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WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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MEMORANDUM

SUBJECT: Science review of WPC-001 report of completed efficacy study of an Oil of Lemon Eucalyptus-based personal repellent against mosquitoes.

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REF: Carroll, S. (2007) Test of Lemon Eucalyptus Personal Insect Repellent (EPA reg. # 305-62). Unpublished study conducted by Carroll-Loye Biological Research under Project No. WPC-001. 225 pages. (MRID 47217601)

ACTION REQUESTED

Provide scientific review of the completed study, MRID 47217601, WPC-001 Pump Spray formulation, to evaluate its scientific validity and assess its consistency with changes recommended by EPA and HSRB to the revised protocol.

CONCLUSIONS

I have reviewed Carroll-Loye product performance study, MRID 47217601, WPC-001 Pump Spray formulation, containing 30% w/w of the active ingredient Lemon of Oil Eucalyptus, and concluded that the study, WPC-001, provides data showing the performance of the tested material.

Although the study was conducted inconsistently with certain provisions of the revised protocol

as listed below, the reported study, MRID 47217601,WPC-001 Pump Spray, generates reliable data for evaluating the repellency of the tested formulation against mosquitoes.

In the test site in Glenn County in a mature forest area, the reported complete protection time (CPT) ranged from 4.00 to 8.25 hours (Mean CPT = 6.1 ± 1.5 hours; 95% CI 5.0-7.2 hours). Subjects experienced an average of 2.9 ±1.3 LIBes. All subjects received confirmed LIBes. The Kaplan-Meier median CPT was 6.25 hours. In the Butte County lakeside grassland site, the reported complete protection time (CPT) ranged from 3.25 to 5.75 hours (Mean CPT = 4.2 ± 0.8 hours; 95% CI 3.6-4.8 hours). Subjects received an average of 2.2 ± 0.4 LIBes. All subjects received confirmed LIBes. The Kaplan-Meier median CPT = 4.00 hours.

A. Reported Protocol Deviations:

1. *With the Study Director consent, subjects not always covered treated limbs between exposures.* This deviation probably reduced abrasion of the test material by the coveralls, without significantly increasing the risk of biting from mosquitoes.

2. *During dosimetry, the number of practice applications proved excessive and unnecessary, and so, they were reduced from 2 to 1 for most subjects.* Subject exposure to the test material was reduced without significantly affecting quality of results.

3. *Three experienced personnel (subjects) were permitted to join CLBR technicians to assist them in treating test subjects for efficacy testing.* This procedure improved synchrony with which applications were made. Technicians monitored auxiliary personnel to ensure conformance with the study protocol.

4. *Treatments were applied in the laboratory well in advance of arrival to the test sites. At Glenn County site exposures began 3 hours post-application. At Butte County site exposures began 2½ hours post applications, except for one subject (subject 69), who was treated with the repellent 1 hour and 45 minutes prior to exposure.* Subjects were instructed to avoid abrasion of treated leg surfaces. Subjects experienced no LIBes during the first hour of testing, suggesting that the data record accurately represents the temporal distribution protection.

5. *Subjects did not maintain specified interpersonal distances during early efficacy exposure intervals on July 12, 2007, due to cluster distribution of mosquitoes. The problem was solved at midday through the discovery of larger mosquito patches. Because all subjects were wearing the same repellent any interaction among them with regard to repellent performance that might have been augmented by excessive proximity are unlikely to complicate the interpretation of results.* Reviewer comment: The researcher needs to clarify the distribution and interpersonal distances among subjects in the field since other (different) repellent formulations were tested simultaneously at the same site on July 12..

6. *Temperature data was collected inaccurately in the first 3 hours on July 12.* The thermometer was set to display the minimum temperature (17 °C) rather than the actual temperature. The setting was corrected at 1:00 p.m. (32 °C). No extraordinary events occurred during that period for which temperature might have been informative, nor were the expected

temperatures likely outside the range of other recorded during the study.

7. *There is a rounding error in dosage calculation for subject 13.* The value 0.57481 was rounded to 0.58 instead of 0.57. That 0.01 ml difference is not consequential for the results or their interpretation.

B. Additional Unreported Protocol Deviations:

1. No blinding of study: *"Experimental design will be partially randomized by subject. Because the treated condition will be evident to experimenters and subjects, and a single test material is under study, neither group [researchers and subjects] will be effectively blinded."* Reviewer comment: More than one formulation was tested simultaneously on same days and locations. The study report does not specify the actual experimental design for these simultaneous tests.

2. Inclusion criteria for selecting human study subjects. Study subjects "*had not used repellents the day prior to enrolling in the study.*" On July 13, subjects 37 and 43 had used LipoDEET 3434 on the previous day, subjects 40 and 60 had used Duranon on the previous day, and subject 63 had used Lipo DEET 302 on the previous day, all as subjects in SCI-001. On July 15, subject 8 had used Duranon on the previous day, subject 15 had used LipoDEET 3434 on the previous day, subjects 71 and 72 had used LipoDEET302 on the previous day, and subjects 20 and 21 had used Ultrathon on the previous day, again all as subjects in SCI-001.

Table 1. Subjects that tested different repellents the day before testing OLE formulation.

Subjects	Gender **F**emale **M**ale	Leg: **R**ight **L**eft	Other formulations used on previous day	Test dates/ location Formulations tested	
			July 12 / Grassland Glenn Co.	July 13 Glenn Co	July 15 Butte Co.
37	F	L	Lipo3434 (L)	OLE (L)	
40	F	?	Duranon (?)	OLE (R)	
43	M	L	Lipo3434 (L)	OLE (L)	
60	F	L	Duranon (L)	OLE)L)	
63	M	L	Duranon (L)	OLE (L)	
			July 14/ Grassland-Glenn Co.		
15	M	L	Lipo 3434		OLE (L)
20	F	R	Ultrathon (R)		OLE (R)
21	M	L	Ultrathon (L)		OLE (L)
71	M	?	Lipo302 (?)		OLE (L)
72	M	R	Lipo 302 (R)		

C. <u>The following are the protocol amendments adopted in the performance of WPC-001 study</u>:

§5.2 Rationale and Main Endpoint. "comparison article" was removed from this section. Consistent with §6.3 Comparison article: "none."

§9.1.3 Exclusion Criteria, all subjects. Addition of §9.1.3.12. This section addresses the exclusion criteria in case that subjects withdraw prematurely from the test.

§9.1.4 Number of Subjects and Rationale for Sample Size: paragraph 6 concludes that a sample size of 10 is not a robust measure of sample distribution, but neither square root or log transformation are suitable, depending on results. Transformed data will be converted back to original scale for reporting on familiar units. This may be appropriate for planned regression analyses of dosimetry data (§11.3.1).

§9.1.5.1and §9.1.5.3 are replaced with text for §9.1.5 Individual subject's influences on repellent performance, and risks from participation, in relation to the choice of subjects; and §9.1.6.1 Sampling frame of study subjects.

§10.4.7 Procedure for Assessing Variable, is amended by addition of §10.4.7 PCR Virus Assay.

§11.3.2 Repellency is amended by addition of concluding paragraphs addressing justification for sample size of 10 subjects, and data analysis including the use of the Kaplan-Meir survival test.

Data capture forms were amended by removing space for subjects' names, and adding blank spaces for recording distance of circumferences for measuring limbs' surface area in order to maintain exact placement of dosimeters.

The reported study adopted the following HSRB specific recommendations:

1. The dosimetry test was conducted outdoors for the pump spray formulation. "Applications were made out of doors, immediately adjacent to the laboratory." (pg. 7).

2. The study provides justification for sample size, and discussion of statistical procedures for analysis of dosimetry and repellency data.

   "Ten human subjects were used in measurements of self-dosing behavior. Likewise, ten human subjects exposed the test material to mosquitoes for efficacy evaluation. A sample size of ten subjects was chosen for efficacy testing 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." pg. 6.

4. Risk from exposure to formulation was further minimized by reducing the number of unnecessary exposures from 3 to 1 during preliminary practice prior to initiation of dosimetry test.

"After practicing applying the pump spray once to each limb to get a feel for its dispensing properties, subjects completed a series of three self application replicates to each lower leg." pg. 7.

5. Risk from exposure to mosquito's bites and mosquito borne diseases were adequately minimized as summarized below:

   a) The efficacy endpoint is the first confirmed landing with intent to bite (FCLIBe)

   b) Exposure periods were limited to 1 minute every 15 minutes.

      "Exposures took place at 15 minutes intervals, which began 180 minutes after applications of the test material at the Forest site, and 150 minutes after applications at the Lakeside grassland site (except on subject 69 at 105 minutes). A technician advised subjects when the 1 minute [exposure] period began and ended...at the end of each 1 minute exposure period, subjects moved into a screen house." pg. 9.

      "A technician recorded data for each subject on a data sheet every 15 minutes, after each exposure." pg. 10.

   c) Prior to testing, field sites were monitored for detection of mosquito-borne pathogens. Post testing, collected mosquitoes were screened for viruses.

      "At time of testing, no mosquito pool or sentinel chicken flocks in either of the counties in which testing was conducted had been positive for WNV in 2007." (pg. 7).

      Viral Assays were conducted post test at University of California Center for Vector-borne disease. Taqman multiplex RT-PCR assays that screened for WNV, Western Equine Encephalitis, and St. Louis Encephalitis were conducted post test on collected mosquitoes. (pg. 7).

      "None of the submitted species or pools was positive for any of the viruses." (pg. 14).

   d) Exposure to mosquitoes

      "All subjects wore head nets and surgical gloves in addition to Tyvek coveralls, and each carried a mechanical aspirator." pg. 9.

   e) In the field, test subjects were arrayed in pairs to facilitate removal of mosquitoes "with intent to bite" and data collection.

      "Treated subjects were partnered into groups of two. Each member of a partner pair was instructed to monitor their own exposed limb and that of their partner for

mosquito landings during one-minute periods of exposure to mosquitoes (a "buddy system")" pg. 9.

f) The number of negative control subjects is limited to 2 experienced personnel, attended by 2 assistants.

"Ambient LIBe pressure was measured by 2 experienced personnel on the same schedule as that for repellent exposure. These negative control subjects were attended by 2 assistants who use mechanical aspirator ..." page 11.

The study report does not mention pre-training of subjects in the laboratory to handle mosquitoes using mechanical aspirators in the field.

STUDY SUMMARY:

MRID 47217601

Dosimetry: was conducted outdoors adjacent to the Arthropod Behavior Laboratory at Carroll-Loye biological Research on July 10, 2007. (pg. 7). Subject 17 participated in Dosimetry on July 11.

Page 7, ...to determined dose, the surface area of lower limbs was determined for individual subjects based on length and average of 4 evenly spaced circumferences taken from each limb. Only legs were used in the study because biting intensity was higher on legs than arms.

Page 8, ...subject practiced application once and repeated applications 3 times. Four circumference bracelets (dosimeters) were replaced with new ones after each application. Dosimeters and the pump spray container were weighted before and after each application.

Page 8, Dose calculation:

"Mean dose weight was calculated for each subject based on weight increment of the dosimeters, multiplied by the quotient of the limb surface area divided by the dosimeter surface area. The calculations yielded a dosing rate of grams / sq. cm. per subject. The grand mean of all 10 subjects was used as the dosage rate for efficacy testing. Applications were done volumetrically based on the specific gravity of the formulation (0.90 g/ml; Appendix 10), and individually adjusted to the limb surface area of each subject.

Page 9, Dose rate = 0.00047 ml/sq.cm
Application was conducted using 3 ml syringes (0.01 ml measurement increments) and spread evenly with 2 finger tips in surgical gloves.

Field tests: were conducted in the field in Glenn and Butte Counties, California.

Glenn County on July 12 and 13, 2007. Habitat description: native forest.

Butte County on July 15, 2007. Habitat description: grassland habitat, with scattered shrubs and small trees around small lake.

Page 7, sites were chosen based on mosquito and virus surveillance data compiled weekly by the California State Department of Public Health. No mosquito pools or sentinel chicken flocks had been positive for WNV in 2007.

Environmental conditions:

Were recorded hourly, and conditions were humid and mild. At forest site, temperature ranged from 19 to 33 ° C; RH was from 31 to 91 %. Wind speed, from 0.0 to 1.7 mph, and ambient light from 1220 to 17,920 lux. At Lakeside grassland, temperature ranged from 23 to 33 ° C; RH was from 42 to 63 %. Wind speed, from 0.5 to 2.1 mph, and ambient light from 6160 to 7590 lux.

Test subjects:

Ten subjects for dosimetry testing, and ten treated subjects at each site for efficacy testing.

Studies at Glenn County, forest site, were conducted with 4 subjects on July 12 (3 female and 1 male), and with 6 others on July 13 (3 females and 3 males). The test at Butte County lakeside site was conducted with 10 subjects on July 15 (3 females and 7 males)).

Page 7, mosquito identification: landing mosquitoes were collected using mechanical aspirators, and transported to the lab alive for ID and screening for viruses (WNV, Western Equine Encephalitis, and St. Louis Encephalitis). Virus screening was performed at University of California Center for Vector-borne disease employing Taqman multiplex RT-PCR assays.

Page 8, subjects were treated in advance and instructed to minimize abrasion of the treated skin.

Statistics

Page 7, Justification for sample size: "to give a reasonably large statistical population size without exposing too many individuals to the minor but present risk of exposure to biting arthropods."

Page 10, Data Analyses.

"Dosimetry analyses, based on subjects' means, consisted of nonparametric rank and correlation tests, and parametric regression."

Endpoint: CPT, the period between application and First Confirmed LIBe. The mean CPT was calculated for all 10 subjects at each site and presented with a 95% confidence interval. Kaplan-Meier median CPT, and CPT survival plots were also generated.

Page 11 and 12, **Results**

Dosimetry:
The mean dose rate = 0.00043 ± 0.00021g/sq. cm
Applied dose for testing efficacy = 0.00047 ml/ sq. cm (70% lower than industry standard). The average dose applied per subject leg was 0.50 ± 0.22 ml.

Table 2 Dosimetry results

MRID 47217601	
Repellent Tested	Oil of lemon Eucalyptus (30% OLE)
Total mean product dose applied per subject (g)	0.47
Mean product dose applied (g/600cm^2)	0.26
Mean product dose applied (g/cm^2)	0.000425 (0.00043)
Mean OLE dose (g/cm^2)	0.00013
No. Subjects	10

Efficacy:
At the Glenn County site the reported complete protection time (CPT) ranged from 4.00 to 8.25 hours (Mean CPT = 6.1 ± 1.5 hours; 95% CI 5.0-7.2 hours). Subjects experienced an average of 2.9 ±1.3 LIBes. All subjects received confirmed LIBes. The Kaplan-Meier median CPT was 6.25 hours. For the Butte County site the reported complete protection time (CPT) ranged from 3.25 to 5.75 hours (Mean CPT = 4.2 ± 0.8 hours; 95% CI 3.6-4.8 hours). Subjects received an average of 2.2 ± 0.4 LIBes. All subjects received confirmed LIBes. The Kaplan-Meier median CPT = 4.00 hours.

Table 3. Summary of repellency field trial results

MRID 47217601	
Repellent Tested	Oil of Lemon Eucalyptus (30 % OLE)
Pooled Median CPT (hrs) (Glenn and Butte Counties, July 12, 13, 15))	4.75
Pooled Mean CPT (hrs) (Glenn and Butte Counties, July 12, 13, 15)	5.22±1.50
Mean OLE dose (g/cm^2)	0.00013
Mean product dose (g/cm^2)	0.00043
Glenn County Site July 12-13 Median CPT (hrs) Mean CPT (hrs)	 6.25 6.1 ± 1,5
Butte County Site July 1 Median CPT (hrs) Mean CPT (hrs)	 4.0 4.2 ± 0.8

Ambient LiBing was 1 per minute at both sites in all periods. (Appendix 1).

Mosquito species at Glenn Co, *Aedes melanimon, **Ae. Vexans*** **most predominant species**, and *Anopheles freeborni*
Mosquito species at Butte Co, *Aedes melanimon, Ae. Vexans, **Anopheles freeborni*** **least predominant species**, and *Culex tarsalis.* (Appendix 12).

Concluding Remarks:

The Human Study Review Board stated in their science assessment of WPC-001, October 25, 2007, that the data generated by this study is usable, and that the identified protocol deviations are unlikely to have a significant effect on test results.