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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

September 23, 2008

SUBJECT: Science Review of Human Study of Mosquito Repellent Performance

FROM: Kevin J. Sweeney, Senior Entomologist
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TO: Marion Johnson, Chief
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RE: Carroll, S. (2008). Efficacy Test of KBR 3023 (Picaridin:Icaridin)-based Personal Insect Repellents (20% Cream and 20% Spray) with Mosquitoes under Field Conditions. Document dated August 5, 2008. Unpublished document prepared by Carroll-Loye Biological Research under Protocol ID LNX-001 MRID 47506401 339pp.

ACTION REQUESTED

Conduct a science review of a completed mosquito field study. Determine the adequacy of the methods employed and the scientific validity of the reported data. Evaluate and assess if the tested products repel adult mosquitoes for up to eight hours. These data were required by the EPA as a registration condition for the following products: EPA Reg. No. 39967-50 KBR 3023 All-Family Insect Repellent Cream (20% picaridin cream) and EPA Reg. No. 39967-53 KBR 3023 All-Family Insect Repellent Spray (20% picaridin pump-spray).

CONCLUSIONS

Scientific aspects of the research were assessed in terms of the recommendations of the draft EPA Guidelines §810.3700 and of the EPA Human Studies Review Board. Study MRID 47506401 was conducted in accordance with Good Laboratory Practices as described in 40 CFR §160, and provides scientific data that are acceptable. Based on the experimental results, KBR 3023 Insect Repellent Cream repelled mosquitoes for 12 hours while KBR 3023 All-Family Insect Repellent Spray repelled mosquitoes for

approximately 14 hours. These results support the hypothesis that each of these products repel mosquitoes for up to 8 hours. The Human Studies Review Board will be asked to comment on this study.

SCIENCE REVIEW

Study Objectives: To determine the Complete Protection Time (CPT) of two registered mosquito repellent formulations containing picaridin against adult mosquitoes under field conditions. The study shall establish the mean time to first confirmed landing for each formulation under field conditions to support the conditionally accepted repellency claim "Repels mosquitoes for up to 8 hours."

Materials & Methods:

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

Study Dates: Repellent product tests were conducted on June 7, 2008 at Site 1-Butte County and on June 15, 2008 at Site 2- Glenn County. Dosimetry testing was conducted on May 14-23, 2008 in the Arthropod Behavior Laboratory at Carroll-Loye Biological Research.

Repellents Tested: The repellents tested were EPA registered products consisting of EPA Reg. No. 39967-50 KBR 3023 All-Family Insect Repellent Cream (20% picaridin cream – Repellent 'A') and EPA Reg. No 39967-53 KBR 3023 All-Family Insect Repellent Spray (20% picaridin pump-spray –Repellent 'B'). Based on the dosimetry data the application of picaridin, when expressed in terms of mg per cm^2 of skin surface, was approximately three times greater for the cream product when compared to the pump-spray product.

Tested positive control/comparison repellent: None

Untreated Control: Two experienced negative control subjects (one male and one female) established and monitored the ambient Landing with Intent to Bite (LIBe) pressure at the same intervals as for repellent exposure; one minute every 15 minutes. There were no statistical comparisons to the untreated controls.

Number of Test Subjects/Treatment Regime: A total of 45 subjects (selected from a pool of 112 subjects that were diverse in age and ethnicity) participated in this study. There were ten test subjects (five male and five female) in the dosimetry phase. In the test phase, ten subjects participated in each product treatment test on each day.

Protocol used including amendments: Protocol LNX-001 was used as amended on June 8 and August 14, 2007. The amended protocol can be found in Appendix 7 of the study.

Protocol Deviations:

Failure to sign Informed Consent Form. On March 31, 2008 Carroll-Loye Biological Research reported to the IIRB that a staff member failed to sign the Informed Consent form for one of two test subjects enrolled March 23 2008 prior to scanning the document for electronic submission to the IIRB.

Use of historical limb measurement data: Page 276 of the report reports a deviation in which the study director used historical measurements of subjects whom previously participated in the last two years instead of measuring each subject before testing as specified in the protocol. This was reported to the IIRB on July 6, 2008.

Failure to follow-up on receipt of Amendment 2 by the IIRB. The protocol was executed without approval of Amendment 2 because it was not received by the IIRB. On July 20, 2008 this deviation was reported to the IIRB.

Reporting of treatment times on June 7, 2008 (see study page 113): This deviation was not reported to the IIRB before or after study execution. This is a deviation from the approved protocol and can be compared to the data collected on June 15, 2008 (see study page 114) where Dr. Carroll followed the approved protocol. On June 7 the treatment time for all subjects was reported as 8:00 a.m. EPA asked Carroll-Loye Biological Research on August 8, 2008 to explain the discrepancy. Dr. Carroll responded on September 5, 2008:

> *"Organizing efficacy data collection, and analyzing those data for CPT, is simplest if we have the same application time for all subjects. While this is not strictly possible or practical, we strive to organize applications to come close to that ideal. Our past practice has been to carry out all applications over periods ranging from about 10 to 20 minutes, using the rule of thumb that the mean time of application is a good approximation for all subjects. This approach is justified by the small fraction of a typical CPT that such introduced variation represents, variation that should not influence our estimated CPT mean, or likely, variance. Subjects are asked to arrive well before the target application time so that limb washing and Tyvek suit donning can be completed first. Ideally, we will have subjects lined up and ready for application, permitting each to be applied within ± 5-10 minutes of the target application time.*
>
> *We have considered such time frames to be narrow enough to record all subjects as receiving applications simultaneously. However, given the marginal value of greater precision, in LNX-001 we proposed to record individual application times. On the first day of the study, however, we inadvertently adhered to our conventional procedure and recorded a mean application time of 0800 for a*

series of applications made by four technicians between, approximately, 0745-0810. On the second test day, we recorded actual application times, and for even greater completeness, included technician initials for the first time as well.

Our practice on the first day was thus a minor protocol deviation that we did not note as such at the time, and hence did not report to the IRB. The implications of this deviation for the data set or the subjects rights and safety appear small.

On September 10, 2008 Mr. King of Carroll-Loye research reported that this additional deviation had been reported to the IRB.

The significance of these deviations and any possible impact on CPT values for that day is discussed in the "Discussion, Conclusions, and Recommendations" section of this review.

Experimental design: The test sites represented different ecological habitats that had similar mosquito fauna and population size present. Abiotic factors were recorded hourly including temperature, wind speed, relative humidity and light intensity. Ten subjects each were randomly assigned to one of two repellent treatments per site for a total of ten subjects per treatment at each site. The sample size of ten treated subjects per test material per field trial is larger than is required by EPA guidelines —large enough to ensure robust averages across subjects. Repellent doses were prepared for each subject based on the historic surface area of the forearm for the June 7 test date and based on the historic surface area of the lower leg for the June 15 test date. The dosing rate for the test subjects was based on the results of a dosimetry analysis performed for each product in May with a sample of ten subjects participating in the study. In each case, half the subjects on the test date were treated on the right limb and the other half on the left limb. On June 7 and 15 repellent treatments made to limbs were distributed as follows: four right arms and six left arms were treated with Repellent A while Repellent B was applied to six right arms and four left arms on each test date. Each treatment was applied to an equal number of males and females. The product application rate for repellent B was 0.00251 ml product/cm^2 while Repellent A was applied at the rate of 0.00097 ml product/cm^2. Subjects were treated before going to the field. On June 7 the pretreatment was reported as two hours before exposure. On June 15 the pre-treatment ranged from a little more than two hours to a little less than three hours. On both dates untreated control subjects 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. Site 1 was sampled on June 7 while Site 2 was sampled on June 15, 2008. Mosquitoes were assayed for West Nile Fever virus, Western Equine Encephalitis virus and St. Louis Encephalitis virus. Assays for malaria parasites were not conducted despite the presence of a vector because the disease is not endemic to the United States.

Data analysis: Subjects remained in the test until the repellent failed as determined by the first confirmed bite (FCB), or until the end of the 14-hour test period, whichever came first. 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. Mean CPT for each repellent was reported as mean CPT $\pm$ SD with the respective 95% confidence interval; and the Kaplan-Meier median CPT values were reported when calculable. The mean number of landings with intent to bite (LIBe) was also reported for each product treatment.

Response to Comments in EPA Protocol Review dated May 24, 2007. Science deficiencies noted in my review are listed below together with modifications in the subject protocol/study:

- *No explicit hypothesis is stated.*

  The HSRB deemed a hypothesis to be unnecessary, and no change was made in the protocol.

- *No explanation is given for employing a negative control in the dosimetry assay as stated in §6.2.1 on p. 11*.

  This is addressed in the Protocol Amendment dated June 8, 2008. Section 6.2.2 reads: 'Dosimetry testing requires an untreated control for the possibility that dosimeters will gain significant weight from contact with untreated skin.'

- *Information on diagnostic statistical tests for normality, or information on how to analyze non-normally distributed data is lacking.*

- *Justification is needed for use of Kaplan-Meier statistical analysis*

  Some of the information mentioned in bullets 3 and 4 is included in the study data analysis discussion, but the protocol was not amended to address these points. In the submitted study the report simply states how the data were analyzed and reported. No justification is given for the application of non-parametric statistical procedures.

- *The procedure by which limb surface area will be measured is not described in detail. The exact location of the 4 dosimeters should be recorded for later placement at the same limb location, and their length before and after application of the test material should coincide.*

  The method used for measurement was not changed in the protocol to include recording the specific location of intermediate circumferences. However, the data sheets mentioned the exact locations for pump-spray and aerosol studies.

The protocol should be amended to include these locations for all test products.

Results:

Results were reported in table form and the degradation of the repellent was plotted for illustrative purposes in Figures 1a and 1b in the study report. Site-specific data for each repellent were not pooled. As presented in Table 1 below, the mean CPT values for both products with their associated standard deviations were near their respective 95% confidence intervals. The variance in the experiment was small. Median CPT values were nearly the same as the mean CPT values for the 20% spray. Median values could not be calculated for the 20% cream product. Based on the dosimetry data the application of picaridin, when expressed in terms of mg per cm^2 of skin surface, was approximately three times greater for the cream product when compared to the pump-spray product yet the CPT values differed little. The mosquito species composition and population size were similar between sites (Table 2 below) but not identical.

Table 1
Repellent Lab Trial Results
(See Table 10 and 11 in MRID 47506401)

	EPA Reg. No 39967-53 (20% picaridin cream) Test Substance 'A'	EPA Reg. No. 39967-50 (20% picaridin spray) Test Substance 'B'
Site 1 Butte Mean CPT $\pm$ SD (hrs)	14.0 + 0.0 (14.0-14.0)	11.6 $\pm$ 1.8 (10.4 - 12.9)
Site 1 Butte Median CPT (hrs)	------	11.3
Site 1 Butte Mean LIBe	0.1 $\pm$ 0.3	2.0 $\pm$ 1.4
Site 2 Glenn Mean CPT $\pm$ SD (hrs)	13.5 $\pm$ 1.1 (12.7-14.3)	11.6 $\pm$ 1.5 (10.5 -12.7)
Site 2 Glenn Median CPT (hrs)	-------	11.7
Site 2 Glenn Mean LIBe	1.9 $\pm$ 1.4	2.4 $\pm$ 0.5

Table 2[1]
Mosquito species and relative population abundance
(See Appendix 6 in MRID 47506401)

Species	Site 1: Butte County June 7, 2008		Site 2: Glenn County June 15, 2008		Disease Vector?	Pathogen Detected?
	No. Mosquitoes	% Abundance	No. Mosquitoes	% Abundance		
Aedes melanimon	149	68	96	50	WEE	No
Ae. vexans	29	13	26	14	No	No
Ae. increpitus	11	5	0	0	No	No
Ae. sierrensis	7	3	5	3	No	No
Ae. nigromaculis	1	<1	0	0	No	No
Ae. sticitcus	0	0	4	2	No	No
Culex tarsalis	17	8	23	12	WNV SLE	No
Anopheles freeborni	5	2	37	19	Malaria	No
An. franciscanus	1	<1	0	0	No	No
Total	220	≈100%	191	≈100%	—	None

[1]No viruses were isolated from any of the collected mosquitoes.

Discussion

The methods employed in these studies were adequate to produce scientifically reliable data. They were based on study protocol LNX-001 as amended on June 8 and August 14, 2007 and on April 25, 2008, in accordance with EPA and HSRB recommendations before testing began. Three protocol deviations were reported to and accepted by the IRB, and described in the study report. One additional deviation was noted in EPA's review.

I do not believe that the use of historical limb measurement data had a significant impact on the scientific outcome of the experiment because it did not have an impact on study results. Limb measurements were unlikely to change for each subject.

The two other deviations reported were non-substantive in nature and did not affect the design or conduct of the research, or the resulting data.

The unreported deviation involved recording a common mean treatment time on June 7 instead of reporting the exact treatment time for each subject as was specified in

the protocol and done on June 15. This deviation had no significant effect on the science outcome of the experiment based on an examination of the treatment data collected on June 15, 2008 (pp. 114 in the study). For "Repellent 'A', the 20% cream, the mean CPT value is 7:28 a.m. ± 10 minutes. Six subjects were treated after that time and four before with treatments beginning at 7:12 a.m. and ending at 7:41 a.m. 'Repellent B', the 20% spray, was applied to the subjects between 7:10 a.m. and 7:50 a.m. The mean time of application for all ten subjects was 7:38 a.m. ± 14 minutes. Four subjects were treated before that time and six after that time. The CPT reported in this study for June 15 would be overestimated by about 0.2 hours if a mean application time was employed as the starting point for CPT calculations. Therefore, use of a mean start time on June 7 from which to calculate the CPT for each treated subject did not have significant influence on the mean CPT value reported for June 7 nor did it harm subjects.

Conclusions

The data collected from this experiment show that EPA Reg. No. 39967-50 KBR 3023 All-Family Insect Repellent Cream (20% picaridin cream) and EPA Reg. No. 39967-53 KBR 3023 All-Family Insect Repellent Spray (20% picaridin pump-spray) provided a CPT of 14 hours and 12 hours, respectively, against mosquitoes under field testing conditions.

Recommendation: The study is scientifically sound and acceptable. The study shows that each product repelled mosquitoes for more than eight hours. The HSRB will be asked to assess the noted deviations in light of the Human Studies Rule.

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