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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

September 27, 2007

MEMORANDUM

SUBJECT: Science review of DEET study reports of completed efficacy studies for mosquitoes.

FROM: Kevin J. Sweeney, Senior Entomologist
Insecticides Branch
Registration Division (7505P)

TO: Marion Johnson, Chief
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RE: Carroll, S. (2007). Test of Dermaegis LipoDEET 302 Personal Insect Repellent, EPA Reg. No. 82810-1 (MRID 47211901).

Carroll, S. (2007). Test of Coulston's Duranon Personal Insect Repellent, EPA Reg. No. 50404-8 (MRID 47211801).

Carroll, S. (2007). Test of Dermaegis LipoDEET 3434 personal Insect Repellent (MRID 47208401).

ACTION REQUESTED

Conduct a science review of three completed studies. Evaluate and assess efficacy of the subject repellent formulations. Determine the adequacy of the methods employed and the scientific validity of the reported data.

CONCLUSIONS

Studies MRID 47211801, MRID 47211901, and MRID 47208401 were conducted in accordance with Good Laboratory Practices but provided scientific data that may not be reliable because the experimental design, which deviated significantly from the SCI-001 protocol, introduced

additional sources of variation into the experiment that were difficult to account for in the data analysis. Based on the experimental results, LipoDEET 302 (30% DEET) (EPA Reg. No. 82810-1) and LipoDEET 3434 (34.34% DEET) (not registered) performed as well as 3M Ultrathon (34.34% DEET) (EPA Reg. No. 58007-1) while Duranon did not. The Human Studies Review Board and consulting repellent experts will be asked to comment on these data sets and related science issues.

SCIENCE REVIEW

Study Objectives:

To determine the Complete Protection Time (CPT) for three repellent formulations, Dermaegis LipoDEET 302 Personal Insect Repellent (EPA Reg. No. 82810-1), Duranon Personal Insect Repellent (EPA Reg. No. 50404-8), and LipoDEET 3434 (not registered and not pending registration).

Materials & Methods:

Study locations: Two State of California locations were used in this study. **Test Site 1 "lakeside grassland"**, was located in Butte County and will be referred to as "Butte County" in the remainder of this review. **Test Site 2 "forest understory"** was located in Glenn County and will be referred to as "Glenn County" in the rest of this review and data tables.

Date(s) of each study: The dosimetry phase was conducted on July 3-5, 2007. Repellent testing was conducted on July 7-8 and 15, 2007, in Butte County and on July 12-14, 2007, in Glenn County.

Repellents Tested: The repellents tested were all DEET based formulations. Dermaegis LipoDEET 302 Personal Insect Repellent (30% DEET) (EPA Reg. No. 82810-1), Duranon Personal Insect Repellent (20% DEET) (EPA Reg. No. 50404-8), and LipoDEET 3434 (34.34% DEET) (not registered and not pending registration). All products were lotions.

Tested positive control/comparison repellent: 3M Ultrathon (34.34% DEET) (EPA Reg. No. 58007-1)

Untreated Control: Two untreated subjects served as "untreated controls" on each test date to monitor ambient mosquito landing pressure.

Protocol: Protocol SCI-001 was used and was included in Appendix 8 of the study.

Experimental design: The study was conducted at two sites on July 7-8 and 12-15, 2007. The test sites represented different ecological habitats, which differed in the mosquito fauna and population size present. Ten subjects each were randomly assigned to one of four repellent treatments per site for a total of 10 subjects per treatment at each site. Subjects did not know the identity of a repellent treatment at the time of testing. However, only 12-15 subjects were tested

each day instead of the expected 40—field testing was conducted at each site on three different days. Repellent doses were prepared for each subject based on the surface area of the lower leg. The dosing rate was based on the results of a dosimetry analysis performed for each product in early July with a sample of ten subjects participating in the study. Untreated control subject and subjects treated with repellent were exposed to mosquitoes for one minute every 15 minutes until the repellent failed. Mosquitoes landing with intent to bite were recorded and aspirated into containers. Collected mosquitoes were identified and pooled for viral detection assays employing the Polymerase Chain Reaction (PCR) methodology.

Data analyses Subjects remained in the test until the repellent failed as determined by the first confirmed landing with intent to bite. The time at which the repellent failed equaled the Complete Protection Time (CPT), and a CPT was recorded for each subject. The CPT for treated subjects where product failure did not occur equaled the test period length. Collected data were analyzed by Kaplan-Meier survival analysis to determine the median CPT. The mean CPT for each repellent was also calculated. The CPT results for each repellent were reported as median CPT and mean CPT$\pm$ SD based on a 95% confidence interval. Duranon vs. 3M Ultrathon were compared using an Analysis of Variance (ANOVA) to test if Duranon differed significantly from the positive control/comparison test article 3M Ultrathon.

Protocol Deviations:

Seven protocol deviations were reported in Appendix 8 of each study. Of these, the most significant is the pre-treatment of subjects with repellent 150-210 minutes before field exposure. An additional deviation was reported in MRID 47208401- the substitution of an unregistered formulation LipoDEET 3434 for the previously approved test substance – Insect-Guard II (EPA Reg. No. 54287-8).

Protocol deviations that were not reported as amendments included: applying different repellents to the same subject every 24 hours; conducting a test each day with less than ten subjects per treatment; pooling results for each repellent collected on different test days; and testing the same repellent at different times of the day on different test days. Treatment allocation was also different than that described in Protocol SCI-001.

Results:

Table 1.
Dosimetry results (see Table 2 in each study)

Report Volume	MRID 47211901		47211801	47208401
Repellent Tested	LipoDEET 302[1] (30% DEET)	3M Ultrathon[1,3] (34.34%DEET)	Coulston's Duranon[1] (20% DEET)	LipoDEET 3434[2] (34.34%DEET)
Total mean product dose applied per subject (g)	1.91	1.47	1.74	1.75
Mean product dose applied ($g/600cm^2$)	1.008	0.7596	0.90084	0.9276
Mean product dose applied (g/cm^2)	0.001608	0.001266	0.001514	0.001546
Mean DEET dose (g/cm^2)	0.000482	0.000434	0.000303	0.000531
No. Subjects	10	10	10	10

[1] The specific gravity of these repellent formulations equaled approximately 1g/ml.
[2] No data provided to support study claim that the specific gravity equals approximately 1g/ml.
[3] The same set of Ultrathon treatment data was reported in each of the three report volumes

Table 2.
Number of subjects treated at each site on each test day
(See Appendices 1 & 2 in each study).

Report Volume		MRID 47211901		MRID 47211801	MRID 47208401	Total Subjects/day
Test Date	Test Site	LipoDEET 302 (30% DEET)	3M Ultrathon[1] (34.34%DEET)	Coulston's Duranon (20% DEET)	LipoDEET 3434 (34.34%DEET)	
Jul 7	Butte County	4	3	3	4	14
Jul 8		2	3	5	3	13
Jul 12	Glenn County	3	4	4	4	15
Jul 13		3	3	3	3	12
Jul 14		4	3	3	3	13
Jul 15	Butte Cty	4	4	2	3	13
Total Subjects/ formulation		20(10/site)	20(10/site)	20(10/site)	20(10/site)	80

[1] The same set of Ultrathon treatment data was reported in each of the three report volumes. These results are reported here only once to avoid confusion about total number of subjects tested on each date.

Table 3.
Mosquito species and relative population abundances
(See Table 3 and Appendix 9 in each study).[1]

Species collected	No. Mosquitoes Butte Site	% Abundance Butte Site	No. Mosquitoes Glenn Site	% Abundance Glenn Site	Disease vector?	Disease pathogen detected?
Ae. melanimon	178	75%	20	7.9%	WEE	No
Ae. vexans	23	9.2%	216	86.1%	No	No
An. freeborni	2	0.8%	15	6%	Malaria	Malaria is not endemic
Cx. tarsalis	36	15%	0	0%	WNV SLE	No No
Total	239	100%	251	100%	---------	No

[1]There were no virus isolations from any of the collected mosquitoes.

Table 4.
Summary of repellency field trial results (see study table 4 and appendix 1)

	MRID 47211901		MRID 47211801	MRID 47208401
	LipoDEET 302 (30% DEET)	3M Ultrathon[1] (34.34%DEET)	Coulston's Duranon (20% DEET)	LipoDEET 3434 (34.34%DEET)
Pooled Median CPT[2] (Butte & Glenn)	10.1	10.4	8.8	9.9
Pooled Mean CPT (Butte & Glenn)	9.9 $\pm$ 1.55	10.1 $\pm$ 2.3	8.83 $\pm$ 1.6	10.5 $\pm$ 1.6
Mean DEET dose (g/cm^2)	0.000482	0.000434	0.000303	0.000531
Mean product dose (g/cm^2)	0.001608	0.001266	0.001514	0.001546
Butte site Median CPT (hrs)	10	10.25	8.4	9.75
Butte site Mean CPT (hrs)	10.3 $\pm$ 1.3	10.1 $\pm$ 2.3	8.4 $\pm$ 1.9	10.6 + 1.3
Glenn site Median CPT (hrs)	10.25	10.5	9.25	10
Glenn site Mean CPT (hrs)	9.5 $\pm$ 1.8	10 $\pm$ 2.2	9.5 $\pm$ 1.3	10.4 +1.9

[1]The same set of Ultrathon treatment data was reported in each of the three report volumes.
[2]Pooled median CPT values are provided here as a comparison to the mean CPT values.

Adequacy of the Methods and Experimental Design Employed

The methods employed in all three studies were adequate to produce scientifically reliable data but changes made to the experimental design introduced sources of variation not discussed in SCI-001 or in protocol amendments. The study protocol (SCI-001) was revised in accordance with EPA and HSRB scientific recommendations before testing began and below I discussed the incorporation of the HSRB recommendations from the January 2007 Meeting Report together with the significance of changes to the experimental design to the collected data.

1. *Experimental Design. "…No where is it justified the randomization to left and right limbs..."* Limb treatment was not random. The same limb was treated on most subjects.

Data were collected at more than one site but not on the same day with the same number of subjects (Table 2 above). For example, ten subjects were treated with LipoDEET 302 in this experiment but of these - four subjects were tested on July 7, two on July 8 and four on July 15. This is referred to in each study (pp. 7) "…distributed the repellents as evenly as possible across the days at each site." These results were pooled and analyzed. Many subjects were treated with a different repellent from one day to the next on the same limb while some subjects were treated with same repellent on more than one day on the same limb. Temporal distribution was also different "…On one day at each site, testing was initiated later in the morning, thus permitting sampling to extend well into the dusk hours when different mosquitoes might become active."

2. *Sample Size. "Including 10 subjects per treatment is probably sufficient, but the justification provided by the investigators is not convincing."*

Sample size justification was not addressed beyond the initial protocol presented to the HSRB in January 2007. Sample was 20 replicates for each treatment, consisting of ten subjects (n =10) per test site per treatment.

3. *Sample Size Consideration for Subject Drop-outs.* Subjects did not drop out of this study and missing values were not a source of variation. However, the revised protocol does not address this concern directly.

4. *Dose. "Typical consumer dose and known toxicity benchmarks should be clearly identified."* The study fully addressed this recommendation. The dosimetry results are presented in Table 1 above. The mean repellent dose applied for each product was nearly the same and approximated the industry standard of 1g/600 cm^2 with the exception of the Ultrathon where the product dose was less.

5. *Statistical Analysis and Assumption of Normality of CPT Measurements. "In choosing statistical analysis the investigator must select the appropriate model for the distribution of the data that will be used."* The statistical analysis was revised to include Kaplan-Meier analysis (non-parametric test) in order to determine the median CPT value for LipoDeet 3434, LipoDEET 302, Duranon, and Ultrathon, respectively. Normality was not assumed due to the small sample sizes, thus, normalized CPT values were not reported. Computerized statistical analyses were

conducted on the dosimetry and experimental data employing SAS JMP Version 5.0.1.2 (SAS Institute, Cary NC). An analysis that determined if the three tested repellents differed from one another or if there was a "date effect" (treatments took place on different days) was not conducted. The study director justified the lack of the "date effect" analysis due to "small sample size for each treatment per day". These small sample sizes resulted from deviations from the experimental design of Protocol SCI-001.

Degradation of repellent efficacy over time was evaluated with the Kaplan-Meier analysis. Survival plots graphically depict the results in each of the studies. No reference to a "linear model" is made in the study or in the revised SCI-001 protocol. An ANOVA was applied to non-transformed CPT values to determine if the CPT values of Duranon and Ultrathon repellents differed significantly ($p < 0.05$; Table 5 of MRID 47211801). This analysis examined the influence of the two repellents, Duranon and Ultrathon, the test sites and the interaction (combined effect) of repellent*site. In every case, the degrees of freedom (df) was only equal to "1" (n = two sites; n= two repellents; df = n-1). The other two studies did not discuss or report this analysis. Instead, pooled mean CPT (see Table 5 in MRID 47208401) values or a discussion on the lack of median CPT differences ("Conclusion" in MRID 47211901) are the basis of comparison between the LipoDEET 302 or LipoDEET 3434 and Ultrathon. The investigator also does not explain why all the survival plots begin at six hours, but does mention that the plots would appear more similar had the entire testing period been plotted.

Discussion of Results and Conclusions

The final science recommendation from the HSRB January 2007 report was:

Interpretation of the results. "Results from the study need to be interpreted judiciously. Given the large variability in individual attractiveness to mosquitoes, the small sample size seriously limits conclusions that the sample is representative of the population of individuals who might eventually be users of these products…"

The study results are summarized in Tables 3 and 4 above. As was expected of sites with differing ecological characteristics, Table 3 shows that the mosquito species composition at each site was very different. Testing in Butte County evaluated each repellent against *Ae. melanimon* while testing in Glenn County evaluated repellent success against *Ae. vexans*. The repellency results presented in Table 4 show, based on median CPT values in which all bites are incorporated into the survival analysis, that Ultrathon was the best performing repellent product followed by LipoDEET 302, LipoDEET 3434 and Duranon. Ultrathon delivered the lowest dose of DEET per square cm of skin surface.

Generally, repellent studies are conducted in a fashion that allows a full treatment (inclusive of all replicates) to be evaluated on each day of the experiment at each experimental site. Treatments are often repeated on different days and results compared for any effects due to conditions on the day of the experiment. In the experiments conducted according to Protocol SCI-001, this was not the case. At a minimum, the same 10 Ultrathon treated subjects should have been evaluated each day at each site, as would be appropriate for a positive control

treatment to detect or confirm the lack of site and/or date effects. If there were no date or site effects, then an argument for use of less than 10 replicates per treatment for the other repellents might have been acceptable. Conversely, significant differences in Ultrathon CPT values due to date or site effects would invalidate the reduced replicates approach and pooling of data from different days for each treatment.

The variance in the experiment is better understood by examining the mean CPT values, each one with a large standard deviation around the calculated mean. When the mean CPT values for the tested repellents are compared, the confidence intervals overlap. Thus, there is no significant difference between mean CPT values - between sites or treatments - except that Ultrathon outperformed Duranon in these tests. LipoDEET 302 and LipoDEET 3434 were essentially equivalent to Ultrathon.

In conclusion, the data collected from this experiment shows that LipoDEET 302 (30% DEET) (EPA Reg. No. 82810-1) and LipoDEET 3434 (34.34% DEET) using the formulation tested in this study (not registered), performed the same as 3M Ultrathon (34.34% DEET) (EPA Reg. No. 58007-1). On the other hand, Duranon (20% DEET) (EPA Reg. No. 50404-8) did not perform as well as Ultrathon or the other repellents tested.

The scientific validity of the results is questionable given the large variance in the CPT values and use of an experimental design that did not enable the investigator to account for any effects due to date or site due to small sample sizes. EPA will consult the HSRB and consulting experts for their opinions on the scientific validity of the collected data.