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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

May 22, 2008

SUBJECT: Science Review of Human Study of Mosquito Repellent Performance

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

TO: Marion Johnson, Chief
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RE: Spero, N. (2008) Evaluation of the Efficacy of Personal Repellents Against Mosquitoes in the Laboratory for Avon Skin-So-Soft SSS Bug Guard Plus Picaridin Insect Repellent (EPA Reg. No. 806-29), Avon Skin-So-Soft SSS Bug Guard Plus Picaridin Insect Repellent Spray (EPA Reg. No. 806-31). Document dated April 4, 2008. Unpublished document prepared by Insect Control & Research, Inc., under Protocol ID G0590607001A117 MRID 47397701 Volume 2 98pp. (Supporting documentation from the Volume 3 entitled "Additional Information to Fulfill 40 CFR 26.1303." 49 p. was reviewed as needed.)

ACTION REQUESTED

Conduct a science review of a completed laboratory study. Determine the adequacy of the methods employed and the scientific validity of the reported data. Evaluate and assess the subject repellent products' data to determine if these products repel the West Nile virus vector, *Culex quinquefasciatus*. If either or both of these products repel the subject vector species, what is the duration of repellency expressed as CPT? Does the CPT derived from the data collected in this study support the ICR hypothesis that the subject repellent products provide complete protection for 8 hours?

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 47397701 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, the subject products repelled mosquitoes for about 9.5 hours. This result supports the hypothesis that these products repel vectors of West Nile virus for 8 hours. The Human Studies Review Board will be asked to comment on these data.

SCIENCE REVIEW

Study Objectives: To determine the Complete Protection Time (CPT) of two registered mosquito repellent formulations containing picaridin against *Culex quinquefasciatus* under laboratory conditions. The study shall establish the mean time to first confirmed bite for each formulation under laboratory conditions to support proposed label amendments to add claims of efficacy against mosquitoes that can vector WNV.

Materials & Methods:

Study location: The study was conducted in the laboratory of Insect Control and Research, Inc. located at 1330 Dillon Heights Avenue in Baltimore, Maryland.

Study Date: Repellent product testing was conducted on March 4, 2008. There was no dosimetry phase.

Repellents Tested: The repellents tested were EPA registered 10% picaridin products Avon Skin-So-Soft SSS Bug Guard Plus Picaridin Insect Repellent (EPA Reg. No. 806-29) and Avon Skin-So-Soft SSS Bug Guard Plus Picaridin Insect Repellent Spray (EPA Reg. No. 806-31).

Tested positive control/comparison repellent: None

Untreated Control: One negative control male subject, selected at random, monitored the continuous aggression of the test mosquitoes in each of the six test cages every 30 minutes during the test. Aggression was defined as five landings in 60 seconds on the exposed 250 cm^2 of skin on the untreated subject. Landing mosquitoes were shaken off the arm to prevent biting. Landings were used as an indicator of aggressiveness in order to maintain the population of host-seeking mosquitoes.

Number of Test Subjects/Treatment Regime: Twelve subjects participated in repellent testing. Each subject had 0.42 ml of Repellent 'A' applied to 250cm^2 of skin surface on one forearm and 0.42 ml of Repellent 'B' applied to 250cm^2 of skin surface on the other. The dose of each repellent product was 1.67mg/cm^2, resulting in a product application of 417.5 mg to each arm for a total of 835 mg/subject.

Protocol used including amendments and deviations: Protocol A117 was used as amended February 8, 2008. The amended protocol can be found in Appendix 1 of the study. Pages 6-8 of Volume 3 include the ICR responses to the EPA and HSRB

recommendations and indicate where changes were made in the protocol. The only protocol deviation is listed on page 6 of the study.

Experimental design: The study was conducted in the laboratory with laboratory-reared colonies of the mosquito *Culex quinquefasciatus*. Six test cages, each measuring 2 x 2 x 2 feet, had a population 200 mosquitoes in each cage. A total of 13 subjects participated in this study—twelve test subjects and one untreated control subject. The sample size was greater than the minimum recommended by the EPA Testing Guidelines. The number of subjects used in this study was determined from a power analysis based on a meta-analysis of studies of this type as described in Rutledge and Gupta 1999 (see V2:Appendix 1 and V3: 42-49), which provides power tables for determining the number of subjects needed to determine protection times up to 8 hours for skin-applied insect repellent studies with varying confidence limits and two-tail levels of significance. Using information from these power tables, 11 subjects are necessary in order to use a CPT value with a 95% confidence interval.. Using one more treated test subject than required helped to ensure a minimum "*N*" of eleven, even if a subject withdrew or was excluded. No statistical comparisons to the untreated control were made in the study. The acceptable level of aggressiveness was 5 landings within 60 seconds; this level was not attained in the first evaluation, and 200 more mosquitoes were added to each of the six test cages in accordance with the amended protocol.

As recommended by the HSRB, test subject attractiveness to mosquitoes was an inclusion criterion At least 5 mosquitoes landed on the untreated forearms of each test subject within 60 seconds when inserted into cages with populations of *Cx. quinquefasciatus* mosquitoes.

Treated subjects exposed each of their treated forearms for 5 minutes at 30 minute intervals to caged mosquitoes of established aggressiveness until they experienced a confirming bite on both arms or until the end of the 10-hour test period—whichever came first. (This is a change from the original protocol, which specified an 8-hour exposure time.) Twelve subjects were treated with both test formulations—one on each forearm. No positive or negative control treatment was evaluated or included in the test design. The test was subject-blinded.

The Study Director reported a change to the treatment of test subject groups as a protocol deviation. Instead of treating six groups of two each, subjects were treated in two groups of six each. This change did not affect the experimental outcomes but should have been done by protocol amendment rather than by protocol deviation.

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 10-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. The mean CPT for each repellent

was reported as mean CPT± SD based on a 95% confidence interval. Median values were not reported.

Results:

Table 1
Repellent Lab Trial Results

Report volume	MRID 47397701 (Volume 2)	
Repellent tested	EPA Reg. No. 806-29 (10% picaridin) Test Substance 'A'	EPA Reg. No 806-30 (10% picaridin) Test Substance 'B'
Mean CPT (hrs.)	9.417 ± 1.094	9.667 ± 0.421

Discussion, Conclusions, and Recommendation:

The methods employed in these studies were adequate to produce scientifically reliable data. They were based on the study protocol A117 as amended in accordance with EPA and HSRB recommendations before testing began. Protocol deviations were reported and IRB approvals obtained.

Of particular significance in the amended protocol are the changes to the data analysis section (See V2: 19-27). The revised analysis presents a justification for sample size selection and an explanation of how the CPT results will be interpreted based on the statistical power of the chosen sample size. The ICR explanation adequately addresses past concerns expressed by the HSRB regarding the statistical power associated with sample size for insect repellent studies. The power associated with sample size for insect repellent studies is based primarily on the work of Rutledge and Gupta (1999). Their meta-analysis of mosquito repellent studies provided power tables for determining the number of subjects needed to determine protection times up to 8 hours with varying levels of statistical significance. Their recommendations were applied to the experimental design and data analysis of the ICR repellent study. As a result, the data collected in this study provided an accurate estimate of protection within a 95% confidence interval.

The data collected from this experiment show that Avon Skin-So-Soft SSS Bug Guard Plus Picaridin Insect Repellent (EPA Reg. No. 806-29) and Avon Skin-So-Soft SSS Bug Guard Plus Picaridin Insect Repellent Spray (EPA Reg. No. 806-31) provide a CPT of 9.5 hours against the vector of West Nile virus, *Culex quinquefasciatus*. These results confirm the values presented by Rutledge and Gupta (1999) and based on the statistical power associated with the sample size in this experiment the results support the ICR hypothesis that the CPT for each product is 8 hours against *Culex quinquefasciatus*, the primary vector of West Nile virus.

Recommendation: The study is scientifically sound and acceptable.