FIELD EFFICACY TEST OF A PMD AND LEMONGRASS OIL-BASED REPELLENT 'NO MAS' AGAINST MOSQUITOES

Data Requirement: OPPTS 810.3700 US EPA

Author: Scott P. Carroll, Ph.D.

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Performing Laboratory: Carroll-Loye Biological Research
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Davis, CA 95616

Laboratory Project ID: NO MAS 003

Standards Applied: U. S. EPA Good Laboratory Practice Regulations (40 CFR 160); 40 CFR 26 subparts K, L and M; FIFRA § 12(a)(2)(P); California State EPA Department of Pesticide Regulation study monitoring (California Code of Regulations Title 3, Section 6710).

Statement of No Data Confidentiality Claims

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d) (1) (A), (B), or (C).

Company: Del Cielo

Company Agent: Sam Darling

Title: Owner/Operator and Director

Date: August 14, 2011

Signature: 

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Compliance for the final Carroll-Loye Biological Research Report for Sam Darling entitled: FIELD EFFICACY TEST OF PMD AND LEMONGRASS OIL-BASED REPELLENT 'NO MAS' AGAINST MOSQUITOES

This study meets the requirements of U.S. EPA Good Laboratory Practice Regulations; Pesticide Programs (40 CFR 160).

12 August 2011

Scott P. Carroll, Ph.D. Date
Study Director

August 14, 2011

Sponsor and Study Submitter Date
Sam Darling
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QUALITY ASSURANCE STATEMENT

Carroll-Loye Biological Research, GLP study for Del Cielo, Protocol Number NO MAS 003, Entitled "Field Efficacy Test of PMD and Lemongrass Oil-Based Repellent "NO MAS" Against Mosquitoes" was inspected during various stages of the study. The data presented in the final report represent an accurate record of the raw data and the experimental findings. Records of results of facility inspections, study and final report audits are kept on file at Sierra Research Laboratories. The phases of the study inspected, dates and the findings were reported to the study director and management is as follows:

Phase Inspected	Date	Description
Protocol Review	22 July 2011	Protocol Review
In-Life Inspection and Audit	23 July 2011	Test Day O – Test Substance Application and Efficacy Evaluations in the Field – Data Collection
Raw Data Audit	08-09 August 2011	Audit of Raw Data
Final Report Audit	10-12 August 2011	Final Report Audit and QAU Statement

William A. Donahue Jr. August 12, 2011

William A. Donahue, Jr., Ph.D. **Date**
Quality Assurance Unit

Carroll-Loye Biological Research Personnel for Study NO MAS 003:

Scott Carroll, Ph.D.
Study Director – Oversight of the study, data analysis and interpretation, report authoring.

William K. Johnson, M.S.
Laboratory Director – Managing the application and observation technicians, preparing Test Materials for application, application of test materials, data recording and entry.

Shawn B. King, M.S.
Director of Operations – Managing and assisting other staff, assisting with logistics, communicating with QAU, report editing and production.

Jenella Loye, Ph.D
Acting Field Manager, Chief Financial Officer and Office Manager – Managing field study logistics

Trevor M. Fowles, B.S.; Ralph Washington Jr., B.S.; Crystal Perreira, B.S., Susan Yonts, M.A.
Application and Observation Technician – Application of test materials, guiding and observing subjects during field exposures, observing LIBe events and reporting to the Laboratory Director.

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Quality Assurance Unit.

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Study Objective and Information Summary

The objective of the study was to determine the Complete Protection Time of No Mas repellent, when applied at a typical consumer dose, against wild populations of the mosquitoes including but not limited to species of the genera *Culex*, *Anopheles*, and *Aedes*, to provide data under the Data-Call-In requirements (EPA Reg. No. 3126-LRN0) of United States Environmental Protection Agency Guideline OPPTS 810.3700.

This mosquito repellent study was sponsored by Mr. Sam Darling of the Del Cielo foundation (Salt Spring Island, British Columbia, Canada), to provide efficacy data in support of a pesticide registration application to the United States Environmental Protection Agency. The test material, based on the active ingredients *p*-menthane-3,8-diol (PMD) and lemongrass oil (citral), is No Mas, a topical lotion repellent.

The study Protocol was reviewed and approved by Independent Investigational Review Board, Inc., and reviewed favorably by the US Environmental Protection Agency and its Human Studies Review Board, and by the California Environmental Protection Agency.

We conducted a dosimetry study in advance of efficacy testing in order to estimate typical consumer dosing behavior. The resulting average dosing rates, of 1.20 $\mu l/cm^2$ on arms and 1.04 $\mu l/cm^2$ on legs, were then employed as the rates for the subjects in the field efficacy study. These results were also used to estimate the Margin of Exposure (MOE) relative to acute dermal toxicity limit dose in No Mas (>5000 mg/kg, see toxicity test reports), resulting in Margin of Exposure (MOE) values of >583 (arms) and >287 (legs) for the repellent. We judged these margins to be sufficiently great to justify dermal exposure of the subjects to the test materials during efficacy testing.

Efficacy was tested in two different habitats under expected environmental conditions for consumers using the product. In each habitat, ten human subjects (five female, five male) each exposed a No Mas repellent-treated limb to mosquitoes for one minute every 15 minutes, until product failure or cessation of the test. Simultaneously, one male and one female untreated

control subject exposed arms or legs in the same manner, in order to assess mosquito biting pressure. Both controls experienced landings within one minute of exposure throughout each test day, indicating that mosquitoes were suitably active for the efficacy study.

Under field conditions, the repellent provided substantial and prolonged protection against the mosquito species (*Aedes melanimon, Ae. vexans, Ae. nigromaculis, Culex tarsalis,* and *Anopheles freeborni*). Mean Complete Protection Time (CPT) for No Mas was 9.8 hours at Site 1 and 10.1 hours at Site 2.

In summary, No Mas repellent at 16% PMD and 2% lemongrass oil concentrations provided prolonged periods of Complete Protection against several species of mosquitoes, including species significant to public health.

Protocol References:

- Carroll-Loye protocol ID number and title: NO MAS 003, 'Field Efficacy test of PMD and Lemongrass Oil-Based repellent 'No Mas' Against Mosquitoes.'
- IRB: Independent Investigational Review Board Inc., Plantation, FL.
- IRB Approval date for protocol/Informed Consent Form: 16 Nov 2010.
- Human Studies Review Board review date for protocol: 27 Oct 2010.
- California Environmental Protection Agency approval: 21 Mar 2011.
- Deviations from the protocol and their consequences are given in Appendix 7.

1) Test Material Table 1: No Mas Test Material

Test Material name (Active Ingredient conc.)	No Mas (p-menthane-3,8-diol 16%, lemongrass oil 2%)
Manufacturer	Sam Darling (Del Cielo)
Lot Number/Batch ID	NO MAS repellent #030
Manufacturing Standards Applied	Good Manufacturing Practice standards, with records available to EPA.
Transport	Commercial Courier, express, insulated container
Chain of Custody	Documented
Specific gravity	0.9524
Delivery system	Lotion
Active ingredient(s) (%)	p-menthane-3,8-diol 16%, lemongrass oil 2%
Inert ingredients	Proprietary, available to US EPA
Stability	Stable
Storage conditions specified	Cool dry place away from flame
Storage conditions applied	Locking, closed cabinet at room temperature (16-24°C) protected from light and moisture sources
Cosmetic properties	White lotion
Acute toxicity for No Mas	The Acute Oral LD50 is greater than 5000 mg/kg in female rats. All animals survived following administration of the test substance. EPA Toxicity Category IV. The Acute Dermal LD50 is greater than 5000 mg/kg in male and female rats. All animals survived exposure to the test substance. EPA Toxicity Category IV.
Irritation and sensitization class For No Mas	Primary Dermal Irritation Index (PDII): 4.4, Moderately Irritating (rabbit). The test substance was Moderately Irritating at 72 hours. EPA Toxicity Category III. Moderately irritating to the eye (rabbit, Draize). Guinea Pig dermal sensitization (Buehler method) determined not a skin sensitizer. a.i. PMD (at 100% concentration; see MSDS) = none for ingestion or inhalation, possible mild skin irritation a.i. Lemongrass Oil (at 100% concentration; see MSDS) = no irritation for dermal or ocular contact, possible sensitization through dermal contact.
Hazard label requirements	For No Mas Repellent (from sample label): Hazards to Humans: Causes moderate eye irritation. Avoid contact with eyes or clothing. If skin irritation occurs or a rash develops, discontinue use. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. **NOTE:** Directions for Use contain specific instructions for avoiding eye contamination. See sample label for details.
Reference materials	Sample label, MSDS and Toxicology/Safety documents in Appendix 8

2) Methods

a) Test Sites and Dates

We measured average subject application rates of the test materials (dosimetry) in order to determine the dose of the repellent to be applied in the repellency phase of the study.

Dosimetry testing was conducted in the Arthropod Behavior Laboratory at Carroll-Loye Biological Research on 5-7 July 2011.

Field tests of repellent efficacy were conducted at two field sites in the Central Valley of California chosen to represent different habitat types. Sites were also chosen based on mosquito and virus surveillance data compiled weekly by the California State Department of Public Health. The sites differed in vegetational structure, water bodies and the composition and relative abundance of foraging mosquito species present (Tables 2 and 7). Site 1 is mature floodplain forest surrounding some marshy areas with standing water, while Site 2 is a relatively open landscape with hedgerows of willows growing along an active stream.

Table 2. Field sites of repellent efficacy study.

Site no.	Date	County	Habitat type
1	23 July 2011	Glenn	Tall floodplain oak forest
2	24 July 2011	Butte	Open irrigated fields near stream

b) Environmental Conditions

Ambient temperature (°C), relative humidity, light intensity (lux), wind speed (MPH, 10 minute average) were measured at approximately 1-hr intervals. Skies were completely free of cloud cover for the duration of the test.

c) Human Study Subjects

A total of 32 subjects participated in the study. They were selected randomly from a pool of 92 subjects. Their demography is described in Table 3.

Table 3. Demography of test subjects, both test dates combined.

Group	Pool	Participated in Efficacy Testing (including Controls, excluding Alternates)	Participated in Efficacy Testing (including Controls, and Alternates)	Participated in Dosimetry	All Subjects in Study
Male	49%	50%	47%	50%	50%
Female	51%	50%	53%	50%	50%
Caucasian	71%	63%	67%	80%	72%
Asian	13%	13%	13%	0%	13%
Hispanic	8%	17%	13%	10%	9%
African-American	4%	8%	7%	10%	6%
Middle-Eastern	4%	0%	0%	0%	0%

For each of the two Test Sites, 5 female and 5 male treated subjects, each exposed one treated limb to wild populations of mosquitoes for one minute every 15 minutes until Test Material failure or cessation of the test. In addition, at each Site, two untreated subjects, 1 female and 1 male, exposed untreated limbs in the same manner coincidentally with treated subjects as an assay for mosquito biting pressure. A sample size of ten subjects was chosen to give a reasonably large statistical population size while avoiding exposing too many individuals to the minor but present risks associated with exposure to biting arthropods. The subjects had the following attributes: they were 18-55 years old, reported themselves to be in good physical condition, were not students or employees of the Study Director, did not believe themselves to be hypersensitive to mosquitoes or phobic of mosquitoes, completed the consenting process including signing the IRB-approved Informed Consent Form, had not used repellents within 1 day prior to the repellency study, and refrained from using alcoholic beverages or perfumed products or smoking beginning at 9 PM the night before, and during, the

test. Females were negative in pregnancy tests conducted immediately before they participated in efficacy testing, and stated that they were not lactating.

d) Mosquitoes and Mosquito-Borne Diseases

Mosquitoes were engaged as encountered in nature. At the time of testing, no mosquito pools collected at either Site 1 or Site 2 within 2 weeks prior to the test days had been positive for West Nile Virus, Western Equine Encephalitis Virus, or St. Louis Encephalitis Virus (see Appendix 6).

The field testing was conducted in wild areas. While appropriate disease vectoring mosquito species and avifauna are probably more abundant, in combination, in these areas than anywhere else in the western United States, West Nile Virus has not been reported from these areas in more than five years of weekly warm season surveillance by the Butte/Glenn County Mosquito and Vector Control District. Rather, it is more commonly detected in urban areas.

Mosquitoes that landed on the exposed limbs of control or treated subjects were collected by subjects and technicians using mechanical aspirators. Note that a small proportion of mosquitoes evaded capture. To expand the sample, some additional mosquitoes were aspirated from the surfaces of Tyvek suits worn by subjects. Collected mosquitoes were either pooled within genus by subject (if control), isolated individually (if treated subject), or pooled generically (if captured from area other than a test limb), given an initial identification and labeled by a technician, and treated by refrigerated knockdown until transported to the Carroll-Loye Biological Research laboratory. There, their identity was verified individually with a stereomicroscope by the Study Director (Ph.D. Biologist).

e) Viral Assays

After being identified, individual or grouped mosquitoes were shifted into glass vials with glass beads for subsequent viral assays, and held at approximately -80°C. They were then hand-delivered cold to the University of California Center for Vector-borne disease for Taqman multiplex RT-

PCR assays that screened for West Nile Virus, Western Equine Encephalitis Virus, and St. Louis Encephalitis Virus. Refer to Appendix 6 for details of viral assay results.

f) Dosage Determination and Margin of Exposure

No Mas repellent dosage rates of 1.20 (arms) and 1.04 (legs) μl/cm^2 was determined by dosimetry.

The individual subject means for the dosimetry from 10 subjects was combined to yield a grand mean to be used as the dosage rate for efficacy testing. The 10 dosimetry subjects were 5 female (nos. 4, 23, 84, 105, 116) and 5 male (nos. 13, 14, 15, 51, 64) subjects. To determine dosage, we measured lower limb surface area for individual subjects based on the length and a set of four circumferences taken from each limb. The amount of No Mas lotion applied to limbs was quantified in a series of three applications. The amount applied was the weight difference in the dispensing tube before and after application (calibrated Sartorius GC 2502).

Estimated dosing based dosimetry grand means, relative to the acute dermal toxicity limit dose of No Mas repellent (>5000 mg/kg, see appendix 8), yielded Margin of Exposure (MOE) values that we judged sufficiently great to justify dermal exposure of the subjects to the test materials during efficacy testing.

In efficacy testing, applications were made volumetrically, based on the limb surface areas of each subject and the specific gravity of the repellent (provided in the Confidential Statement of Formulation on file at US EPA). Despite the individual variation in dosing rate inevitable in actual consumer use, we used the same, average dosing rate in all subjects. The chief advantage of this approach is that it may guard against early failures in subjects who might otherwise “under-dose” for the test conditions. In consumer use, those who under-dose might be expected to re-apply repellent when protection fails, and to perhaps learn about adequate dosing from experience. That accommodation cannot take place in standard repellent efficacy trials. Consequently, the average values from dosimetry studies were chosen as a reasonable approximation of sensible dosing behavior.

g) Test Materials and their application (see Appendix 3 and Appendix 7)

Test Material were received at CLBR on 10 May 2011, with Chain-of-Custody documented. It was stored at the Carroll-Loye Offices in a closed cabinet at room temperature (16-24°C) within specifications provided by the sponsor.

A single Test Material was investigated, and there was no blinding in this study as the control condition was untreated. Individual doses were prepared for each subject on the basis of the surface area of their forearm or lower leg. Before repellent was applied, subjects washed their limbs to be treated carefully with a fragrance-free cleanser in tap water, rinsed them with tap water, then rinsed them again with 35% ethanol in water, and then dried them with clean cotton towels. Repellent was then applied by CLBR technicians and staff, using 1 ml syringes (0.01 ml measurement increment), and one fingertip in a surgical glove to spread the material as evenly as possible. For subjects with limbs large enough to require doses exceeding 1 ml, the total dose was measured into, and dispensed from, two syringes.

For the test at Site 1, treatments were applied at the CLBR laboratory in early morning, prior to travel to the field. For Site 2, applications were made after travel to the field (within the screen enclosure) and hence later in the day. This latter approach permitted testing against evening-active species of mosquitoes present at Site 2.

The treatment allocation and efficacy test dosing are given in Appendix 3.

h) Exposure to Mosquitoes

During exposure in the field, all subjects wore head nets and surgical gloves in addition to Tyvek coveralls, and each carried a mechanical aspirator to remove landing mosquitoes from exposed skin before biting could occur. Treated subjects were partnered into groups of two, and each subject moved in a group of others from the shelter to the exposure area, where all were equally exposed to resident mosquitoes. Each member of a partner pair was instructed to monitor the front of their own exposed limb and the back of the exposed limb of their partner for mosquito landings during one-minute

periods of exposure to mosquitoes (a “buddy system”). Exposures were as follows:

- Untreated Subjects: exposure just prior to treated subject exposures to assay for mosquito foraging activity prior to repellent challenge
- Exposure interval, all subjects: 15 min.
- Exposure duration per interval, all subjects: 1 minute
- Time between application and first exposure: varied by individual; approximately 3.2 hours at Site 1, approximately 6 minutes at Site 2
- 5 female treated subjects at each Site, numbers 28, 92, 105, 118, 125 at Site 1 and numbers 4, 39, 76, 81, 85 at Site 2
- 5 male treated subjects at each site, numbers 29, 41, 64, 106, 123 at Site one and numbers 14, 63, 88, 120, 121 at Site 2

Hand-held timers and a clock were used to ensure adherence to specified protocols for exposure duration and frequency. Technical personnel monitored the results of each exposure in the subject group, and were equipped with mechanical aspirators to remove mosquitoes from subjects should mosquitoes be present. All LIBes were reported to a scientist who recorded the events by subject code and the clock time of exposure interval. At the end of most exposure periods, subjects moved into a screen house.

Ambient LIBe pressure was assessed by two experienced subjects on the same schedule as that for repellent exposure. Each of these negative control subjects was attended by two assistants who used aspirators to quickly remove any LIBing mosquitoes. Both controls exposed a limb then covered the limb as soon as LIBes occurred.

A stopping rule for exposures was invoked when a subject experienced a landing following another in either of the two prior exposure periods. Subjects were withdrawn from further exposure to biting insects when such an event occurred. At Site 1, testing continued until repellent failure on all subjects. At Site 2, testing was stopped at the onset of darkness, when mosquito populations became inactive.

i) Data recording

Technicians dubbed “data Captains” recorded each LIBe observation (time, subject number, number of LIBEs during exposure minute, and whether

mosquitoes were captured) for each exposure. Those records were reported directly to the CLBR Laboratory Manager on each return to the shelter. The Laboratory Manager then recorded those results on the LIBe data form.

j) Data Analysis

Dosimetry data were entered into an Excel 2004 (Macintosh) spreadsheet for calculations of surface area and dosing means. Those means were double-checked with a handheld calculator. Dosimetry analyses were based on subject means. Data were entered into an Excel 2004 (Macintosh) spreadsheet. All descriptive statistics were generated with the software 'SAS JMP' Version 5.0.1.2 (SAS Institute, Cary NC), with the exception of Weibull means and confidence intervals. Those were generated by fitting a Weibull model in the statistical software 'R', with censoring, and taking exp (Lambda).

We calculated Complete Protection Time (CPT) as the interval between application and the First Confirmed Landing with Intent to Bite (FCLIBe). The FCLIBe was defined as the first LIBe that was followed by another within one-half hour, i.e., within either of the subsequent two exposure periods. This measure is analogous to that of First Confirmed Landing, which is commonly used in measures of repellency to blood-feeding flies, including mosquitoes. CPT measured in this way gives a single time value for each subject. Mean CPTs (Weibull and Normal) were calculated across all 10 subjects at each study site, and are presented herein with 95% confidence interval limits. Kaplan-Meier CPT survival plots were also generated, and Kaplan-Meier median CPTs were calculated.

5) Results

a) Dosimetry (see also Appendix 3)

The amount of No Mas applied was measured as the mass of the material leaving the dispensing vessel (plastic squeeze bottle). The No Mas dosing rate was based on a specific gravity of 0.9524 (i.e., 0.9524 kg/liter). Dosimetry data are given by subject in Table 4.

The grand mean (±sd) of subject mean doses for No Mas on arms was 0.57± 0.18 g. The grand mean (±sd) of subject mean doses on legs was 1.13± 0.40g. For efficacy testing, arms were treated at Site 1, and legs were treated at Site 2, matching findings from pre-test observations of site differences in mosquito propensity to attack those body regions. Accordingly, dosing for Site 1 was based on the grand mean of arm dosimetry (1.14mg [1.20 µl] per cm^2), and dosing for Site 2 was based on the grand mean of leg dosimetry (0.99 mg [1.04 µl] per cm^2). Mean grams per subject for efficacy testing (i.e., on each participating subject's single treated limb) are given in Appendix 3.

Table 4. No Mas dosimetry: mean mg applied per cm^2 by 10 subjects. Each subject applied repellent to each limb three times. Means for each limb are calculated from those three values per limb.

Subject code	Left arm	Right arm	Arm mean	Left leg	Right leg	Leg mean
4	1.21	0.99	1.10	0.97	1.16	1.07
13	1.37	1.30	1.34	0.94	0.87	0.91
14	1.09	1.24	1.17	1.47	1.26	1.37
15	1.47	0.86	1.17	1.18	0.97	1.08
23	1.26	1.22	1.24	0.65	0.70	0.68
51	1.20	1.17	1.19	1.38	1.06	1.22
64	1.60	1.60	1.60	1.28	1.25	1.27
84	1.11	1.09	1.10	0.68	0.70	0.69
105	0.63	0.56	0.60	0.43	0.43	0.43
116	0.85	0.95	0.90	1.19	1.14	1.17

Margins of Exposure (MOEs) relative to the acute dermal toxicity limit dose of No Mas (>5000 mg/kg, see Appendix 8) were estimated for the chosen dosage rates (Table 5). The model target subject was a 70 kg adult. The resulting MOE values were close to those estimated in the study protocol, and were deemed sufficient to permit risking prolonged dermal exposure of subjects to the test materials during efficacy testing.

Table 5. Margin of exposure estimation for No Mas: mean grams of test material and active ingredients to be applied based on efficacy test subject limb surface areas, and the resulting exposure values.

Limb	Average g test material applied	Rate in 70 kg human (mg/kg)	Margin of exposure
Arm	0.60	8.57	>583
Leg	1.22	17.43	>287

b) Environmental Conditions

Efficacy data were collected under suitable environmental conditions. Environmental conditions during field exposures are summarized in Table 6. Environmental data are detailed in Appendix 5.

Table 6. Summary of field temperature, relative humidity, light, and wind speed conditions for both test sites.

Variable	Range	
	Site 1	*Site 2*
Temperature	25–37 °C	21–34 °C
Relative humidity	37–67 %	34–71 %
Light intensity	2110–13,300 lux	0–12,200 lux
Wind speed (10 min average)	0–0.9 mph	0.2–1.5 mph

c) Ambient LIB (mosquito Landing with Intent to Bite) Pressure

At each test site, the dual untreated control subjects experienced a minimum of 1 LIBe per exposure in all exposure periods (see data sets, Appendix 4a and 4b). While in most cases a value of ‘1’ was used to indicate suitable mosquito activity, in some observations,

greater values indicate that more than one foraging mosquito landed simultaneously.

d) Mosquito Species Present

LIBing mosquitoes were collected from the exposed limbs of treated and control subjects by aspiration. Those samples were pooled by subject for viral screening. Added to those pools for some subjects were small numbers of additional LIBing mosquitoes that were collected from other body areas that were briefly and inadvertently exposed (e.g., when headnets or gloves gave incomplete coverage). Some additional miscellaneous mosquitoes were also collected from the screen shelter at each Site. The collected mosquitoes are identified and summed for each Site in Table 7.

Table 7. Number of mosquitoes of each species collected, including those collected from control and treated subjects, during the efficacy trial at each site.

	Site 1					Site 2				
		Controls					Controls			
Species	Total	1	2	Treated	Misc.	Total	1	2	Treated	Misc.
Aedes melanimon	61	20	25	7	9	76	37	31	7	1
Aedes vexans	40	11	7	8	14	5	1	4	0	0
Aedes nigromaculis	0	0	0	0	0	1	0	0	1	0
Culex tarsalis	0	0	0	0	0	4	2	2	0	0
Anopheles freeborni	0	0	0	0	0	2	1	1	0	0

A total of 5 species of mosquitoes were foraging at the field sites. As is typical of mosquito repellent tests in the United States, species in the genus *Aedes* were especially aggressive in pursuing human subjects. *Culex tarsalis* and *Anopheles freeborni* also commonly approached subjects at Site 2 from dusk until 2200.

e) Viral Assays

Collected mosquitoes were assayed for diagnostic molecular evidence of the viral pathogens that cause West Nile Fever, Western Equine Encephalitis, and St. Louis Encephalitis. The assayed mosquitoes, in 19 separate samples, included specimens collected from treated limbs (LIBes pooled by subject) and pooled specimens from each control. None of the submitted specimens or pools tested positive for any of the assayed viruses (Appendix 6).

f) Subject Observations During Testing

None of the subjects experienced an adverse reaction to the Test Materials during dosimetry or efficacy testing. There were no observed or reported medical incidents for any subjects during or within 14 days following either dosimetry or efficacy phases of the study.

g) Efficacy: Influence of Test Material on Probability of a Mosquito LIBe

To better understand the results presented in this section, note that no statistical comparisons between the two test sites are made or inferred in this report.

In every case, mosquitoes were strongly affected by No Mas repellent. Weibull mean CPTs (appropriate for survival data) were 9.8 hrs for Site 1 and 10.1 hours for Site 2. Table 8 gives these means with their 95% confidence intervals, along with those for a model assuming a normal underlying distribution for comparison. For survival data, i.e., time to an event when the event is inevitable, the Weibull distribution is generally more fitting than the normal distribution, and Weibull plotting confirmed this for these data sets.

Note that the normal mean for Site 2, at which 6 of 10 subjects did not fail, is calculated by assigning the time of study termination as the time of failure. The normal descriptive statistics therefore substantially underestimate No Mas performance at Site 2.

Kaplan-Meier medians for these survival data were also computed (Table 8). Again, their applicability was limited by the relative failure to fail at Site 2. Further, the Kalpan-Meier confidence intervals simply reflect the minimum and maximum CPTs recorded, and so are not particularly sophisticated predictors for either data set.

Table 8. Complete Protection Time against mosquitoes afforded by No Mas at two study Sites. Presented are means and medians, with lower and upper bounds of 95% confidence intervals.

Site/Parameter	Parameter value[1]	Lower 95%	Upper 95%
Site 1			
Weibull mean	9.8	9.0	10.6
Normal mean	9.2	8.1	10.2
Kaplan-Meier median	9.6	6.4	10.5
Site 2			
Weibull mean	10.1	8.2	12.5
Normal mean[2]	8.5	7.8	9.2
Kaplan-Meier median	-	6.8	-

[1]Parameters for Site 2 are computed from 4 actual and 6 estimated CPTs.

[2]Normal mean for Site 2 is based on assigning the time of study termination as the time of failure for the 6 of 10 subjects that did not fail.

Individual subject results are detailed in Table 9, and the raw data are presented, in temporal sequence, in Appendix 4. The average number of total LIBes experienced by individual subjects was 2.4 at Site 1 and 1.2 at Site 2.

Kaplan-Meier survival plots for the repellency of No Mas against mosquitoes are given for both sites in Figure 1. All subjects received confirmed LIBes at Site 1, while only four of 10 received confirming LIBes at Site 2.

Table 9. No Mas against mosquitoes: Complete Protection Times (CPTs[1]) in hr (in descending order), whether a confirmed LIBe (CLIBe) occurred, and number of LIBe's by subject.

Subject number	CPT	CLIBe?	Total LIBes
Site 1			
125	11.17	Yes	2
106	10.85	Yes	2
28	10.47	Yes	2
118	9.60	Yes	3
123	9.60	Yes	2
41	8.95	Yes	4
105	8.80	Yes	2
92	8.42	Yes	2
29	7.72	Yes	2
64	6.40	Yes	3
Site 2			
4	9.25	No	0
81	9.17	No	0
39	9.12	No	0
76	9.08	No	1
85	9.05	No	0
88	9.02	No	0
14	8.38	Yes	2
120	8.08	Yes	5
63	6.77	Yes	2
121	6.77	Yes	2

[1]CPTs for the six subjects at Site 2 that did not receive Confirmed LIBes is based on assigning the time of study termination as the time of failure; they are therefore estimated minimum CPTs.

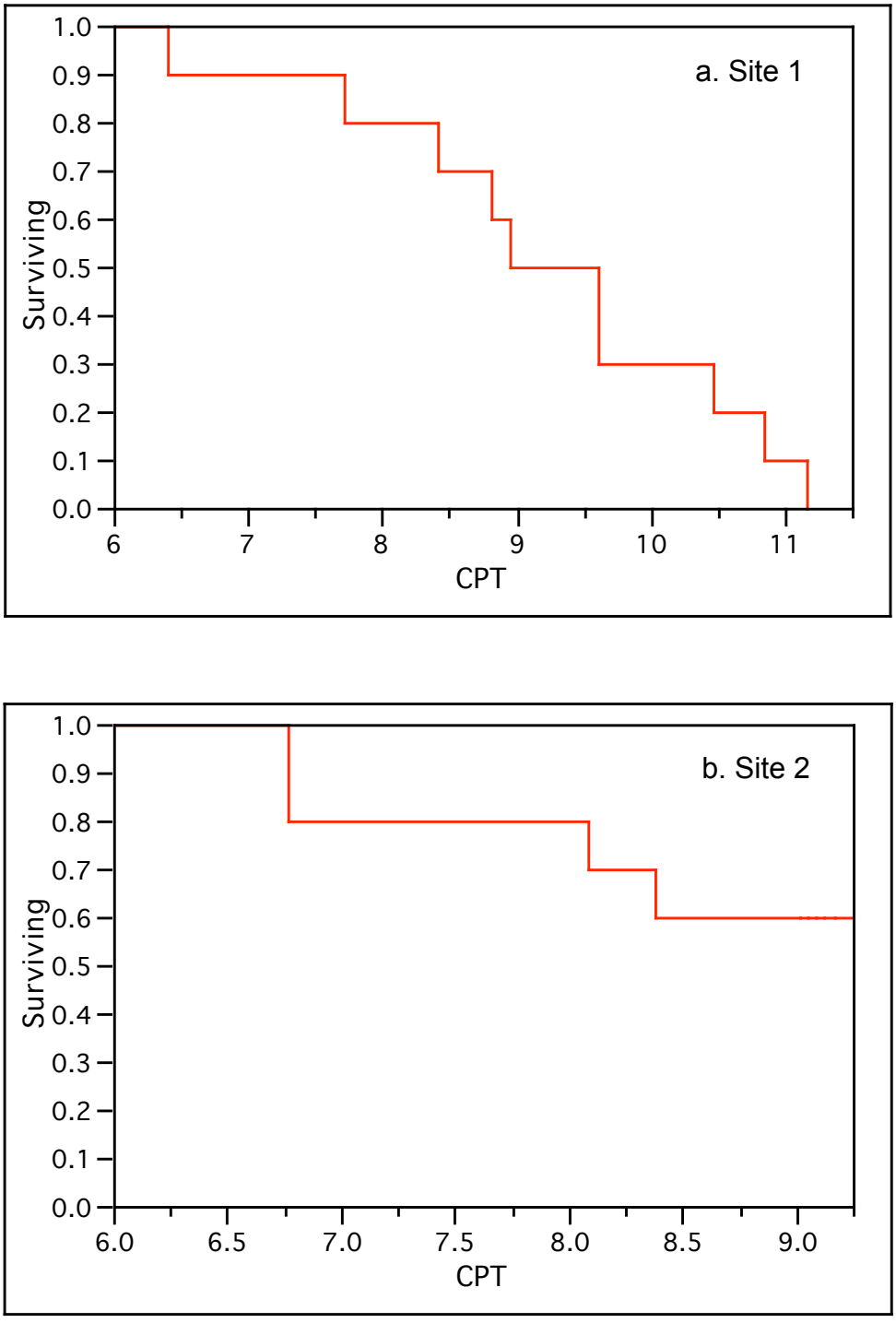

Figure 1. Survival plot of Complete Protection Time (CPT) for No Mas against mosquitoes. a. Site 1; b. Site 2.

Summary and Conclusions

This mosquito repellent study investigated the duration of efficacy of 'No Mas', a topical lotion based on the active ingredients *p*-menthane-3,8-diol (PMD) and lemongrass oil (citral), when applied at model consumer doses. The investigation was sponsored by the product's developer to produce the efficacy data required for a pesticide registration application to the United States Environmental Protection Agency. The efficacy study was conducted on two test days, one in flooded forest and the other in moist field/streamside habitat, against naturally-occurring populations of *Culex*, *Anopheles*, and *Aedes* mosquitoes.

The study Protocol was reviewed and approved by Independent Investigational Review Board, Inc., and reviewed favorably by the US Environmental Protection Agency and its Human Studies Review Board, and by the California Environmental Protection Agency.

We began with a laboratory study of dosing behavior, which showed the Margin of Exposure, relative to acute dermal toxicity limit doses for No Mas, to be >583 (1.20 $\mu l/cm^2$ on arms) and >287 (1.04 $\mu l/cm^2$ on legs). We judged these margins to be sufficiently great to justify dermal exposure of the subjects to the test materials at these dosing rates during efficacy testing.

On each field test day, ten unique human subjects (five females, five males) each exposed a No Mas repellent-treated limb to mosquitoes for one minute every 15 minutes, until product failure or cessation of the test. Simultaneously, one male and one female untreated control subject exposed arms or legs in the same manner, in order to assess mosquito biting pressure. Both control subjects experienced landings within one minute of exposure throughout each test day, indicating that mosquitoes were suitably active for the efficacy study.

Under field conditions, the repellent provided substantial and prolonged protection against the mosquito species (*Aedes melanimon, Ae. vexans, Ae. nigromaculis, Culex tarsalis,* and *Anopheles freeborni*). Mean Complete Protection Time (CPT) for No Mas was 9.8 hours at Site 1 and 10.1 hours at Site 2.

In summary, No Mas repellent provided prolonged periods of Complete Protection against several species of mosquitoes, including species significant to public health.