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-004 reports of completed efficacy studies for mosquitoes.

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 Unpublished study conducted by Carroll-Loye Biological Research under Project No. EMD-004.1. 143 p. (MRID 46979003)

Carroll, S. (2006) Test of Personal Insect Repellents (Pump Spray). Unpublished study conducted by Carroll-Loye Biological Research under Project No. EMD-004.2. 140 p. (MRID 46979004)

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 46979003 EMD-004.1 lotion formulation and MRID 46979004 EMD-004.2 pump spray formulation.

CONCLUSIONS

The reported studies MRIDs 46979003 and 46979004 are scientifically sound and able to generate reliable data for evaluating the repellency of the formulations tested against mosquitoes. 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 FCLIBe

    g. Reduction in number of negative controls to 2 research assistants for assessment of biting pressure

    h. Test sites were monitored for incidence of West Nile virus (WNV) prior to conducting the test

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

    j. Conduct the dosimetry test outdoors

    k. Determine effective dose

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

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

1) The efficacy endpoint was changed to first confirmed landing with intent to bite (FCLIBe)

2) Exposure periods were limited to 1 minute every 15 minutes

3) The incidence of disease vectors was monitored by a sentinel chicken flock a month and a week prior to conducting the test. The results of these surveys were negative to the presence of WNV in the study sites.

4) Test subjects were pre-trained in the laboratory to handle mosquitoes using mechanical aspirators. The mosquitoes used for pre-training were laboratory reared pathogen-free mosquitoes.

5) In the field, test subjects were arrayed in pairs to facilitate removal of mosquitoes "with intent to bite" and data collection.

6) Reduction in number of negative control subjects to 2 experienced personnel, attended by 2 assistants.

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. Aerosol dosimetry results were not compared to those for Lotion and Pump Spray as described by Dr. Carroll to Dr. Fuentes in an email message of December 11, 2006.

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.

c. No efficacy test was conducted in Florida. The study sites were 2 different mosquito habitats in Butte and Glen Counties, CA. Butte site was a forest, and Glenn County was a pasture/marshland. No WNV or related viruses had been recently isolated from these locations.

d. Four of the 10 test subjects withdrew from the study before receiving any landing for reasons unrelated to the test. [The test was not repeated and the early departing subjects were not replaced.] This underestimated product performance and yielded a conservative measure of product performance.

e. Treated limbs were not always covered if the test subject avoided mosquitoes by stepping into screen house.

REVIEW OF MOSQUITO 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

Study sites were 2 different habitats in the state of California, where the mosquito population was monitored for the presence of pathogen vectors prior to conducting the study, and found negative to WNV. The efficacy endpoint was changed to FCLIB. Sample size of 10 replications (subjects) per treatment was justified and used to estimate the average CPT. Test results yielded a conservative value due to early departure of 4 test subjects prior to completion of the test and before receiving any landings, and by

including these subjects in the statistical estimation of the average CPT assuming that their time of participation in the study coincided with the withdrawal criterion. (Excluding those subjects from the estimation of the mean would have resulted in smaller sample size and loss of statistical power, which is not advisable). Risk minimization included pre-test training on handling mosquitoes in the laboratory, intermittent exposures of 1 minute to mosquitoes in the field every 15 minutes, test subjects arrayed in pairs to assist each other with data collection, and negative control subjects reduced to 2 experienced personnel, attended by 2 assistants. Test sites were monitored prior to testing for lack pathogen vectors in those localities. Results generated from these efficacy studies were analyzed as proposed in the revised protocol. Data was analyzed using descriptive statistics (SAS JMP version 5.0.1.2 SAS Institute, Cary NC). Mean CPT was calculated standard deviation. The results generated by this data analysis are conservative values taking into account 10 replications (test subjects), including 4 participants which departed early from the test before reaching the stopping criterion. The reported complete protection time (CPT) for the lotion formulation in the forest site ranges from 6 to 8.5 hours (Mean CPT = 7.3 ± 0.93); in the marsh/pasture site, ranged from 7.75 to 10 hours; the average CPT = 8.5 ± 0.84). The CPT from the pump formulation in the forest site ranges from 5 to 8 hours; the average CPT = 7.1 ± 0.99; in the marsh/pasture site, ranged from 7.7 to 10 hours; the average CPT = 8.4 ± 0.84 hours.

2. Review comments

I have reviewed Carroll-Loye product performance studies EMD-004, MRID 46979003 lotion formulation and MRID 46979004 pump formulation, against mosquitoes in the field, and concluded that the reported studies EMD-004 contain information sufficient for evaluating the repellent properties of these formulations containing the active ingredient IR 3535 against mosquitoes. The Agency agrees with the reported complete protection time (CPT) for the lotion formulation in the forest site ranged from 6 to 8.5 hours (Mean CPT = 7.3 ± 0.93) and in the marsh/pasture site the CPT ranged from 7.75 to 10 hours to give an average CPT = 8.5 ± 0.84). The Agency also accepts that the CPT for the pump formulation in the forest site ranged from 5 to 8 hours with an average CPT = 7.1 ± 0.99 and in the marsh/pasture site the CPT ranged from 7.7 to 10 hours with an average CPT = 8.4 ± 0.84 hours.

The reported studies are scientifically sound and acceptable. They are able to provide reliable data, and they were conducted consistently with the following changes recommended by HSRB and EPA to the revised EMD-004 protocols:

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

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

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

d. Risk from exposure to formulations was minimized by reducing the number of

unnecessary exposures from 3 to 1 during dose determination.

e. Risk from exposure to mosquitoes in the field was adequately minimized as summarized below in the Repellency phase.

Dosimetry phase:

The reported study adequately addresses recommendations regarding individual dosimetry of each formulation, and safety of the study dose and test material, respectively. The dosage rate for the lotion formulation is 0.0010 ml ± 0.0003 g or 0.0011 ml of test material per square cm of skin surface. The dosage rate for the pump formulation is 0.00059 ± 0.00013 g. or 0.0006 ml /sq.cm of skin surface. The average dosage rate is reported as the value of the grant mean of subject means. The dosimetry study was conducted indoors and outdoors.

Repellency phase:

Study sites were 2 different habitats in the state of California, where the mosquito population was monitored for the presence of pathogen vectors prior to conducting the study, and found negative to WNV. The efficacy endpoint was changed to FCLIB. Sample size of 10 replications (subjects) per treatment was justified and used to estimate the average CPT. Test results yielded a conservative value due to early departure of 4 test subjects prior to completion of the test and before receiving any landings, and by including these subjects in the statistical estimation of the average CPT assuming that their time of participation in the study coincided with the withdrawal criterion. (Excluding those subjects from the estimation of the mean would have resulted in smaller sample size and loss of statistical power, which is not advisable). Risk minimization included pre-test training on handling mosquitoes in the laboratory, intermittent exposures of 1 minute to mosquitoes in the field every 15 minutes, test subjects arrayed in pairs to assist each other with data collection, and negative control subjects reduced to 2 experienced personnel, attended by 2 assistants. Test sites were monitored prior to testing for lack pathogen vectors in those localities. Results generated from these efficacy studies were analyzed as proposed in the revised protocol.

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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