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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

December 14, 2006

MEMORANDUM

SUBJECT: Science review of EMD-003 reports of completed efficacy studies for ticks.

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

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

REF: Carroll, S. (2006) Test of Personal Insect Repellents (Lotion). Unpublished study conducted by Carroll-Loye Biological Research under Project No. EMD-003.1. 148 p. (MRID 46979001)

Carroll, S. (2006) Test of Personal Insect Repellents (Pump Spray). Unpublished study conducted by Carroll-Loye Biological Research under Project No. EMD-003.2. 146 p. (MRID 46979002)

ACTION REQUESTED

Assess the validity of revised protocols for efficacy studies on IR3535 pump and lotion formulations, and assess their consistency with changes recommended by EPA and HSRB. Provide scientific review including determining Complete Protection Time (CPT) of the completed studies, MRID 46979001 EMD-003.1 lotion formulation and MRID 46979002 EMD-003.2 pump spray formulation.

CONCLUSIONS

The reported studies MRIDs 46979001 and 46979002 are scientifically sound and able to generate reliable data for evaluating the repellency of the formulations tested against ticks. These studies were conducted consistently with changes made to the revised protocol as recommended by EPA and HSRB.

REVIEW OF PROTOCOLS

1. HSRB recommendations for improvements to the protocols:

    a. Add of preliminary phase to estimate typical consumer dose

    b. Discuss risk and risk minimization

    c. Discuss sample size and statistical analysis

    d. Eliminate positive controls

    e. Add pre-test training for dosimetry and product performance testing

    f. Change repellency endpoint to First Confirmed Crossing (FCC)

    g. Equal arrangement of experimental and control subjects

    h. Test each formulation individually because differences in volatility, viscosity, container, mode of application, etc. between different formulations could affect dosimetry

    i. Conduct the dosimetry test outdoors for the pump formulation

    j. Determine effective dose

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

2. Protocol changes adopted in the performance of EMD studies:

    a. Each formulation was tested individually for determination of dosage rate.

    b. Dr. Carroll verified via e-mail dated 12-11-2006 that dosimetry test for the pump formulation was also conducted outdoors although that information is not included in the study report.

    c. Information concerning acute low toxicity of the test material is available.

    d. The study provides justification for sample size, and discussion of statistical procedures for analysis of dosimetry and repellency data.

    e. 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.

f. Risk from exposure to formulations was further minimized by reducing the number of unnecessary exposures from 3 to 1 during dosimetry.

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

1) The efficacy endpoint was changed to FCC.

2) Exposure periods were limited to 3 minutes every 15 minutes

3) Pre-test training in handling disease-free ticks in the lab was provided.

4) Ticks were descended from field caught adults and reared on quarantined rodents screened to be pathogen free.

3. Deviations from the protocol:

a. Exposure of subjects to test material was reduced from 3 trials to 1 application without compromising reliability of data. Data sheets were modified from those previously appended in the protocol. The aerosol formulation was removed for the trial.

b. Subjects collected their own data in coordination with verifying technicians. Although subjects were familiar with data entry protocols, they were not GLP trained. However, data quality was not compromised by obvious ambiguity in their records.

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

REVIEW OF EFFICACY STUDIES

1. Study summaries:

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 was proposed to use a total of 48 bracelets, 8 per each arm and leg. Each subject repeated the application procedure 3 times for each limb. Seven females and 5 males participated. A mean dosage per unit skin surface per subject was calculated on the basis of 3 repeated applications per subject. The dosage rates were 0.0011 ± 0.0003 g or 0.0011 ml of lotion formulation per square cm of skin surface, and 0.00059 ± 0.0003 g. or 0.0006 ml of the pump formulation per square cm of skin surface. These values are the grant means of subjects' means. Individual doses were prepared for each test subject on the basis of the surface area of

their forearm.

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

b. Product performance

Ten subjects were randomly assigned to formulation treatments and exposed to lab-reared, pathogen free nymphal deer tricks, *Ixodes scapularis*, for 1 minute at 15 minutes intervals to assess lasting efficacy of IR3535–based formulations tested separately. 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, and the limb 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.

The study endpoint changed to First Confirmed Crossing (FCC), which is analogous to the First Confirmed Bite method for estimating Complete Protection Time (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. Mean (CPT), within 95% confidence interval, was calculated across 10 replications (subjects, designated as replications), and reported with its associated std. deviations.

The results for the lotion formulation, shows that half of the subjects were protected from 10 to more than 12 hours with 1, 2 or no crossings at all, while the other half of the subjects experienced from 2 to 4 crossings during exposure periods that lasted from 5 to 8 hours approximately. So, the reported protection time for the

lotion formulation ranges from 5 to 12 hours (Mean CPT = 9.1 ± 2.5). The results from the pump formulation show a longer CPT than that observed for the lotion. For the pump formulation, CPT ranged from 6.5 to 15 hours; average CPT was 12.1 ± 2.8.

2. Reviewer comments

I have reviewed Carroll-Loye product performance studies MRIDs 46979001 and 46979002 and concluded that the studies EMD-003 and EMD-004 contain information sufficient for evaluating the repellent properties of these formulations containing the active ingredient IR 3535 against ticks. The reported protection time for the lotion formulation ranges from 5 to 12 hours (Mean CPT = 9.1 ± 2.5). The results from the pump formulation show a longer CPT than that observed for the lotion. For the pump formulation, CPT ranged from 6.5 to 15 hours; average CPT was 12.1 ± 2.8.

a. The reported studies, MRIDs: 469790-01 EMD-003 lotion formulation and 469790-02 EMD-003 pump formulation are scientifically sound and able to generate reliable data for evaluating the repellency of the formulations tested against ticks,

b. The study results show the lotion and pump formulations have a minimum lasting efficacy of hours, respectively. Efficacy against nymphal ticks, *Ixodes scapularis*, is expressed as average CPT = 9.1 hours (± 2.5) for the lotion formulation, and an average CPT = 12.1 hours ((±2.8) for the pump formulation.

c. The studies were conducted consistently with changes made to the revised protocol as recommended by EPA and HSRB.

Dosimetry is one of the strengths of the revised protocol, which is used to verify subjects' safety. The reported studies tested each formulation individually for dosimetry as recommended by the HSRB.

Risk minimization of subjects' exposure to formulations 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 dosimetry and pre-test training in handling ticks using laboratory reared, pathogen-free tick nymphs as proposed in the revised protocol. The study reports also address availability of data on acute toxicity and safety of the test material. The test material has been tested for acute toxicity on animals. These data show low toxicity. MSDS documentation is included in the study protocol. Inert ingredients are on EPA inert list 4 (relatively safe for all users) with few ingredients in list 3 that cause ocular irritation (e.g. alcohols). 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 for product performance was conducted as discussed in the revised protocol. However, for dosimetry studies, the reports neither present results for statistical comparisons between formulations nor for individual subject differences in application behavior and possible dosing interaction with the 3 formulations by using Friedman ANOVA as proposed in the revised protocol. It has been be explained why the studies reports do not present the results generated by the proposed analysis; Dr. Carroll stated via e-mail dated 12-11-2006, that "*Dosimetry comparisons among the formulations was omitted because it was peripheral to the chief objective of determining dosage for efficacy assessment. The inclusion of such a comparative analysis in the protocol is an obsolete remnant of the initial conception that grew from early discussions of dosimetry with BPPD personnel. Other minor analytical changes also reflect the development and improvement of our thinking as the work progressed.*"

Protocol deviations while conducting the studies are adequately documented and discussed in the studies reports.

3. Overall conclusions:

The Carroll-Loye studies, EMD-003 and EMD-004, contain information sufficient for assessing the repellent properties of various formulations containing the active ingredient IR 3535 against ticks and mosquitoes. The CPT for the lotion formulation is 9.1 hours and the CPT for the pump formulation is 12.1 hours.

Attachment: Email message from Dr. Scott Carroll to Dr. Clara Fuentes, December 11, 2006.

Attachment

Scott P Carroll
<spcarroll@ucdavis.edu>
12/11/2006 02:28 AM

To Clara Fuentes/DC/USEPA/US@EPA
cc
Subject Re: Efficacy test reports for EMD aerosol repellent

Dear Clara,

I wanted to clarify a few things based on your helpful comments on our studies. These clarifications will apply to the studies with all three actives (lotion, pump spray and aerosol). I will send you the draft mosquito aerosol report shortly (minus the 100+ pages of appendices, which are being collated now for submission this week). Ticks and aersol will follow. Note that I have now had time to expand the dosimetry analyses, and will add that to each of the other reports to really round them out scientifically.

1) Dosimetry applications were made out-of doors for pump spray and aerosol. I have now stated that in the methods sections.

2) Dosimetry *practice* application sessions were reduced from 3 to 1 because they were excessive and unneeded, but dosimetry applications for data collection remained at 3.

3) The analyses to compare dosimetry between the products were not conducted, as you astutely pointed out. To address that change, we have included the 'deviation statement' in Appendix 5:

> 6. Aerosol dosimetry results were not compared to those for Lotion and Pump Spray.
>
> *Dosimetry comparisons among the formulations was omitted because it was peripheral to the chief objective of determining dosage for efficacy assessment. The inclusion of such a comparative analysis in the protocol is an obsolete remnant of the initial conception that grew from early discussions of dosimetry with BPPD personnel. Other minor analytical changes also reflect the development and improvement of our thinking as the work progressed.*

4) The ticks have been in captivity for many generations, over about 10 years. Thus the screening against pathogens would have had ample opportunity to detect any. The nice thing is that even if a female with, e.g., the Lyme spirochaete somehow got through, only about 1 in 300 eggs gets it. So that if all the rodents in the colony are disease free, and thus not a source, by the second generation, only 1 egg in 300, divided by 300 again, would have Lyme (1 in 90,000). So by the time you have just a few generations, the probability goes so close to zero that the risk eliminated. I am sorry I left that ambiguous in the protocol, as I had meant it to be very clear, of course.

5) Thanks for your other comments. I am also clarifying those, e.g., why were ticks sometimes placed back at the starting point when they had already rejected the repellent? In part that was double checking, since the rejection criterion could be construed as a bit ambiguous (e.g., what is the exact angle the tick has to change its walking to in order to count as a clear rejection), but it was also something that the participants really wanted to do, out of curiosity. Had a tick ever crossed on such a second trial, that would have been something to discuss. The main point should be, I think, that this addition of trials only challenged the repellent further, without actually, as it turned out, changing the results. I should probably include this explanation as part of a deviation statement (or just eliminate that observation since it did not affect the results).

I hope that this response and update is useful at this point.

More very soon, and thanks very much again,
Scott

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