
25	.	LOGLOG	361.000	.

==========

The SAS System

---------- Site=2 ----------

The LIFETEST Procedure

Quartile Estimates

Percent	Point Estimate	95% Confidence Interval Transform	[Lower	Upper)
75	584.000	LOGLOG	491.000	647.000
50	519.000	LOGLOG	454.000	584.000
25	458.000	LOGLOG	444.000	519.000

SAS Code

```
*=======================================================*
* Programmer: James Nguyen, USEPA                       *
*                                                       *
* Project: Mosquito Studies                             *
*                                                       *
* Study: MIM-006                                        *
*                                                       *
* Date: January 2022                                    *
*=======================================================*;
options formdlim="=" nodate nonumber ls=100 ps=100;

data MIM_006;
      input Site SubID TimeFCL censor;
      datalines;
1      104    361 0
1      103    562 1
1      72     562 1
1      11     556 1
1      30     420 0
1      4      556 1
1      76     551 1
1      23     550 1
1      150    551 1
1      55     546 1
```

```
1       70      546 1
1       91      545 1
1       63      540 1
2       7       584 0
2       33      527 0
2       62      647 0
2       69      491 0
2       73      548 0
2       98      519 0
2       122     462 0
2       123     458 0
2       132     444 0
2       167     595 0
2       178     454 0
2       181     448 0
2       193     627 1
;
run;

*==> EPA's model;
ods graphics on;
ods select Quartiles SurvivalPlot;
Proc lifetest data = MIM_006 plots=survival (cl) CONFTYPE=loglog;
       by site;
       Time TimeFCL*censor(1);
run;

*==> EPA attempted to replicate the registrant's analysis;
ods select Quartiles SurvivalPlot ;
Proc lifetest data = MIM_006 plots=survival (cl) CONFTYPE=log;
       by site;
       Time TimeFCL*censor(1);
run;
```

Attachment 6

Responsiveness to EPA and HSRB Science Comments on Draft Protocol

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

	EPA Recommendations	Action Taken by Study Sponsor
1.	ICF should be revised concerning covering treated skin between exposure periods. Subjects should not be instructed to cover treated skin between exposure periods since this practice is likely to disturb the repellent.	The ICF states (Appendix 1), "If you are a treated subject and more than one mosquito lands on your repellent applied treated skin during one of the five-minute periods, or in two of three consecutive exposure periods (that is, 30 or 60 minutes apart), you should cover the skin and not expose it again." The covering of skin as described here occurs at FCL, and no more exposure periods followed this covering. Thus, this change is acceptable.
2.	Revise item #13 in §3.3.2, Exclusion Criteria (p. 18) that reads, "Has participated in another field repellency test day of this study in the previous 72 hours." The limit of 72 hours between testing days should not apply to the proposed study since each subject will not participate in more than one field test. This sentence should be removed from the protocol	The sentence was removed from the protocol (Appendix 1, p. 142 of 362).
3.	Assigning treatments to a treated subject's non-dominant arm is acceptable. However, non-dominant limb is not applicable to lower legs. Therefore, applications to lower legs should be randomly applied, and randomization process should be described.	The protocol was not revised to specify a randomization process for applications to lower legs (Appendix 1). However, measurements of legs were not included in the final study, so this comment is not applicable.
4.	The following paragraph, extracted from §4.8.4 on p. 31, needs revision: "Treatments may be applied at the field site or shortly before travel from the lab to the field site. Because field testing conditions are likely to be more fatiguing to subjects than conditions in the laboratory prior to departure to the field or conditions during transport to the field, if the Test Material is anticipated to remain effective for many hours, applications may be made several hours in advance of the first exposure period to reduce the probability of subjects needing to withdraw due to exhaustion before receiving a confirmed landing. Exposures of the second treated limb will begin at the next exposure period, in order to produce a replacement estimate of Complete Protection Time." It is not recommended to prolong delaying first field exposure unless the registrant established criterion for ensuring CPT recorded after prolonged first exposure delay is accurate and unlikely to have occurred at an earlier timepoint. In addition, "exposures of "the second treated limb" does not apply to this experimental design, so the statement should be deleted.	This paragraph was replaced with the text below to justify that CPT is unlikely to occur at an earlier timepoint: "Research conditions may vary within and among field sites. Depending on conditions on the day of the test, limb washing, receiving head nets and latex-free gloves, and repellent applications to treated subjects may be completed before travel to the field site, or at the field site. The Study Director may allow for repellent applications before traveling to a field site in order to support protocol execution or subject safety. The delay between application and exposure may range from about 15 minutes to up to about 2.5 hours. Such a delay is feasible for the test material because other EPA-registered 7.75% undecanone products are labeled for 4.5 hours of protection against *Aedes aegypti* mosquitoes, and OLE products show similar repellency to DEET when tested at the same concentrations (e.g., Goodyer et al. 2020[8]), likewise suggesting more than 2.5 hours of protection. Accordingly, the test material is not likely to

		fail within this delayed exposure timeframe. To guard against inflating protection times, however, in the unlikely event that a treated subject receives a confirming landing in the first or second exposure after the exposure delay, that subject will be excluded from the study and replaced with an alternate." The justification for rationalizing why CPT is unlikely to occur before first exposure is acceptable. However, removing and replacing a treated subjects that receive a confirming landing in the first or second exposure after the exposure delay is unacceptable. This removal of subject data would provide an inaccurate representation of CPT recorded after first exposure delay. **However, this situation did not occur during repellency testing (Note to File p. 276 of 380 in MRID 519127-01). Therefore, the situation is not applicable to the study**.
5.	The study protocol should indicate that the following CPT data from treated subjects will be reported in tabulated form to include: 1) time (hours:minutes) when product was applied per subject; 2) start time of each field exposure per subject; 3) length of time (hours:minutes) between product application and first exposure per subject; 4) time when first landing occurred per subject; 5) time when second (confirmatory) landing occurred per subject; and 6) total number of hours:minutes from time of product application to time of first confirmed landing or CPT time-point per subject.	It was indicated that these data will be reported in tabulated form (Appendix 1, p. 63 of 362).
6.	The study protocol should indicate that data from control subjects will be reported in tabulated form to include: 1) start time (hours:minutes) of each field exposure; 2) time and number of mosquitoes landing on each control; 3) total number of mosquitoes landing in five minutes per control; 4) total time (minutes) for five landings to occur (when five landings happen in less than five minutes).	It was indicated that these data will be reported in tabulated form (Appendix 1, p. 63 of 362). **However, data were not summarized and presented in tables in study report (MRID 517071-01). Data were reported from raw data collection sheets. Start time of each exposure period is not reported in raw data sheets; only number of exposure periods and total landing mosquitoes per exposure period are reported. Time of each landing is not reported. Time to reach five landings is not reported**.
7.	Replace "LIBes" with "Landings" on the heading of raw data collection sheets for Controls. Furthermore, the purpose of the metric "repulsion" = 0 at the bottom of the data collection sheets for control is unclear and should be removed.	The raw data sheets were revised as recommended (Appendix 1, p. 70 of 362).
8.	Add to exclusion criteria in §3.3.2 that those subjects that are proved to be unattractive to mosquitoes will be excluded from further participation in repellency testing.	This recommendation was implemented (p. 48 of 362 I n MRID 517071-01) (See Appendix 1 in this review).

9.	Amend §4.7.2 on p. 29 to propose that only mosquitoes that land on exposed skin of both control and treated subjects will be collected and that those landing on protective clothing of subjects will not be collected. It is recommended that the same standard for collecting mosquitoes that land only on exposed skin of treated and control subjects be maintained. Adding a new metric to measure background foraging activity is beyond the objective of monitoring landing pressure by control subjects. Furthermore, trying to collect all landings might potentially distract subjects and staff members from paying closer attention to mosquitoes landing on exposed skin, which could potentially result in inaccurate measures of CPT and landing pressure.	It is proposed in section 4.7.3 (Appendix 1, p. 58 of 362) that, "Mosquitoes will be collected from two distinct types of landing sites: on untreated subjects' skin and on treated subjects' skin." This aligns with the recommendation and is acceptable.
10.	Amend the rationale/justification for sample size and remove last paragraph on pp. 20 -21. Justification for sample size is based on EPA simulation for determination of sample size, provided in Protocol Appendix 8. A sample size of 13 would be adequate to ensure that the study includes enough subjects to return reliable results without including more subjects than necessary.	The statements in the 1st paragraph of section 4.1 of the protocol regarding sample size determinations from an EPA power analysis are acceptable (Appendix 1, p. 51 of 362) (See Appendix 1 in this review). **However, the last paragraph in section 4.1; p. 52 of 362 in MRID 517071-01) was not removed from the protocol as recommended. This last paragraph, as well as the second paragraph in 4.1, includes sample size statements regarding historical guidelines, standards, and other assertions that are extraneous to the proposed study**. **Although these two paragraphs have not been removed despite past recommendations, including a comment made in the Science Review of a Registrant's response to EPA 75-Day deficiency letter (10-28-2020), sample size for repellency testing was determined as recommended.**
11.	Specify that data from withdrawn subjects who are not replaced should be counted as censored data for statistical analysis. A sentence in the protocol (Appendix 1, p. 63 of 362) states, "Data from withdrawn or removed subjects who are not replaced with alternates will be treated as censored data for statistical analysis."	A sentence in the protocol (Appendix 1, p. 63 of 362) states, "Data from withdrawn or removed subjects who are not replaced with alternates will be treated as censored data for statistical analysis." This recommendation was adopted.
12.	Remove the statement "attempted to bite" on line 1584, §4.8.4 on p. 32, and replace it with "landing".	The study sponsor edited the statement to state, "**Each subject will report the number of mosquitoes that land on their own treated skin during that five-minute period when asked by a researcher** who will note it on a data sheet." This amendment aligns with the general recommendation (made in the past) of replacing iterations of 'bite' with iterations of 'landing' throughout the protocol. However, the use of the term 'biting' was still used regarding control subject data in protocol sections 4.2 (p. 52 of 362). 4.7.4 (p. 59 of 362), and 4.8.4 (p. 62 of 362). Also, the phrase "the EPA-recommended substitution for direct quantification of biting pressure" in section 4.2

		(p. 52 of 362) is extraneous to the study protocol and should have been removed. **However, landings, not mosquito bites, were used for assessment of subject attractiveness to mosquitoes, aspirator practice test, and as efficacy endpoint in the field.**
13.	Multiple technicians will conduct product applications to minimize time of application and make time of application more consistent among subjects, however variability in application can be attributed to multiple technicians applying the required volume per subject. It is recommended to ensure consistency in the application method.	The protocol describes the use of three different researcher in the product application procedures (Appendix 1, p. 57 of 362). Details were not provided on if/how the product was applied in a consistent manner on all treated subjects. **Note to file on p. 258 of 362 and on p. 18 of 362 in MRID 517071-01 (study report) describes roles of technicians applying and supervising applications to ensure consistency.**
14.	There should be a plan for skipping exposures due to bad weather. In addition, it is recommended to check weather conditions in advance to test day to avoid scheduling testing under bad weather.	The protocol states (Appendix 1, p. 58 of 362), "Every attempt will be made to schedule field test dates to coincide with weather conducive to study conduct. There is a small chance that weather conditions such as rain, high winds, or unexpected cold will require that the test be canceled or rescheduled. Researchers will inform subjects of any scheduling changes." This change is acceptable.
15.	Trap type should be added to description of methods for site monitoring.	The trap type is specified in the protocol (Appendix 1, p. 39 of 362).
16.	In addition to specifying how the Study Director will coordinate with local agencies, please consider adding (as appendices) the SOPs for the mosquito control districts of the testing locations/sites to further clarify the methods of site surveillance.	The protocol specifies that (Appendix 1, p. 58 of 362), "CLBR will check with local disease control officials for reported cases, and with local vector control if they are assaying mosquito populations for Anopheles-vectored disease organisms." The suggested appendices were not included in the protocol**. However, the information is provided in Note to File on p. 309 of MRID 519127-01.**

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

	HSRB Recommendations	Action Taken by Study Sponsor
1.	Monitor field sites prior to testing to confirm the presence and enough of a diversity of mosquito species.	The protocol includes statements regarding mosquito species show below. **Appendix 1, p. 58 of 362:** "Locations may be in or adjacent to the Central Valley of California or southern California (depending on season) or at another geographic location depending on the availability of wild mosquito populations of suitable activity and species composition (see Table 1). Test site information will be furnished to EPA on request once it is clear when testing will be permitted, since season influences the availability of test arthropods on both regional and local scales." **Appendix 1, p. 58 of 362**: "Sites will be chosen on the basis of having present the three genera, *Aedes, Culex,* and *Anopheles*, as determined by advance surveillance using mosquito traps. We will test repellency against those genera, likely including some of the species listed in Table 1." **Note: Table 1 lists multiple sites across various states with a wide variety of mosquito species. Two sites adjacent to each other in Glenn and Butte counties, California, were selected for the study, on the basis that species from the three genera were present, and mosquito borne pathogens were not detected a month before field testing (with only one exception for one case of WNV detected on Culex tarsalis collected on August 10, 2021 (48 days prior to efficacy testing at Site 1).**
2.	Add additional information to the study protocol clarifying how the application of the material "…dispensed from tuberculin (1 ml) syringes by researchers wearing surgical gloves who apply it to treated subjects by spreading evenly over the area to be treated using one finger in a light rubbing motion" represents (or is consistent with) the end-user application of pump spray. EPA noted this method of application is consistent with the dosage and distribution on skin with the bag-in-valve applicator of the product. This justification for using the study application should be explicitly stated in the protocol.	There was no justification or clarification provided in the protocol to address this comment. **However, this comment was addressed by the Agency, and explanation for applying standard dose, derived from dosimetry studies, is provided on p. 53 in MRID 517071-01 (p. 24 §4.6 of MIM-006 protocol) (see Appendix 1 in this review)**
3.	The protocol states: "Given that we consider end user safety essential for the fundamental ethics of conducting this study, the Sponsor proposes generating and providing dermal absorption data for MNK as applied in the form of end product we will be testing, if feasible, or of the active ingredient. No	This text regarding a dermal absorption study was not removed from the protocol and can be found on p. 117 of 362 in MRID 517071-01 and on p.123 of 380 in MRID 519127-01 (p. 88 of MIM-006 protocol) (see Appendix 1 in this review). **The old text remaining in the protocol is inconsistent with updated statement that endpoint for toxicological**

	subject recruitment will begin until the dermal absorption study is completed and reviewed by EPA, and the study shows that the dermal absorption rate of MNK is such that the estimated MOE will be at an acceptable level." Based on information provided by the EPA, it appears that a dermal absorption study will not be occurring. The information about this proposed study should be removed from the protocol.	**risk is based on skin irritation data (p. 145 of 362 in MRID 517071-01; p. 116 of MIM-006 protocol) (see Appendix 1).**
4.	Discuss how the recruitment and randomization strategy will work if the testing locations test sites are geographically remote Page 14, Sampling Frame in Section 3.2 of the protocol). This information could also be included or referenced earlier in the protocol to further avoid confusion regarding recruitment strategy and study conduct across disparate geographical locations.	Details regarding the recruitment strategy in scenarios of testing sites being geographically remote were not provided in Section 3.2 (Appendix 1, pp. 47-48 of 362). Section 4.7 specifies that pools of approximately 30 separate candidates for each field trial would be recruited if field trials are geographically remote. Individual candidates were proposed to be chosen, while alternating sexes, for follow up consenting interview scheduling. The consenting process was to be continued until we 20 enrolled subjects were obtained, 10 of each gender. Subjects were to be assigned to the first trial by randomly choosing one of the two genders, randomly choosing 5 subjects from that gender, and then repeating the process for the other gender. This was providing a list of 10 subjects (5 males and 5 females). The remaining 10 subjects were to be assigned to the second trial. **However, these details are no longer relevant since the final review was done in two sites that were geographically proximate.**
5.	Section 1.2 of the protocol makes a number of factual assertions (e.g., CDC notes substantial consumer interest in new and effective insect repellant products) but provides no citations for these statements. This should be corrected to be consistent with other parts of the protocol where analogous statements are backed up with citations.	The statement regarding 'substantial consumer interest' was removed from section 1.2 of the protocol (Appendix 1, p. 35 of 362). However, no citation was provided for the claim that DEET-based repellent may produce mild to serious side effects (Appendix 1, p. 35 of 362), nor for claims that the CDC estimate that about 4 out of 5 people who are infected with WNV do not develop any type of illness made in the protocol (Appendix 1, p. 39 of 362) and the ICF (Appendix 1, p. 173 of 362).
6.	It is stated that "Alternate subjects may return later to replace subjects that initiate testing but withdraw before useful data are generated." It is unclear if this is feasible for testing done in remote locations. If this procedure is used, please indicate how alternate subjects be notified that they are serving as a replacement.	The study was not done in remote locations. The updated protocol (Appendix 1, p. 51 of 362) states, "Alternate subjects will remain at the test site so that alternate subjects may replace subjects that initiate testing but withdraw, are removed, or are excluded before they receive a confirmed landing or the Study Director stops the test."
7.	Clarify that the "number of mosquitoes that attempted to bite their own treated skin during that five-minute period" is the actually the number of landings on treated skin in that five-minute period. Additionally, the note "in a typical test of a reasonably effective repellent, dozens of '0' landing values will be recorded for each '1' or '2'" is unclear (line 1587). The recording of a '1'	The original text described in the comment came from Section 4.8.4. The corresponding paragraph in the updated protocol states, "**Each subject will report the number of mosquitoes that land on their own treated skin during that five-minute period when asked by a researcher who will note it on a data sheet.** For perspective, note that in a typical test of a reasonably effective repellent, during

	or '2' is unclear; please clarify which will be recorded and under what circumstance.	most exposure periods, potentially for the first several hours, there are no landings on treated subjects, and they do not experience close contact with mosquitoes. The probability of eventual direct contact, if any occurs before the cessation of exposure due to darkness or subject withdrawal, removal, or exclusion, increases at a slow rate."
8.	Clarify that if at least 50% of subjects have data that are right-censored, the mCPT will not be computed. 'm' could be clarified to be 'median'.	The study sponsor included this statement in the protocol (Appendix 1, p. 62 of 362): "If 50% or more of the subjects' data are right censored, mCPT will not be reported." The abbreviation 'mPCT' was clarified to refer to a median (Appendix 1, p. 51 of 362)."
9.	Clarification should be added that the analyses will be conducted for each of the field studies separately.	The study sponsor followed this recommendation (Appendix 1, p. 64 of 362).
10.	Clarify how long of a pause will be considered for a second landing to be confirming for the first landing.	See comment #2 in Table 1 above.

Attachment 7
Product Label

Mimikai™ Lilly Pilly Repellent Master Label

EPA Reg. No. 93616-*

Alternate names: Lilly Pilly, Lilly Pilly Repellent, Mimikai Repellent, Mimikai Insect Repellent; Mimikai Mosquito Repellent; Mimikai Tick Repellent

MASTER LABEL includes:

Sublabel A: Mimikai™ Lilly Pilly Insect Repellent labeling for liquid pump spray packaging

Sublabel B: Mimikai™ Lilly Pilly Insect Repellent labeling for pressurized (bag-on-valve) packaging

Optional Label Claims

[Bracketed Text] = Optional language
{Braced Text} = Administrative, will not be on labels

Mimikai, Inc.
c/o Bergeson & Campbell, P.C.
2200 Pennsylvania Ave., NW, Suite 100
Washington DC 20037

{Sublabel A: Liquid Pump Spray Packaging}

Mimikai™ Lilly Pilly Insect Repellent

KEEP OUT OF REACH OF CHILDREN

[See directions for use on the side of container[s].]

Active Ingredients:

Oil of Lemon Eucalyptus* (CAS No. 1245629-80-4)	11.00%
2-Undecanone** (CAS No. 112-12-9)	7.75%
Other Ingredients	81.25%
Total	100.00%

*Approx. 65% *p*-menthane-3,8-diol

{**U.S. Pat. No. XXXXXXXX; Alternative Patent info: Patent Pending} NET X fl oz (XXmL)
EPA Reg. No. 93616-*
EPA Est. No. [as indicated on container]

READ ENTIRE LABEL BEFORE EACH USE

First Aid
Call a poison control center or doctor [or healthcare professional] for treatment advice. Have the product container or label with you when calling a poison control center (1-800-222-1222) or doctor [or healthcare professional] or going for treatment.

PHYSICAL OR CHEMICAL HAZARDS
COMBUSTIBLE. Keep away from heat or open flame.

DIRECTIONS FOR USE

It is a violation of federal law to use this product in a manner inconsistent with its labeling.
Read and follow all directions and precautions on this product label.

To repel [insects] [mosquitos] [and] [ticks], apply to all areas of exposed skin and clothing.
For best results, spread evenly with hand to moisten all exposed skin.
Do not spray directly on face.
To apply to face, dispense on palm of hand and spread on face and neck.
Do not apply over cuts, wounds, irritated or sunburned skin.

STORAGE AND DISPOSAL:
Store the product in a cool, dry place, out of reach of children. If empty: nonrefillable container. Place in rubbish or please recycle. Call your local solid waste agency [or 1-800-CLEANUP] for disposal instructions. Never place unused product down any indoor or outdoor drain.

[Distributed by:]
MIMIKAI INC.
1564 Green Valley Road, Danville, CA 94526
customerservice@mimikai.com

{Sublabel B: Pressurized (bag-on-valve) Packaging}

Mimikai™ Lilly Pilly Insect Repellent

KEEP OUT OF REACH OF CHILDREN

[See directions for use on the side of the container[s].]

Active Ingredients:

Oil of Lemon Eucalyptus* (CAS No. 1245629-80-4)	11.00%
2-Undecanone** (CAS No. 112-12-9)	7.75%
Other Ingredients	81.25%
Total	100.00%

*Approx. 65% *p*-menthane-3,8-diol

{**U.S. Pat. No. XXXXXXXX; Alternative Patent info: Patent Pending} NET X fl oz (XXmL)
EPA Reg. No. 93616-*
EPA Est. No.[as indicated on container]

READ ENTIRE LABEL BEFORE EACH USE

First Aid

Call a poison control center or doctor [or healthcare professional] for treatment advice. Have the product container or label with you when calling a poison control center (1-800-222-1222) or doctor [or healthcare professional] or going for treatment.

PHYSICAL OR CHEMICAL HAZARDS

COMBUSTIBLE. Keep away from heat or open flame.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.
Read and follow all directions and precautions on this product label.

To repel [insects] [mosquitos] [and] [ticks], apply to all areas of exposed skin and clothing.
For best results, spread evenly with hand to moisten all exposed skin.
Do not spray directly on face.
To apply to face, dispense on palm of hand and spread on face and neck.
Do not apply over cuts, wounds, irritated or sunburned skin.

STORAGE AND DISPOSAL:

Store the product in a cool, dry place, out of reach of children. If empty: nonrefillable container. Place in rubbish or please recycle. Call your local solid waste agency [or 1800 CLEANUP] for disposal instructions. Never place unused product down any indoor or outdoor drain.

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{Mimikai Lilly Pilly Repellent Optional Marketing Claims}

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{General}

[Mimikai™ believes in nature's genius and a way that lets us live in harmony, even with the bugs that bug us. Mimikai™ has created products that are effective and beautifully balanced.] [Mimikai's patented technology is a university collaboration, backed by science.]

{Fragrance}

[Mild] Scented Body Mist
A scent you will love
Clean fragrance
Clean scent
Fragrance made with essential oils
Fragranced with essential oils: [Frankincense], [Orange Flower], [Carrot Seed]
No synthetic fragrance
Smells great
Synthetic Fragrance Free

{Duration/ Repels}

[4] [Four] hours proven protection [against ticks]
[8] [Eight] hours proven protection against mosquitoes
[Also] Repels [annoying] mosquitoes
[Also] Repels [annoying] ticks
[Gives] protection for the entire family against ticks and mosquitoes while camping, hiking, and enjoying other outdoor activities
[Instant] [Complete] [mosquito] [tick] [defense] [protection]
[Protect(s)(ion) against] [Repels] (the) mosquitoes that may [transmit] [carry] [West Nile Virus] [Malaria] [St. Louis Encephalitis] [for up to (eight) (8) hours]
[Protect(s)(ion) against] [Repels] [deer] [the] ticks that may[transmit] [carry] [Lyme Disease] [Rocky Mountain Spotted Fever] [Tick Paralysis] [Encephalitis] [Ehrlichiosis] [Powassan Virus] [for up to (four) (4 hours]
[Protect[s]] [Defend] [Shield] your family from mosquito[s] [bites]
[Protect[s]] [Defend] [Shield] your family from tick[s] [bites]
[Provides] Protection for the whole family
[Strong] [Maximum] [High quality] [Reliable] [Dependable] [Long lasting] [Effective] protection [from ticks] [against ticks] [from mosquitoes] [against mosquitoes]
Defends your [whole] family against mosquito bites
Defends your [whole] family against tick bites
Delivers up to [4] [four] hours of repellency of ticks
Delivers up to [8] [eight] hours of repellency of mosquitoes
Effective, dependable mosquito protection
Effective, dependable mosquito repellency for up to eight hours
Effective, dependable tick protection
Effective, dependable tick repellency for up to four hours
Family Care Insect Repellent Spray
Keeps mosquitos away from you and your family
Keeps ticks away from you and your family
Long lasting mosquito protection
Long lasting protection from mosquito
Long lasting protection from ticks
Long lasting tick protection
Mosquito protection

Mosquitoes hate [Mimikai™] plant extract repellent.
Proven protection from ticks/mosquitoes
Provides protection from mosquitoes and ticks
Provides protection from Mosquitoes and Ticks [including mosquitos that cause West Nile Virus, and ticks that cause Lyme disease, Rocky Mountain Spotted Fever, Tick paralysis, Encephalitis, Ehrlichiosis, Powassan Virus]
Repel don't kill
Repels [lone star ticks] [deer ticks] [and] [brown dog ticks] [for more than 4 hours]
Repels [mosquitoes and ticks] [ticks] [mosquito] [so you can] [to help you] enjoy life outdoors.
Repels mosquitoes and ticks [from treated skin and clothing]
Repels mosquitoes for up to [8] [eight] hours
Repels mosquitos [including those] [which] [that] may [transmit] [carry] [spread] [West Nile Virus] [Malaria] [and] [St. Louis Encephalitis] [for up to eight hours]
Repels ticks [including those] [which] [that] may [transmit] [carry] [spread] Lyme Disease, Rocky Mountain Spotted Fever, Tick Paralysis, Encephalitis, Ehrlichiosis, Powassan Virus] [for up to four hours]
Repels ticks and mosquitoes [for up to 4 (four) hours] [from treated skin and clothing]
Repels ticks for up to [4] [four] hours
Repels up to [4] [four] hours against ticks
Repels up to [8] [eight] hours against mosquitoes
Tick protection

{Actives}
[Contains] (made with) 2-Undecanone
[Contains] (made with) Oil of Lemon Eucalyptus
A plant-based formula.
Active Ingredient 2-Undecanone
Active ingredient derived from [the leaves of] Lemon Eucalyptus tree
Active Ingredient derived from plant extracts
Active Ingredient extract of Lemon Eucalyptus
Contains 2-Undecanone
Contains Lemon Eucalyptus Oil
Contains plant extracts [of Lemon Eucalyptus]
Duel Active Technology
Exclusive Dual Active Technology
Exclusive Mimikai™ Technology
Naturally-based active ingredient.
Plant-based Active Ingredient
Powered by 2-Undecanone
Powered by Citriodiol® OLE Nature's Repellent (logo)
Powered by Mimikai™
Unique plant-based active combination

{Other}
[Citriodiol®] [The active ingredient] has a carbon negative footprint [because it is made directly from the oil of Eucalyptus citriodora trees].
[Simple] [Smooth] [Accurate] [Effortless]
180+ Sprays
Based on Biomimicry
Beat the bite
Botanically based active ingredients

Clean
Developed at [NC State] [North Carolina State University] [NCSU], trusted in the field.
Developed in a lab, trusted in the field.
Easy application
Easy to apply
Eucalyptus citriodora trees [sequester] [absorb] [large amounts of] carbon [from the atmosphere] which [benefits the planet] [and] make Citriodiol® [the active ingredient] [in this product] carbon negative.
Goes on easy
Great for the whole family
Make peace with mosquitos
Make peace with ticks
Mosquitoes hate [product name] plant extract repellent as much as chemical repellents.
Mosquito Protection 360°
New formula {to be removed 6 months after first shipment}
No mess [and] [convenient]
No rubbing required
Non-drying
Non-toxic and not an irritant when used as directed
Not sticky or greasy
Plant Derived
Plant-based active ingredient
Powered by Plants
Quick and even coverage
Scientifically Proven Effective
Suitable for application to all ages
Technology 5 years in the making
Tick Protection 360°
Unique plant-based active ingredient
We Love, Mosquitoes Hate
Won't harm your gear
Works well on the whole family

{Claims specific to Sublabel B:}
BOV Aerosol technology [no propellants]
Contains no [propellants] [propellant chemicals]
Continuous spray
Easy continuous spray application
Fine mist spray
NEW {valid for 6 months from date of first product shipment with pressurized packaging}
No pumping required
Not an Aerosol
Propelled with air not chemicals
Spray in any direction at any angle
Sprays at any angle
Sprays more evenly and consistently
Sprays upside down