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

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

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SUBJECT: Science Review of Human Study of Tick Repellent Performance

FROM: Kevin J. Sweeney, Senior Entomologist
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TO: Marion Johnson, Chief
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RE: Carroll, S. (2010) Efficacy Test of KBR 3023 (Picaridin; Icaridin)-based Personal Insect Repellents (20% Cream and 20% Spray) with Ticks under Laboratory Conditions. Unpublished study prepared by Carroll-Loye Biological Research under Project No. LNX-003. 168 p. (MRID 48053801)

ACTION REQUESTED

Conduct a science review of a completed tick repellency study. Determine the adequacy of the methods employed and the scientific validity of the reported data. Determine the duration of tick repellency from the test data for the tested products. As explained in the study the collected repellency data will be used to support the efficacy data requirements for the following conditionally registered products: EPA Reg. No 39967-50 (KBR 3023 All-Family Insect Repellent Cream) and EPA Reg. No. 39967-53 (KBR 3023 All-Family Insect Repellent Spray). Both products contain 20% picaridin.

CONCLUSION

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 LNX-003 was conducted in accordance with Good Laboratory Practices as described in 40 CFR §160, and provides scientific data that are acceptable. The Human Studies Review Board will be asked to comment on this study.

SCIENCE REVIEW

Study Objectives: To measure the Complete Protection Time (CPT) of two conditionally registered tick repellent products containing 20% picaridin against two species of nymphal

ticks under laboratory conditions. The study shall establish the mean and median times to First Confirmed Crossing (FCC) for each formulation for each tick species tested.

Materials & Methods:

Study location: This study was performed in the Arthropod Behavior Laboratory at Carroll-Loye Biological Research in Davis, California.

Study Dates: The study was initiated on January 15, 2010. Repellent product testing was conducted on January 23-24, 2010.

Repellents Tested: EPA Reg. No 39967-50 (KBR 3023 All-Family Insect Repellent Cream) and EPA Reg. No. 39967-53 (KBR 3023 All-Family Insect Repellent Spray). Both products contain 20% picaridin.

Dose rates: The standard dose used for the spray product was based on dosimetry testing with the same materials reported in study LNX-001. The standard dose used for the cream product was determined by pooling data from study LNX-001 with additional dosimetry data collected in the related study LNX-002 (black fly field study). Volumetric dose rates were expressed in micro-liters per square centimeter of treated skin ($\mu l/cm^2$). The dose applied to each subject was calculated based on the measured skin area of the treated limb, and was reported in milliliters (ml). Volumetric doses were converted to mass doses expressed in milligrams using the specific gravity of formulations—0.98 for lotion, and 0.96 for spray. For the spray product each subject received $0.97\mu l/cm^2$ of product, equivalent to 0.9312 mg product/µl. For the cream product, the volumetric dose rate was $1.94\mu l/cm^2$, equivalent to 1.9012 mg product/µl. Because both products contain 20% picaridin, the average picaridin dose was 1/5 the average product dose. For the spray product the mean picaridin dose was 100 mg per subject and 192 mg/subject for the cream product. MOE calculations were based on an assumed 70 kg subject and the acute dermal LD_{50} value for picaridin at the limit dose of greater than 2,000 mg/kg. For the cream product the MOE = 741 and for the spray product the MOE = 1429, both values exceed the target MOE = 100.

Positive control/comparison repellent: There was no positive control.

Untreated Control: Each treated subject served as their own untreated control. Tick questing behavior was confirmed on the untreated arm of each subject before the tick was used for repellency testing.

Number of Test Subjects/Treatment Regime: Sample size was adequate, consistent with a previous protocol reviewed favorably by the HSRB and accepted by EPA, and substantially exceeded the recommendation of the current EPA guideline. A total of 23 subjects (selected from a pool of 119 subjects diverse in age and ethnicity) participated in this study. Three were alternate subjects; twenty were treated. In the test phase, ten subjects participated in each product treatment test on each day. Treatments were randomized within each gender. There were an equal number of male and female test subjects. Each subject participated on

only one day of the test, but testing included both tick species. All ticks, whether or not repelled, were removed from the arm of the subject before they had time to bite.

Protocol Amendments: Protocol LNX-003, dated July 27, 2009, begins on page 97. Amendment 1, dated October 30, 2009, begins on p. 135. This amendment fully addressed the EPA's comments in its review of the protocol, and responded to HSRB comments at the meeting in October 2009.

Protocol Deviation: No protocol deviations were reported for this study.

Experimental design: The experimental design was very similar to recent Carroll-Loye Biological Research studies. Twenty subjects (10 male and 10 female) were randomly assigned by gender to one of two repellent treatments for a total of ten subjects per treatment. The sample size of ten treated subjects per test material is larger than is required by EPA guidelines —large enough to ensure robust averages across subjects.

Subjects treated with the cream formulation are reported as 'A' treatments while subjects treated with the spray formulation are reported as 'B' treatments. One arm of each subject was treated with repellent and the other arm was untreated. Different subjects were used on each day of the study. Repellent doses were prepared for each subject based on the surface areas of the forearm. In each case, half the subjects on the test date were treated on the right limb and the other half on the left limb. The exact time of repellent application was reported.

During efficacy testing each subject confirmed questing and tested one nymphal tick of each species during each 15-minute period over the course of the test. Exposure to each tick was for a period of 3 minutes on each arm. Further exposures to each species were stopped for any subject who experienced a "crossing" by that species into the treated area of the forearm confirmed by another crossing in either of the subsequent two exposure periods. The details of the how a crossing was determined and evaluated are described in the study in Section 4g on pages 15-16. This endpoint was used to calculate the Complete Protection Time (CPT) for each subject. Test day was ignored as a variable because the environmental conditions were the same and the test was conducted under laboratory conditions.

Tick species and life stage: Two vectors of tick-borne disease in the United States were used in this experiment: the blacklegged tick, *Ixodes scapularis*, the vector of Lyme disease, and the American dog tick, *Dermacentor variabilis*, the vector of Rocky Mountain spotted fever. Both species were tested in the nymphal life stage of development. The total number of ticks tested was 2,274.

Tick disease pathogen detection: Ticks used in this experiment were screened for tick-borne disease pathogens and determine to be disease-free by the U.S. Centers of Disease Control and Prevention in Atlanta, Georgia USA.

Data analysis: Subjects remained in the test until the repellent failed as determined by a confirming crossing, or until the end of the 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. The mean CPT was calculated for each tick species for each repellent. This value was reported as mean CPT ± sd with the respective 95% confidence interval. Kaplan-Meier median CPT values were reported where calculable. An estimate of time to 25% failure for each test product against each tick species was also calculated. Degradation of repellency time of the spray product against both tick species was plotted for illustrative purposes in Figures 1 and 2 of the study report.

Results:

Table 1
Repellent Trial Results
(See Tables 5, 6, 7, 8 and Figure 1 in MRID 48053801)

		Reg No. 39967-50 Cream 20%	Reg No. 39967-53 Spray 20%
Ix. scapularis	Mean CPT ± sd (95% CI)	12.6 ± 4.3 h. (9.5 – 15.7 h)	14.1 ± 1.8 h. (12.7 – 15.4 h)
	Kaplan-Meier Median CPT[1]	--	15.0 h.
	Time to 25% failure	>15.4 h.	13.1 h.
	Mean crossings per subject	2.0 ± 1.2	2.4 ± 2.2
D. variabilis	Mean CPT ± sd (95% CI)	15.3 ± 0.3 h. (9.5 – 15.7 h.)	14.0 ± 1.6 h. (12.8 – 15.2 h.)
	Kaplan-Meier Median CPT[1]	--	14.1 h.
	Time to 25% failure	9.7 h.	12.0 h.
	Mean crossings per subject	1.6 ± 2.0	1.2 ± 1.4

[1] Insufficient data to calculate Kaplan-Meier Median CPT for 20% Cream

In spite of extending the study for the extraordinarily long duration of 15.25 h., more than half the subjects did not experience a confirmed crossing. It was not possible to calculate a median time to failure for the 20% cream, and although there was also significant right-censorship of the data for the 20% spray, there were enough data points to support calculation of the K-M median. Mean CPT values were reported because the 1999 repellent guideline calls for them, but the K-M medians (where they could be calculated) and the reported time to 25% failure provided undistorted summary statistics, which better characterize the duration of protection provided.

The 20% cream had a mean CPT = 12.6 h against *Ix. scapularis* and 15.3 h against *D. variabilis*. Most of these data were right-censored and a median could not be calculated from the Kaplan-Meier analysis. However, the time to 25% failure was ≥ 15.4 h for *Ix. scapularis*

and 9.7 h for *D. variabilis*. For the 20% spray product, data collected with *Ix. scapularis* resulted in a median CPT of 15 h while the mean CPT equaled 14.1 h. The mean CPT against *D. variabilis* was 14 h and the median CPT was 14.1 h. Time to 25% failure for this species was 12 h.

The mean number of crossings between species and products was not significantly different. For the insect repellent spray five subjects received confirmed crossings for each tick species. Two of the cream-treated subjects reported confirmed crossings for *D. variabilis* while four reported confirmed crossings for *Ix. scapularis*. Data from subject 67 against *D. variabilis* showed that this subject experienced four crossings in the course of the test, but none were confirmed. Likewise, subject 52 experienced 3 unconfirmed crossings against *Ix. scapularis* for the cream product. For the spray product the mean CPT ≥ 10.2 h against *Ix. scapularis* and ≥ 11.4 h against *D. variabilis*. The mean CPT ≥ 14.5 h for the cream product against *D. variabilis* but a FCC was reported at 3.4 h and 7.5 h against *Ix. scapularis*, with the mean CPT for the remaining eight subjects ≥ 9.7 h.

On page 20 the study reports that "The average total number of crossings per subject was 2.0 or less", which is incorrect. Since each subject was exposed to both species of tick, the average number of crossings per subject is the sum of the figures for each tick species shown in Table 1 above. The correct "average total number of crossings per subject" is thus 3.6 for both the cream and the spray. This mistake does not affect the outcome or the acceptability of the study results, as the dataset is unambiguous and complete.

Conclusions:

The methods employed in these studies were adequate to produce scientifically reliable data. They were based on study protocol LNX-003 as amended in accordance with EPA and HSRB recommendations before testing began. Both products provided a high degree of repellency against each tick species.

Recommendation: The study is scientifically sound and acceptable.