EPA Review of Carroll-Loye Protocol 'No Mas 003'

Proposal for a Field Test of Mosquito Repellency for 'No Mas'

Clara Fuentes, Ph.D.
Kelly Sherman
Office of Pesticide Programs

Overview

- Protocol for a field study of the repellent efficacy of a lotion formulation containing 16% para-menthane-3,8-diol (PMD) and 2% lemongrass oil, called 'No Mas'
- Submitted by Carroll-Loye Biological Research (CLBR) in July 2010
  - Before EPA's Revised Guidelines for Skin Applied Repellents were released in August 2010
- Protocol is similar to a previous CLBR mosquito field study, LNX-001 (protocol reviewed June 2007; completed report reviewed October 2008)
- Research proposed to satisfy EPA registration requirements

Overview 2

- Sponsor is developing this product as a low-cost repellent for distribution in developing countries with vector-borne disease
- Sponsor reports that the product has broad-spectrum efficacy against more than 40 species of mosquitoes, including four of the most important malaria-vectoring anophelines
- The purpose of the present study is to test the product for efficacy against three mosquito genera – *Culex*, *Anopheles*, and *Aedes*

Science Assessment: Carroll-Loye Protocol 'No Mas 003'

Clara Fuentes, Ph.D.

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs

Study Objectives

- Dose Determination Phase
  - To determine the amount of No Mas a consumer might typically apply, and to determine the standard dose (in ml/cm2) for use in the repellency phase
- Repellency Phase
  - To determine the duration and efficacy in the field of No Mas in repelling wild mosquitoes of the genera *Aedes*, *Anopheles*, and *Culex spp.*

Toxicity of the Test Material

- Acute Dermal = LD_{50} >5,000 mg/kg body weight.
- Acute oral = LD_{50} >5,000 mg/kg body weight.
- Not irritating to the skin
- Not a skin sensitizer.

MOE Estimate

- Acute dermal LD_{50} of No Mas is > 5,000 mg/kg
- Estimated maximum dose = 1,000 mg/subject
- Assuming 70 kg subject, equivalent dose rate is 1,000/70 = 14.3 mg/kg
- Margin of Exposure (MOE) > 5,000/14.3, or > 350

Dose Determination

- Ten subjects self-apply No Mas repeatedly to each arm and each leg
- Dose rate (mg/cm^2) determined from weight of lotion applied and skin area of subject's forearm or lower leg
- Grand mean of subject means calculated as estimate of typical consumer dose
- Grand mean dose is converted to volumetric dose (ml/cm^2) for use in Repellency Phase

Repellency Phase Design

- Test sites: 2 different mosquito habitats. The product will be tested once at each of 2 ecologically different habitats in California's Central Valley.
- One treatment consisting of one formulation
- Number of Subjects per site:
  - 5 male and 5 female treated subjects
  - 2 untreated control subjects
  - 2 Alternate subjects
- Exposure of treated and untreated subjects for 1 minute at 15-minute intervals
- Landing pressure must be at least 1 LIBe/minute for untreated controls

Endpoints and Measures

- Endpoint is first confirmed LIBe for each subject or end of test, whichever occurs first
- Measures:
  - Time from application to first exposure
  - Time of each LIBe
  - Complete Protection Time (CPT)–time between application and FCLIBe or end of test

Statistical Analysis Plan

- In addition to individual subject data, study will report:
  - Mean CPT with standard deviation and 95% confidence interval
  - Kaplan-Meier median
  - Time to 25% failure

Measures to Ensure Reliability

- Test material will be applied by laboratory technicians
- All landings will be verified and recorded by a research technician
- Mosquito landing pressure throughout the test will be monitored by 2 untreated subjects
- Subjects' attractiveness to mosquitoes will be determined prior to testing
- Subjects will be trained to handle mosquitoes prior to testing

Compliance with Scientific Standards

The following elements are adequately addressed:

- Available acute toxicity studies with No Mas
  - Adequately characterize toxicological profile of the formulation
  - Support estimate of acceptable Margin of Exposure (MOE)
- Dose determination
- Experimental design of repellency phase
- Statistical analysis plan

Comments and Recommendations (cont.)

- Care is needed to ensure target genera of mosquitoes are present in sufficient numbers at selected field sites to allow achievement of study objective
- Justification for sample size in future protocols should not rely on comparison to superseded 1999 guideline

Ethics Assessment: Carroll-Loye Protocol 'No Mas 003'

Kelly Sherman

Office of the Director
Office of Pesticide Programs

Value to Society

- Proposed study would test the field repellent efficacy of 'No Mas' against three species of mosquitoes
- Product-specific efficacy testing is required to support label claims of repellency against mosquitoes
- A low-cost alternative to other available repellents could benefit many users

Subject Selection

- Participants will be recruited from among previous subjects of CLBR testing who have expressed interest, supplemented by word of mouth
- Inclusion and exclusion factors are well defined and appropriate
- No eligible subjects are expected to be especially vulnerable

Risks to Participants

- Test material will irritate the eyes on contact, and may cause skin irritation in some individuals
- Possible exposure to biting arthropods
- Possible exposure to arthropod-borne disease
- Risks of physical stress in the test environment
- Breach of privacy (pregnancy testing)

Benefits

- No direct benefit to subjects
- Primary direct beneficiary is sponsor
- If materials are proven effective, indirect beneficiaries will include repellent users who prefer this product to other repellents

Risk:Benefit Balance

- Risks have been effectively minimized
- Risks are reasonable in light of the expected societal benefits of the knowledge likely to be gained

Independent Ethics Review

- The Independent Investigational Review Board (IIRB) reviewed and approved the protocol and informed consent materials
- IIRB's complete policies and procedures, entitled "Human Research Protection Program Plan," was provided to the HSRB

Informed Consent

- Description of subject recruiting and consent processes is complete and satisfactory
- Consent forms include all elements required by regulations
- Language and reading level of consent forms is appropriate

Respect for Subjects

- Effective methods for protecting subjects' privacy
- Proposed level of compensation is appropriate
- Subjects will be free to withdraw at any time
- Medical care for research-related injuries will be provided at no cost to subjects

Applicable Ethical Standards

- This is a proposal for third-party research involving intentional exposure of human subjects to a pesticide, with the intention of submitting the resulting data to EPA under the pesticide laws
- The primary ethical standards applicable to the conduct of this research are 40 CFR 26, Subparts K and L, and FIFRA 12(a)(2)(P)
- Attachment 1 to the EPA Review contains a point-by-point evaluation of how this protocol addresses the requirements of 40 CFR 26 Subparts K and L

Findings in EPA Ethics Review

- No specific deficiencies relative to 40 CFR 26, subparts K and L, or to FIFRA §12(a)(2)(P)
- CLBR protocol 'No Mas 003' will meet the applicable requirements of 40 CFR part 26, subparts K and L

'No Mas 003': Charge Questions

If the proposed field repellency study protocol 'No Mas 003' is revised as suggested in EPA's review and if the research is performed as described:

- Is the research likely to generate scientifically reliable data, useful for assessing the efficacy of the tested materials in repelling mosquitoes?
- Is the research likely to meet the applicable requirements of 40 CFR part 26, subparts K and L?