EPA Review of the Carroll-Loye Biological Research Completed Study No Mas 003

Field Efficacy Test of a PMD- and Lemongrass Oil-based Repellent 'No Mas' Against Mosquitoes

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Background

- Completed report of a mosquito repellent field efficacy study
- Test material is called ‘No Mas’
- ‘No Mas’ is a lotion formulation containing 16% PMD and 2% lemongrass oil
- Protocol reviewed by HSRB at October 2010 meeting
- Research conducted in California, at two sites, in July 2011
- Final report submitted to EPA in August 2011

Background 2

- Sponsor is developing ‘No Mas’ as a low-cost repellent for distribution in developing countries with vector-borne disease
- Sponsor reports that the product has broad-spectrum efficacy against more than 40 species of mosquitoes, including four of the most important malaria-vectoring anophelines
- The purpose of the present study was to test the product for efficacy against three mosquito genera – *Culex*, *Anopheles*, and *Aedes*

EPA Science Review of Carroll-Loye Biological Research Completed Study 'No Mas 003'

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Purpose of the Science Review

- Assess the validity of the efficacy data for evaluating the performance of ‘NO MAS’ repellent against mosquitoes in the field
- Provide scientific review of the study and its consistency with approved protocol NO MAS-003

EPA Scientific Assessment

The Carroll-Loye Efficacy Test, NO-MAS 003, MRID 48577201, was conducted in a manner substantially consistent with the study protocol reviewed by EPA and the HSRB

Consistency Between Conduct and Protocol

- Protocol amendments were approved prior to study initiation
- Justification provided for choice of sample size
- Rationale provided for choice of statistical methods to analyze non-normally distributed data

Deviation from the Protocol

- Data forms were reformatted to minimize data entry error and thus enhance accuracy.
- The reported deviation is expected to improve data quality.

Study Objectives

The test's objectives are:

- to characterize the performance of 'NO MAS' against wild populations of mosquito species among the genera *Culex, Anopheles* and *Aedes.*
- to estimate the mean value of Complete Protection time (CPT) within 95% confidence interval
- to provide efficacy data required to support registration of NO MAS formulation

Study Design

Dose determination:

- Standard consumer dose for arms and legs was determined from grand mean of 10 subject means (5 males and 5 females).

Efficacy testing:

- Sample size: 10 treated subjects (5 males and 5 females) per site.
- Test sites: 2 different mosquito habitats
- Number of treatments: 1 lotion formulation
- Negative control: 2 untreated subjects/ site to monitor landing activity during testing.

Dosimetry Endpoint and Standard Consumer Dose

Dosimetry Endpoint: Standard Consumer Dose

Arms = 1.14 mg/cm^2

Legs = 0.99 mg/cm^2

Standard consumer dose used for testing efficacy;

-Arms = 1.14 mg/cm^2 (0.9524 kg/L) =1.20 $\mu l/cm^2$

-Legs = 0.99 mg/cm^2 (0.9524 kg/L) =1.04 $\mu l/cm^2$

Margin of Exposure (MOE)

Margin of Exposure

Arms MOE > 583

Legs MOE > 287

Based on the NOEL of dermal toxicity > 5,000 mg/kg b.w. and standard dose

Efficacy Endpoint and Other Measurements

- Complete Protection Time (CPT)
- Landing pressure (threshold=1 LIBe/minute)
- Exposure delay (min)-time between application and first exposure:
  - Site 1 = 3.2 hours
  - Site 2 = 6 minutes
- Duration of exposure: 1 minute every 15 minutes

Results from Field Test: Time Distribution of CPT by Site

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

Results from Field Test: Mosquito species by Site

Mosquitoes collected at SITE 1			
Mosquito species	**Total collected**	**Total collected by subject**	
		2 Control subjects	**10 Treated subjects**
Aedes melanimon	52	45	7
Aedes vexans	26	18	8
Total number of all species	78	63	15

Mosquitoes collected at SITE 2			
Mosquito species	**Total collected**	**Total collected by subject**	
		2 Control subjects	**10 Treated subjects**
Aedes melanimon	76	68	7
Aedes vexans	5	5	0
Aedes nigromaculis	1	0	1
Culex tarsalis	4	4	0
Anopheles freeborni	2	2	0
Total number of all species	88	79	8

Statistical Analysis

Dosimetry procedure

- Average testing dose was the grand mean (± SD) across 10 subjects' means.

Efficacy Testing

- Sample size: 10 subjects/test site.
- Statistical methods employed to calculate CPT within 95% CI:
  - Weibull Mean
  - Kaplan-Meier median, and
  - Normal Mean

Complete Protection Time Values by Site
(from Carroll-Loye Biological Research)

CPT values within 95% CI by test site

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	--

Statistical Questions for the HSRB

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

1. Parametric (with Weibull distribution) or

2. Non-parametric (Kaplan-Meier)?

Summary Assessment of Reliability

Accuracy measurements

- Test material was applied by laboratory technicians.
- Alternate subjects were enrolled to ensure adequate sample size.
- All landings were verified and recorded by a research technician.
- Pre-training of subjects on how to handle mosquitoes.

Summary Assessment of Reliability (Cont.)

Measurement of uncertainty

Mean CPT was calculated across all 10 subjects/site, and was presented within 95% confidence intervals, assuming a non-normal Weibull distribution.

Compliance with Scientific Standards

The following elements are adequately addressed:

- Prerequisite acute toxicity research to characterize toxicological profile of the formulation and calculate margin of exposure (MOE)
- Dosimetry
- Experimental design
- Verification of subject attractiveness to mosquitoes

Conclusion

- The study is scientificallyacceptable
- The data provides scientifically reliable information because it satisfies the following scientific criteria:
  - It produced important information that cannot be obtained except by research with human subjects
  - It has a defined scientific objective
  - The study design (subject selection, sample size, dosing, QA/QC, etc.) generated adequate data to meet the test objective

EPA Ethics Review of Carroll-Loye Biological Research Completed Study 'No Mas 003'

Kelly Sherman

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Recruiting

- Recruiting process outlined in the protocol was followed
  - 32 subjects were selected randomly from a pool of 92 subjects
  - 10 subjects participated in the dosimetry phase
  - 22 subjects participated in the field testing at one or both sites
  - 6 subjects were enrolled as alternates

Consent Process

- Per protocol, subjects were provided with the MSD sheet, study synopsis, consent form, California Experimental Bill of Rights, and other study-related information
- Subjects signed the consent form and the CA Experimental Bill of Rights prior to enrollment
- No reported or unreported deviations related to the consent process

Subject Demographics

- Dosimetry phase:
  - 5 males, 5 females
- Field testing, at each site:
  - Treated subjects: 5 males, 5 females
  - Untreated controls: 1 male, 1 female
- All subjects over the age of 18
- All female subjects were tested for pregnancy prior to participation

Monitoring

- Research was conducted without incident
- No subjects withdrew from the research
- No adverse events or incidents of concern were reported

No Mas 003: Protocol Review

- Protocol was approved by IIRB, Inc. and submitted to EPA in July 2010
- EPA's science and ethics review found the protocol acceptable with minor changes
- Reviewed by the HSRB on 10/27/10
- HSRB concurred with EPA, recommended minor revisions
- Amendment 1 dated 11/15/10, addressed most EPA and HSRB comments; approved by IIRB 11/16/10
- Amended protocol was approved by CDPR on 3/21/11

No Mas 003: Amendment 1

- Provided additional justification for the chosen sample size and discussion of data analysis approach
- Adjusts wording in the protocol and consent form per recommendations from EPA, HSRB, and CDPR
- Excludes as permissible subjects employees of the sponsor or researchers

Responsiveness to Previous Ethics Reviews

- Most of EPA's/HSRB's comments from the protocol review were addressed in Amendment 1:
  - Employees of the sponsor were excluded as permissible subjects
  - Symptoms of heat stress and equine encephalitis were added to the consent form
  - Most drafting recommendations were incorporated
- Two HSRB comments were not addressed:
  - The acronym PMD was not spelled out when first used
  - "Child/Minor" was not added to the list of exclusion criteria, although the inclusion criteria specify that subjects must be between 18-55 years old

Protocol Deviation

- One deviation: reformatting of dosimetry data form
- This deviation did not affect the rights or safety of the subjects, or compromise informed consent

Completeness of Submission

- The primary study report, MRID 48577201, is complete
- All requirements of 40 CFR 26.1303 for documentation of ethical conduct are satisfied

Substantive Acceptance Standards

- 40 CFR 26.1703
  - Prohibits reliance on data involving intentional exposure of pregnant or nursing women or of children
- 40 CFR 26.1705
  - Prohibits reliance on data unless EPA has adequate information to determine substantial compliance with subparts A through L for 40 CFR 26
- FIFRA 12(a)(2)(P)
  - Makes it unlawful to use a pesticide in human tests without fully informed, fully voluntary consent

Findings

- No Mas 003 did not involve intentional exposure of pregnant or nursing women or of children
- ‘No Mas 003’ was conducted in substantial compliance with all applicable requirements of 40 CFR part 26, subparts K and L
- Subjects were fully informed and participated voluntarily

Conclusion

- If No Mas 003 is determined to be scientifically acceptable, I find no barrier in law or regulation to EPA's reliance on it in actions under FIFRA

No Mas 003 Completed Study: Charge Questions

1. Is the completed study No Mas 003 sufficiently sound, from a scientific perspective, to be used to estimate the duration of complete protection against mosquitoes provided by the tested repellent?

2. Does available information support a determination that the studies were conducted in substantial compliance with 40 CFR part 26, subparts K and L?