January 21, 2007

EPA-HSRB-06-04

Subject: October 18-19, 2006 EPA Human Studies Review Board Meeting Report

Charge to the Board

Scientific Considerations

Does the proposed research described in Study EMD-004 from Carroll-Loye Biological Research appear likely to generate scientifically reliable data, useful for assessing the efficacy of a test substance for repelling mosquitoes?

Board Response to the Charge

Protocol EMD-004 is now revised and contains considerably more detail than the original protocol. Overall the revised protocol is greatly improved from the original and in many respects may be considered exemplary. The protocol describes a test of the efficacy of 3-[N-butyl-N-acetyl]-aminopropionic acid, ethyl ester (IR3535) to repel mosquitoes in field experiments. It describes the use of three formulations (pump spray, aerosol and lotion), and the number of replications (10 for each formulation). The components of the three formulations are stated. There will now be two untreated controls, both of whom are experienced in the field, and no positive controls. Two habitats are proposed for use, in or adjacent to the Central Valley in California and/or in the Florida Keys. The compound has a very low toxicity profile in animal tests. The compound has been used in Europe for over 20 years as a repellent without reports of adverse effects in humans. The new protocol also includes a dosimetry experiment.

General HSRB Scientific Criteria

- The scientific question was stated (i.e., to test the efficacy of IR3535 in repelling mosquitoes).
- Existing data were not adequate to answer the question of efficacy of these new formulations.
- Because existing data were not adequate to answer the question of efficacy, new studies involving human subjects are necessary.
- The potential benefits of the study were clear, i.e., that an effective repellent would be available that would have either greater efficacy and/or fewer drawbacks than what was currently approved.
- It is likely that the benefits would be realized (i.e., efficacy as a repellent) because there was a long positive history of efficacious use with this compound from its European use.
- The risks have been more extensively described, as have the strategies to minimize risk.

- The most likely relevant risk would be disease transmitted by the mosquitoes, if the mosquitoes carried pathogens, and some mosquito-borne diseases (e.g., West Nile virus-mediated disease) are very serious. The revised protocol does indicate that the likelihood is low of the mosquitoes in the two test areas to be carriers of disease organisms that could be transmitted to humans. However, using the fewest number of untreated controls (now indicated to be two persons experienced in removing the mosquitoes before they bite) would provide minimal risk of disease to the participants. The protocol now indicates that all the inert ingredients in the formulations lack toxicity at the exposure levels anticipated.

Study Design Criteria

- The purpose of the study was clearly defined (i.e., efficacy testing).
- There were specific objectives/hypotheses (i.e., that IR3535 in the proposed formulations is an effective repellent) and the study as described can test this hypothesis.
- The sample size is now a definite 10 individuals (with 2 extra recruits in case a subject drops out or fails to attend the test session) with 2 negative controls and no positive controls. The same number of subjects would be tested in both locations (if both locations are tested). The basis for the dose levels and formulations had not been provided; however, there is now a dosimetry experiment prior to the field experiment that would quantify the amount of repellent being used. There were no controls with just the formulation matrix without the repellent; the PI has provided an adequate explanation for this.
- There was a plan allocating individuals to treatments.
- It is anticipated that the findings from this study can probably be generalized beyond the study sample.

Participation Criteria

- There was more extensive justification for the selection of the target population.
- The participants were representative of some of the population of concern; however, there are others in the population unlike these participants who are likely to use these products, but it would either be unethical to test them or would be less appropriate to test them. The participating population is considered appropriate and reasonable.
- The inclusion/exclusion criteria were appropriate.
- The sample was not a vulnerable group.

Measurement Criteria

- The measurements were expected to be accurate and reliable.
- The measurements were appropriate to the question being asked.
- Quality assurances issues are now more appropriately addressed.

Statistical Analysis Criteria

- The data should be able to be analyzed statistically if the efficacy with time was the subject of the analysis and the comparisons are made across time. It is not the

intent of the protocol to compare treated to untreated statistically. The purpose of the two untreated control subjects is to monitor the biting pressure.
- The statistical method seems to be appropriate.
- Measures of uncertainty were now addressed.

Laboratory and Field Conditions
- No laboratory experiments were proposed in this protocol, probably because of the data already available due to the compound's long previous use.
- The field conditions were representative of the intended use.
- The protocol now includes a stop rule plan, medical management plan, and a safety monitor.

HSRB Consensus and Rationale

The revised protocol, EMD-004, contains considerably greater detail than the original and it answers all the scientific questions that were posed by the HSRB in its original review. The PI has been extremely responsive to the original review comments. The revised protocol should generate scientifically valid results of efficacy in repelling mosquitoes.

Ethical Considerations

Charge to the Board

Does the proposed research described in Study EMD-004 from Carroll-Loye Biological Research appear to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board Response to the Charge

Overview of Study

This protocol was originally reviewed at the June 2006 meeting of the HSRB, at which time the Board concluded that the study failed to meet the requirements established in the Environmental Protection Agency's final human studies rule (40 CFR Part 26). In particular, the study did not comport with the applicable requirements of 40 CFR Part 26, subpart K. The Board also recommended that the protocol be revised to include: (1) a more accurate discussion of subject assignment; (2) a more extensive discussion of the risks (with specific information about the risk of vector borne diseases); (3) clarification of proposed compensation for research-related injuries; (4) a clarification of the lack of direct benefit to research subjects; and (5) the inclusion of specific mechanisms to prevent coercive enrollment and to protect subject confidentiality.

A revised, IRB-approved protocol was submitted for review (Carroll 2006b). The research is to be conducted by Carroll-Loye Biological Research, a private laboratory in Davis, California by using healthy volunteers. The revised research protocol submitted

consists of two interdependent studies: 1) a dosimetry study, performed under controlled laboratory conditions, designed to determine the amount of an insect-repelling compound, known as IR3535, that normal subjects would typically apply when provided with one of three compound formulations (lotion, pump or aerosol); and 2) an efficacy study, performed at field sites in Northern California and/or Southern Florida, designed to measure the efficacy of IR3535 as a mosquito repellent. Dosimetry will be determined either by passive dosimetry using self-adhesive roll-gauze (spray and aerosol formulations) or by direct measurement of compound application (lotion formulation). The efficacy of IR3535 as a mosquito repellent would be determined by measuring the ability of the three formulations to prevent mosquito landings (defined as "Lite with Intent to Bite"; LIBe) under field conditions. Mosquitoes will be aspirated mechanically prior to biting. Prior to initiation of the efficacy study, all volunteers will be trained both to recognize a mosquito landing with the intent to bite (LIBe) and to remove such mosquitoes with an aspirator using laboratory-raised, pathogen-free mosquitoes in a controlled laboratory setting.

The dosimetry study, conducted in conjunction with the dosimetry analyses described in protocol EMD-003, would enroll 12 subjects per test formulation, for a total of 36 subjects. The efficacy study would enroll 10 subjects per test formulation, for a total of 30 subjects. Two additional untreated control subjects (experienced field-workers) would be enrolled to determine ambient LIBe pressure under field conditions; such measurements are necessary to determine IR3535's efficacy as a mosquito repellent. Each untreated subject would be attended by two assistants who would aspirate mosquitoes prior to biting, thus minimizing risk of exposure to vector-borne illnesses. Subjects may participate in either or both studies, making the total number of volunteers enrolled no less than 38 but no greater than 68. In addition, three alternate subjects would be enrolled to: 1) replace any subject who withdraws from participating; and 2) protect the confidentiality of any subject excluded from the study as a result of pregnancy or a potentially stigmatizing condition, as described below.

Critique of Study

The Board concurred with the factual observations of the strengths and weaknesses of the study, as detailed in the EPA's Science and Ethics Review (Carley and Fuentes 2006b). With the provision of detailed IRB minutes and the exclusion of children and pregnant women, the proposed research described in Protocol EMD-004 comports with the applicable requirements of 40 CFR Part 26, subparts K and L.

In brief, the risks to study participants are minimal and justified by the likely societal benefits, including data on the efficacy of IR3535 as a mosquito repellent. The nature and likelihood of any side effects or adverse events are described clearly in the informed consent documents. Specifically, the risks to study participants are three-fold: 1) allergic reaction to test materials themselves; 2) exposure to biting arthropods; and 3) possible exposure to arthropod-borne diseases. Plans for the medical management of any side effects or adverse events have been developed, but Carroll-Loye Biological Research

also may wish to designate a specific physician to be contacted in the event that any adverse side effects are seen.

As IR3535 is commercially available and has been used as a repellent in Europe for years with no evidence of toxic effects, the subjects enrolled in this study are unlikely to be at increased risk of experiencing adverse side effects upon exposure to the test materials. Reactions to mosquito bites are usually mild and easily treated with over-the-counter steroidal creams. Excluding subjects who have a history of such severe skin reactions will minimize the risk of a subject experiencing a severe physical reaction to a mosquito bite. In addition, the study protocol is designed specifically to minimize the likelihood that a mosquito will bite, through the use of clear stopping rules, limited exposure periods, and joint observation. Finally, to minimize the risk that study subjects would be exposed to diseases like West Nile Virus, field tests of repellent efficacy would be conducted only in areas where known vector-borne diseases have not been detected by county and state health or vector/mosquito control agencies for at least one month.

At the June 2006 meeting, the Board expressed concern about the potentially coercive nature of study subject recruitment. Although the study is to be conducted by Carroll-Loye Biological Research, a private research laboratory in Davis, California, the Principal Investigator of the study and Co-Owner of the research laboratory, Dr. Scott P. Carroll, also is an adjunct faculty member of the Department of Entomology at the University of California, Davis. As the majority of research participants will be recruited from the University's student population, including from Dr. Carroll's own department, the Board previously recommended that the protocol and consent documents be altered to define clearly the mechanisms in place to prevent coercion. The revised protocol includes several such mechanisms, including the exclusion of any student or employee of the Study Director, a substantial waiting period between recruitment and study enrollment, and an interview by Dr. Carroll, designed to minimize coercive subject recruitment and enrollment. Several HSRB members, however, expressed concern that offering to send subjects recruited in California to a field site in Florida might unduly influence individuals to engage in research activities for which they would not otherwise volunteer; Carroll-Loye Biological Research may wish to restrict recruitment of participants to specific localities or, alternatively, discuss opportunities for out-of-state travel only after subjects have enrolled in the research study.

Finally, in accordance with the newly promulgated provisions in the EPA's final human studies rule (40 CFR §§ 26.1701-1704), children and pregnant women are explicitly excluded from participation, the latter being confirmed by requiring all female volunteers to undergo a self-administered over-the-counter pregnancy test on the day of the study. Previously, the Board raised concerns about the potentially stigmatizing nature of a positive test, and recommended that Carroll-Loye develop additional protections to ensure that the results of over-the-counter pregnancy tests would be kept private. The use of so-called "alternate" subjects is one such safeguard; that study participants may be designated as alternate subjects and automatically excluded from participation allows for potentially pregnant volunteers to withdraw without compromising their confidentiality.

HSRB Consensus and Rationale

The Board concurred with the initial assessment of the Agency that the revised protocol, EMD-004, submitted for review by the Board meets the applicable requirements of §40CFR26, subparts K and L.