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

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

September 29, 2011

MEMORANDUM

SUBJECT: Science Review of Completed Carroll-Loye Mosquito Repellent Field Efficacy Study No Mas 003

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REF: Carroll, S. (2011) Field Efficacy Test of PMD and Lemongrass Oil-Based Repellent, No Mas-003, against Mosquitoes. Efficacy Test Report NO MAS-003. Unpublished document prepared by Carroll-Loye Biological Research. 411 pp.

ACTION REQUESTED

Conduct a science review of the completed study, Field Efficacy Test of PMD and Lemongrass Oil-Based Repellent, No Mas 003, against Mosquitoes" (MRID 48577201) to determine the adequacy of the methods employed and the scientific validity of the reported data, and to assess the consistency with the approved protocol.

The protocol No Mas 003 was initially submitted to EPA for review in August 2010. The protocol and EPA's review dated October 1, 2010, were discussed by the HSRB on October 27, 2010. The HSRB reviewed the protocol favorably, concluding in their December 13, 2010, final report of the October meeting that "the proposed field repellency study protocol No Mas-003, if revised as suggested in EPA's review…and performed as described, is likely to generate

scientifically reliable data, useful for assessing the efficacy of the tested material in repelling mosquitoes."

CONCLUSIONS

Scientific aspects of the research were assessed in terms of the recommendations of EPA Guideline §810.3700 and of the EPA Human Studies Review Board. Study MRID 48577201 is in compliance with Good Laboratory Practices (US EPA Pesticide Programs 40 CFR §160) (pg. 3) and was inspected for Quality Assurance during various stages of the study (pg. 4). This study provides scientific data that are acceptable pending resolution of data analysis for characterization of NO MAS performance. The Human Studies Review Board will be asked to comment on the statistical analysis employed in this study.

SCIENCE REVIEW

Study Objectives: To determine the Complete Protection Time (CPT) of NO MAS repellent when applied at a typical consumer dose, against wild populations of mosquitoes, including but not limited to species of the genera *Culex*, *Anopheles*, and *Aedes*. To provide data required by the EPA as a condition of registration for NO MAS 003 mosquito repellent, containing 16% w/w of p-menthane-3,8-diol (PMD) and 2% w/w of lemongrass as active ingredients, and to estimate the mean and standard deviation for CPT, which is measured as the period of time between product application and the time of First Confirmed Landing with Intend to Bite (FCLIBe).

Materials and Methods:

Study Locations: Field test sites were located in the Central Valley of California. The sites represented different habitats for different mosquito species, and were chosen based on mosquito and virus surveillance data compiled weekly by the California State Department of Public Health. Site 1 was a tall floodplain with mature oak forest surrounding marshy areas and standing water, located in Glenn County. Site 2 was a relatively open irrigated landscape with hedgerows of willow trees growing along an active stream located in Butte County (Table 2 pg. 11). West Nile Virus had not been reported in the test area in more than 5 years of weekly surveillance by Butte/Glenn County Mosquito and Vector Control District. Both sites differ in composition and distribution of mosquito species given in Table 7, pg. 20, in the study report. No mosquitoes collected at either site for 2 weeks prior to testing were positive for West Nile Virus, West Equine Encephalitis virus, or St. Louis Encephalitis virus.

Study Dates: Dosimetry testing was conducted in Arthropod Behavior Laboratory at Carroll-Loye Biological Research on July 5-7, 2011. Repellent product tests were conducted in the field at Sites 1 and 2 on July 23-24, 2011.

Repellent Tested: NO MAS 003 mosquito repellent formulation, containing 16% w/w of p-menthane-3,8-diol (PMD) and 2% w/w of lemongrass as active ingredients.

Tested positive control/comparison repellent: There was no positive control in this study.

Untreated Control: Two experienced negative control subjects (female control subjects 4 and 84 at Sites 1 and 2, respectively, and male control subjects 63 and 52 at Sites 1 and 2, respectively. pp. 57-58) exposed untreated limbs for 1 minute every 15 minutes at the same time as treated subjects to monitor the ambient Landing with Intent to Bite (LIBe) pressure throughout the test. There were no statistical comparisons to the untreated controls.

Mosquito species and life stage: NO MAS repellent was evaluated against natural populations of adult female mosquitoes. The species, number and distribution of collected mosquitoes are listed in Table 7 on page 20 of the study report. The most common species identified in Site 1 and Site 2 was *Aedes melaniomon*, followed by *Ae. vexan*, more common at Site 1 than at site 2. At Site 2, *Ae. vexan*, was equally common to *Culex tarsalis*, followed by *Anopheles freeborni* and *Aedes nigromaculis*. *Anopheles freeborni* and *Ae. nigromaculis* were the least common species only collected at Site 2.

Number of Test Subjects/Treatment Regime: A total of 32 healthy subjects, between the ages of 18 and 55 years old, were randomly selected from a pool of 92 subjects to participate in the study. A total of 10 subjects (female subjects 4, 23, 84, 105, 116 and male subjects 13, 14, 15, 51, 64), were employed for determination of repellency dose. Four of the 10 dosimetry subjects also participated in the repellency test. Dosimetry subjects 64 (male) and 105 (female) participated in the efficacy test at Site 1, and dosimetry subjects 4 (female) and 14 (male) participated in the efficacy test at Site 2.

For field testing, repellent-treated female subjects 28, 92, 105, 118, 125, and repellent-treated male subjects 29, 41, 64, 106, 123 participated in repellency testing at Site 1 (Randomized Treatment Allocation Table, pg. 55, and pg. 16 of study report). Repellent-treated female subjects 4, 39, 76, 81, 85, and repellent-treated male subjects 14, 63, 88, 120, 121 participated in repellency testing at Site 2 (Randomized Treatment Allocation Table, pg. 56 and pg. 16 of study report). Treatment was replicated 10 times (on 10 subjects) per site.

Protocol used including amendments: CLBR Protocol NO MAS 003 (pp. 309-322) obtained prior review from EPA and the HSRB. EPA's science and ethics review was dated 10/01/10 and the HSRB reviewed the protocol at its October 2010 meeting. The HSRB's report dated 12/13/10 concluded that "t"he proposed field repellency study protocol No Mas-003, if revised as suggested in EPA's review…and performed as described, is likely to generate scientifically reliable data, useful for assessing the efficacy of the tested material in repelling mosquitoes." Following HSRB review, the protocol and consent form were modified through Amendment 1 (dated 11/15/10). This amendment incorporated changes responsive to the comments of EPA, HSRB, and California Department of Pesticide Regulation (CDPR), as well as additional corrections initiated by the investigators and/or sponsor. The Independent Investigational Review Board, Inc. granted approval to Amendment 1 on 11/16/10.

Protocol Deviations: One protocol deviation was reported. Data forms were reformatted to minimize data entry error and thus enhance accuracy. Therefore, the reported deviation is expected to improve data quality.

Consistency of study with approved protocol: The study was conducted in accordance with the IIRB approved protocol as augmented by protocol amendment 1 (pp. 309-322). One protocol deviation was reported by the investigators (discussed above). I did not note any unreported deviations.

Responsiveness to Prior EPA and HSRB Comments

Table 1:

EPA and/or HSRB Science-Related Comments on the No Mas 003 Protocol	Addressed prior to initiation of the study?
1. Amend the protocol to remove erroneous reference to spray repellents (e.g. the first sentence on page 79, under "Rationale"; Carroll 2010, 79).	Yes.
2. Consider whether the proposed sample size is large enough to be likely to yield a definitive answer to the research question and its size justified statistically in the protocol.	Yes.
3. In the statistical analysis plan, discuss how non-normally distributed data points will be treated. That is, how the researcher plans to analyze and interpret results from non-normally distributed data points that may follow an unknown distribution.	Yes
4. On page 28, line 41, delete the phrase "for each test material" because this protocol is for testing one single formulation.	Yes
5. Update the sequential exposure table to include 80 sequential exposure intervals.	Yes

Dose determination: A dosimetry determination phase was conducted prior to efficacy testing to estimate typical consumer dose. The amount of lotion applied to limbs (forearms and lower legs) was averaged over 3 applications per subject. The overall average grams of lotion applied on forearms and lower legs across 10 dosimetry subjects was 0.57 ± 0.18 and 1.13 ± 0.40 grams, respectively. Overall average dose rates for arms and legs are 0.00114 and 0.00099 g/sq. cm, respectively, which are calculated from each subject individual limbs' surface area value. The dose rates in g/sq. cm were modified to convert grams to mL based on specific gravity of the repellent (0.9524 kg/L) (Calculations are shown in raw data summary table on pp. 59 – 60).

Dose rates: In the efficacy testing phase, test material applications were made volumetrically (mL) based on specific gravity of the formulation and adjusted to surface areas of repellent treated subjects' limbs so that for efficacy testing at Site 1 and Site 2, all subjects' arms and legs were treated at a volumetric dose rate of 1.20 µl/cm^2 and 1.04 µl/cm^2, respectively, based on the

grand mean of arm and leg dosimetry. The efficacy testing dose rates of 1.20 µl/cm^2 (forearms) and 1.04 µl/cm^2 (lower legs) were used to estimate Margin of Exposure (MOE) values > 583 and >287 for arms and legs, respectively, relative to NO MAS acute dermal toxicity limit dose > 5,000 mg/kg in males and female rats. The resulting values are close to those predicted in the study protocol, presented in Section 4.6, *Margin of Exposure and Dosimetry*, on page 113 of the study protocol, included in study report MRID 48577201. (Toxicological profile of NO MAS repellent in summarized Appendix 1 of this review).

Experimental design: There was no blinding in the study since the test was designed to evaluate one formulation at 2 different field sites, using 2 different groups of 10 treated subjects per site, and 2 experienced control subjects per site to monitor mosquito landing pressure throughout the test. Subjects were trained in the laboratory to use mechanical aspirators and handle mosquitoes prior to field testing. The pre-test training process is described in the Informed Consent Authorization as a Research Study Subject (no repellent applied) on pg. 129, and in Informed Consent Authorization as a Research Study Subject (repellent applied) on pg. 140. Subject preparation for testing was done consistently with the test guideline for Product Performance of Skin-applied Insect Repellents of Insect and Other Arthropods Test Guidelines (OPPTS Test Guideline No. 810.3700).

The NO MAS formulation was tested at each site on 2 consecutive dates. The first trial was conducted on Site 1 on July 23, 2011, and the second trial was conducted at Site 2 on July 24, 2011. For Site 1, the subjects were treated in the CLBR laboratory early in the morning, prior to travel to the field. For Site 2, the subjects were treated upon arrival at the field later in the day. Time between application and first exposure varied among individual subjects approximately 3.2 hours at Site 1, and approximately 6 minutes at Site 2 (pg. 16). Repellent treatments were applied by Carroll-Loye Biological Research (CLBR) technicians and staff, using 1 mL syringes (0.01 ml increments). The material was spread evenly on subjects' skin using one fingertip in a surgical glove. Repellent performance was evaluated on two different groups of ten subjects, five males and five females. To ensure adequate landing pressure throughout the test, two experienced subjects, one male and one female, monitored landings coincidentally with treated subjects exposure periods.

During exposure in the field, treated subjects were arranged in pairs to monitor landings on their own exposed limbs and the back of the exposed limb of their partner. They were equipped with mechanical aspirators to remove landing mosquitoes before they would bite. Exposure to mosquitoes lasted 1 minute every 15 minutes until repellency failure or cessation of the test. The results of each exposure were monitored by technicians, who were equipped with mechanical aspirators to remove mosquitoes landing on subjects. Technicians reported all landings to a scientist in charge of recording each landing by subject and time of occurrence (during exposure interval). A stopping rule was invoked and the subject was withdrawn from further exposure when he or she experienced a confirmed landing; that is, a landing following another within 30 minutes. At Site 1, testing stopped once all subjects had experienced a First Confirmed Landing with Intent to Bite (FCLIBe). At Site 2, the test ended at the onset of darkness when landing activity ceased (Table 9, pg. 23, and raw data tables, pp. 71-72). At that point, only four of the ten subjects had experienced a Confirmed Landing with Intent to Bite.

The study was conducted under suitable environmental conditions summarized in Table 6, pg. 19, of the study report. Mosquito landing pressure throughout the test was adequate in that it was at or above the minimum of one mosquito landing per minute.

Mosquito disease pathogen detection: No collected mosquitoes were found positive for viral pathogens.

Data analysis: Results from each site were analyzed independently using descriptive statistics without making comparisons between sites. The means and medians of CPT, with their associated confidence levels, are tabulated by site in Table 8, pg. 22, of the study report. Survival data were analyzed using both non-parametric (Kaplan-Meier) and parametric (Weibull, Normal) methods. Kaplan-Meier is a non-parametric method which does not assume any parametric distribution of the data. On the other hand, parametric methods such as Weibull and Normal methods make distributional assumptions about the data. The Weibull probability density distribution, which is an exponential function commonly used to model the probabilities of times to an event when the event is inevitable, was used in survival analysis to estimate mean CPT within 95% confidence intervals from a non-normally distributed set of values. The researcher explains that mean and variances modeled by the Weibull distribution better characterize the mean and 95% confidence intervals of complete protection times even in the event of substantial right censoring.

Results:

Table 2. Results as presented in Table 8, pg. 22, of the Study Report, MRID 48577201.

Site/Parameter	CPT (hours)	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	8.5	7.8	9.2
Kaplan-Meier median	--	6.8	--

Table 2 above summarizes the results of the field test by site. The Weibull mean values have a smaller confidence interval when compared to the Kaplan-Meier survival analysis; thus, the investigator asserts that use of the Weibull distribution provides a better estimate of CPT with a higher level of confidence. The Weibull mean CPT values for both sites analyzed independently are nearly the same, 9.8 and 10.1 hours. Individual subject data are tabulated in Table 9, page 23, of the study report.

Table 3. Individual Subject Data as presented in Table 9, pg. 23, of the Study Report, MRID 48577201.

Sites	Subjects	CPT (hrs)	CLIBe (Yes or No?)	Number of LIBe
Site 1	125	11.17	Y	2
	106	10.85	Y	2
	28	10.47	Y	2
	118	9.60	Y	3
	123	9.60	Y	2
	41	8.95	Y	4
	105	8.80	Y	2
	92	8.42	Y	2
	29	7.72	Y	2
	64	6.40	Y	3
Site 2	4	9.25	N	0
	81	9.17	N	0
	39	9.12	N	0
	76	9.08	N	1
	85	9.05	N	0
	88	9.02	N	0
	14	8.38	Y	2
	120	8.08	Y	5
	63	6.77	Y	2
	121	6.77	Y	2

At Site 1, all subjects experienced first confirmed landings ranging from a CPT of 6.40 to 11.17 hours. At Site 2, the normal mean of CPT is generated from a sample of 10 subjects, of which only 4 experienced confirmed landings at around 8 and 6.77 hours. The test ended after 9:25 hours of exposure periods, when mosquito landing pressure declined below acceptable levels. The time when the test ended was assigned as the time of product failure (Table 9 pg. 23). The mean and median protection time for Site 2 were calculated from 4 actual and 6 estimated CPT. The average number of landings experienced by individual subjects was 2.4 at Site 1, and 1.2 at Site 2 (pg.22). Figure 10 on page 24, illustrates Kaplan-Meier survival plots for lasting repellency of NO MAS formulation against mosquitoes tested at both sites.

Question to the HSRB regarding statistical analysis of the data:

Which statistical method is appropriate to calculate the Complete Protection Time for the NO MAS repellent?

1. Parametric (with Weibull distribution or normal distribution); or
2. Non-parametric (Kaplan Meier)

Conclusion:

The methods employed in this study were adequate to produce scientifically reliable data. They were based on study protocol No Mas 003 as amended in accordance with EPA and HSRB recommendations before testing began. No Mas 003 repellent provided a high degree of repellency against against several mosquito species (*Aedes melanimon, A. vexans, A. nigromalis, Culex tarsalis, and Anopheles freeborni*) encountered in the field. The Weibull mean CPT was 9.8 hours at Site 1, and 10.1 hours at Site 2. The reported protocol deviation was non-substantive in nature and did not affect the design or conduct of the research, or the resulting data.

Recommendation: The study is scientifically sound and acceptable.