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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAR 9, 2007

MEMORANDUM

SUBJECT: Science review of EMD-003.3 report of completed efficacy study of IR3535 Aerosol Spray formulation against ticks.

FROM: Clara Fuentes, Ph.D., Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

TO: Linda Hollis, Branch Chief
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

REF: Carroll, S. (2007) Test of Personal Insect Repellents [IR3535 Aerosol Spray]. Unpublished study conducted by Carroll-Loye Biological Research under Project No. EMD-003.3. 143 p. (MRID 47045901)

ACTION REQUESTED

Provide scientific review of the completed study, MRID 47045901, EMD-003.3 Aerosol Spray formulation, to evaluate its scientific validity, and assess its consistency with changes recommended by EPA and HSRB to the revised protocols.

CONCLUSIONS AND RECOMMENDATIONS

I have reviewed Carroll-Loye product performance study, MRID 47045901 EMD-003.3 Aerosol Spray formulation, containing 20% w/w of the active ingredient IR3535, and concluded that the study, EMD-003.3 contains information sufficient for evaluating the repellent properties of this formulation against ticks. The reported complete protection time (CPT) ranges from 4.25 to 14 hours (Mean CPT = 11 ± 2 hours). Twenty five percent failures occurred at 10.75 hours.

The reported study, MRID 47045901 EMD-003.3 Aerosol Spray, is scientifically sound and able to generate reliable data for evaluating the repellency of the formulation tested against ticks. This study was conducted consistently with changes made to the revised protocol as recommended by EPA and HSRB. These changes are listed below:

1. Addition of preliminary phase to estimate typical consumer dose

2. Discussion on risk and risk minimization.

3. Discussion of sample size and statistical analysis

4. Elimination of positive controls

5. Pre-test training for dosimetry and product performance testing

6. Change of repellency endpoint to FCC

7. Equal arrangement of experimental and control subjects.

Next are the HSRB specific recommendations to the amended study protocol:

1. Conduct the dosimetry test outdoors.

2. Determine effective dose.

3. Ensure safety of test material including information on toxicological reference points such as NOAEL/LOAEL.

The following are the protocol changes and recommendations adopted in the performance of EMD-003.3 study:

1. Dosimetry test for the Aerosol Spray formulation was conducted outdoors. "Applications were made outdoors, immediately adjacent to the laboratory." (p. 8)

2. Information concerning acute low toxicity of the test material is available. (Appendix 7. *Study protocol EMD-003*; 6.1.7 *Test Material Safety* (p. 43).

3. The study provides justification for sample size (page 7), and discussion of statistical procedures for analysis of dosimetry and repellency data (p. 11).

4. Treated and untreated arms were equally arrayed with orientation ink dots to assess ticks questing behavior. Questing behavior was assessed by positioning ticks on specifically marked (dotted) regions of the forearms as recommended by EPA and HSRB, and proposed on the revised protocol. (p. 10).

5. Risk from exposure to formulations was further minimized by reducing the number of unnecessary exposures from 3 to 1 during pre-test dosimetry training. "After practicing applying the Aerosol once to each limb to get a feel for its dispensing properties, subjects completed a series of three self-application replicates to each limb" (p. 8).

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

    a) The efficacy endpoint was changed to FCC. (Study Protocol p. 3: Section 5.1 *Objective of Research*)

    b) Exposure periods were limited to 3 minutes every 15 minutes. (p. 11: 8. *Data Recording*).

    c) Pre-test training in handling disease-free ticks in the laboratory was provided. "Subjects had practiced procuring unused ticks from the vials/trays with a small artist's paintbrush in advance of the test." (p. 9).

    d) Ticks were descended from field-caught adults and reared on quarantined rodents screened to be pathogen free (p. 8).

Deviations from the protocol

1. Exposure of subjects to test material was reduced from 3 trials to 1 application during pre-test practice without compromising reliability of dosimetry data.

2. Data sheets were modified from those previously appended in the protocol.

3. Data recording errors do not compromise the quality of the data set.

4. Questing behavior of ticks was not affected by an unexpected $1^{o}C$ raise in temperature during 2 testing periods (from 25 to 26 ^{c}o).

STUDY SUMMARY

A. Establishment of Typical Consumer Dose

The weight of material applied during dosimetry was measured using 2.5 cm wide strips of self adhesive roll gauze bracelets. Bracelets were weighed before and after each application, and the formulation container was also weighed before and after each application. Each subject repeated the application procedure 3 times for each limb during dosimetry testing. Seven females and 5 males participated. A mean dosage per unit skin surface area per subject was calculated on the basis of 3 repeated applications per subject. "The grand mean of subject means was then used as the dosage rate for the efficacy testing." (p.9). The dosage rate was 0.00143 ± 0.0008 g/cm^2. That mean was converted to volume using the specific gravity of the formulation

(0.94) (Appendix 9). The dose applied for testing efficacy was 0.00143 ml/cm^2 of skin surface of the subject's arm (p. 14). Individual doses were prepared for each test subject on the basis of the surface area of their forearm (Appendix 3 *Treatment Allocation and Dosing* p. 31). The sample was comprised of 6 males and 4 females (p.9).

Calculations:

The estimated dosage per trial = **Total captured** x 1/**Proportion covered**,
where:

Total captured = difference in mass (gain or loss of weight) between treated and untreated dosimeters.

Proportion of total surface area covered by dosimeter =
surface area of a set of 4 dosimeters / surface area of the limb

Dosing rate (in weight) / surface area of skin = total estimated weight of applied material per limb surface area. These values were converted to volume by dividing the weight in grams by the specific gravity of the formulation :

Mean dosing rate [g/cm^2] divided by test material specific gravity [g/ml] = Efficacy test dosing rate [ml/cm^2]

B. Product Performance

Ten subjects were assigned to the formulation treatment and exposed to lab-reared, pathogen free nymphal deer tricks, *Ixodes scapularis*, for 3 minute at 15 minutes intervals to assess lasting efficacy of IR3535 –based formulation (p. 11). Each subject was his/her own negative control for prescreening of questing ticks behavior. Only actively questing ticks were selected for efficacy testing. Dose rate was determined for each formulation separately by passive dosimetry as recommended by the HSRB. Dosage applications were made volumetrically, based on specific gravity of the formulation (0.94), and the arm surface areas of individual subjects. Subjects practiced handling ticks using a fine paintbrush prior to testing. To measure the effect of formulation on ticks questing behavior, subjects' treated and untreated arms were equally marked with 3 dots; one dot placed at the wrist (margin of the treatment area), another was placed 3 cm from the wrist dot into treatment area toward the elbow, and another dot was placed 3 cm from the wrist dot on the opposite direction toward the palm, the initiation dot. Subjects worked together in placing ticks at the initiation dot, and collecting data. Each tick was exposed only once to the test material, and brushes used to handle individual ticks were periodically cleaned and replaced with new or clean ones to avoid contact with test material (p. 10).

The study endpoint changed to First Confirmed Crossing (FCC), which is analogous to the First Confirmed Bite method for estimating Complete ProtectionTime (CPT). A crossing is defined as crawling into the treated area within 3 minutes of exposure, and confirmed by another subsequent crossing within 30 minutes, or 2 crossings occurring during two consecutive

exposures. No crossing beyond the treated area was considered as being repelled. Fifty percent of the subjects withdrew before receiving a FCC. "Their CPT values were computed as the time until they withdrew, plus 15 minutes, which yield the minimum CPT that would have been reported had they continued exposures at the 15 minute intervals and experienced crossings in the subsequent 2 periods" (p. 11). Kaplan-Meier survival analysis was employed to analyze efficacy data.

Complete Protection time ranged from 4.25 to 13.5 hours (table 4 p. 15). Average CPT = 10.95 ± 2.8 hours, with a 95% confidence interval of 8.9 to 13 hours. The mean time to failure was 11.73 hours, standard error = 0.94 hours. The median time to failure was 13 hours. The time to 25% failures was 10.75 hours. (p. 16).

REVIEWER COMMENTS

Dosimetry is one of the strengths of the revised protocol, which is used to verify subjects' safety. The reported study tested the Aerosol Spray formulation outdoors for determination of dosage as recommended by the HSRB.

Risk minimization of subjects' exposure to the test material and tick bites during testing were adequately addressed for both dosimetry and product performance tests. Risk minimization approaches include a deviation from the revised protocol by reducing the number of applications of formulation per subject from 3 to 1 during pre-test practice, and pre-test training in handling ticks using laboratory reared, pathogen-free tick nymphs as proposed in the revised protocol. The study report also addresses availability of data on acute toxicity and safety of the test material submitted with the original protocol. The test material has been tested for acute toxicity on animals. These data show low toxicity. MSDS documentation for the active ingredient is included in the original study protocol. Thus, the studies adopted HRSB recommendations concerning information on toxicological reference points such as NOAEL/LOAEL for safety of test material during dosimetry trials.

Repellency endpoint was changed to FCC. Minimization of risk from exposure to tick bites and pathogen transmission were adequately addressed in the study method.

Sample sizes were 12 and 10 subjects for dosimetry and product performance tests, respectively, as discussed in the revised protocol.

Data analysis "Dosimetry analysis, based mainly on subjects means, consisted of non-parametric rank and correlation test, and parametric regression. Those, and other, descriptive statistics were generated with the software 'SA JMP' Version 5.0.12 (SAS Institute, Cary NC)"… "Mean CPT was calculated across all 10 subjects, and is presented with standard deviation and 95% confidence interval information as well." To further account for subjects withdrawing early, the Kaplan-Meier survival analysis was employed. (p. 11)

Protocol deviations while conducting the studies are adequately documented.

Attachment:

Dr. Carroll's e-mail dated 03/01/2007.

From: **Scott P Carroll** spcarroll@ucdavis.edu 03/01/2007 05:13 PM
To: Clara Fuentes/DC/USEPA/US@EPA, Clara Fuentes <fuentesclara@yahoo.com>
Subject: Re: EMD-003.3

Dear Dr. Fuentes,

This email offers my responses to your scientific review of my study report EMD-003.3, aerosol. For minor comments please see the attachment.

First, under Conclusions and Recommendations you state that 'The reported complete protection time (CPT) ranges from 9 to 11 hours.' Note that the range shown in Table 4 is 4.25-14 hours. The text of the report states the maximum duration as 13.5 (I missed the 14 hour value when composing that sentence, as it had flipped to the top of the prior page). However, all of the associated statistical values you present from the report (mean, 95% CI, 25% failure) are correct. You have the 4.25-13.5 range listed on your page 4.

On the second page, under 'Deviations...' you regard deviation 4 as referring to the previous (pump spray and lotion) study reports. That was an error in the aerosol mosquito report, EMD-004.3, where it was deviation #5. However, in my copy of the tick report, there is no deviation 5. Deviation 4 refers to the high temperature recording. Either our copies of the report differ in this regard, or your comment is a holdover from your review of the mosquito report.

Also under deviations, you comment regarding the lack of blinding relates to an important point. As you are aware, because of the change in aerosol availability, it was not tested at the same time as pump spray and lotion, such that experimenter blinding was lost. However, subjects did not specifically know that we were testing aerosol.

On your page 6, I the same deviations questions arise. If your copy and mine are the same, all of those points are eliminated.

Please let me know what you think.

Regards,
Scott

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