Completed IR3535 Insect Repellent Efficacy Studies

Studies EMD-004.1 and EMD 004.2

Charge to the Board

Scientific Considerations

The active ingredient IR 3535 was tested for its ability to repel mosquitoes from the forearms or legs of volunteers by the protocol presented and modified by Carroll-Loye. The protocol had been modified based on the suggestions and input of EPA and HSRB. The results were reported in EMD-004.1 and EMD-004.2

The active ingredient was formulated into two products, a pump spray and a lotion, but data on the originally proposed aerosol was not provided because of an error in the formulation. The products were produced using Good Manufacturing Practices. All experiments were conducted using Good Laboratory Practices. A passive dosimetry experiment was done, as suggested by the HSRB, to determine the amount of product that would be utilized by people using the product as directed. This passive dosimetry experiment was used to determine a grand mean of the 12 individuals tested (3 subsamples each) per product that was then used for all 10 individuals per product participating in the subsequent mosquito repellency tests for each product. (It should be noted that the dosimetry experiment was in common for both this study and the tick repellency study, EMD-003, since the same formulated products were used for both.)

The experiment was a field study and was conducted according to the approved protocol with only very minor deviations, and none of these deviations would have affected the quality of the data or the safety of the subjects. Two locations in California were used, one a dense forest and the other a moist pasture marshland; the two locations had differences in the composition and relative abundance of mosquito species. Neither location showed evidence of the presence of West Nile Virus (WNV). The number of 10 subjects per product was justified in the text as leading to sufficient statistical power while exposing only a small number of people to the potential risks.

Each subject had one limb treated, and the remainder of the body was covered with material impervious to mosquitoes. There were two experienced persons serving as negative controls (i.e., without any repellant product) to confirm mosquito biting pressure (and biting pressure was maintained throughout the period of the study, defined as at least one Landing with Intent to Bite, LIBe, per min). Experimental subjects, in pairs, monitored LIBe's during a one min interval each 15 min, until the First Confirmed LIBe (FCLIBe) was determined. Stopping rules were employed. The Complete Protection Time (CPT) was calculated as the mean for all participants for each product. For the lotion the study identified a range of 6-8.5 hr with a mean CPT of 7.3 hr for the forest, and a range of 7.75 to 10 hr with a mean CPT of 8.5 hr for the marsh. For

the pump spray the study identified a range of 5 to 8 hr with a mean CPT of 7.1 hr for the forest and a range of 7.7 to 10 hr with a CPT of 8.4 hr for the marsh. The CPT is probably conservative as a number of the subjects reported no LIBe's at all, and the experiment was terminated before a FCLIBe was observed.

With respect to the science criteria established earlier by the HSRB for completed studies:

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 because repellent efficacy was determined in carefully designed field experiments.
- The risks are minimal because the formulation products are of very low toxicity, the mosquitoes were aspirated before they had an opportunity to bite, and the regions selected did not have evidence of WNV.
- The most likely relevant risk would be irritation from mosquito bites, but participants were instructed to remove mosquitoes before they were bitten, or the possibility of infection with WNV, but the regions selected had no evidence of the virus.

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).
- The study as described tested this hypothesis.
- The sample size was 10 individuals per product along with 2 experienced individuals to confirm mosquito biting pressure. A dosimetry experiment prior to the field experiment determined the amount of repellent to be tested.
- There was a plan allocating individuals to treatments.
- It is anticipated that the findings from this study can be generalized beyond the study sample.

Participation Criteria

- There was 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 accurate and reliable.
- The measurements were appropriate to the question being asked.
- Quality assurance was addressed; however, some of the quality assurance was not as precise as it should have been.

Statistical Analysis Criteria
- The data can be analyzed to calculate CPT with a range of variability.
- The statistical method will be commented upon in more detail by the Board in its response to protocol SCI-001 below. It should be noted that although there are probably better methods than have been traditionally used to calculate the repellent efficacy, new products will likely need to be compared to existing products and it is imperative that potential users of the products be informed accurately of the relative protection among products. Therefore EPA is urged to make certain that any calculations of efficacy be of a nature that allows products to be compared with some common metrics or values.
- Measures of uncertainty were addressed.

Laboratory and Field Conditions
- Laboratory experiments were not conducted.
- Field experiments were appropriate.
- The study included a stop rule plan, medical management plan, and a safety monitor.

HSRB Consensus and Rationale

In conclusion, the reported studies on the efficacy of lotion and pump spray formulations of IR3535 (studies EMD-004.1 and EMD-004.2) on repelling mosquitoes are sufficiently sound, from a scientific perspective, to be used to assess the repellent efficacy of the two formulations against mosquitoes.

The Board also recognized that recent advances in statistical analyses means that there are probably ways of measuring efficacy of individual products that would be an improvement over traditional techniques. The Board encouraged EPA to proceed in its efforts to examine how a transition to more accurate methods of calculating efficacy can be introduced so that consumers can not only compare relative efficacy of products based on traditional methods but also have better information on the degree of protection

individual products provide. The Board recommended inclusion of a description of the sampling frame and definition of eligible subjects to help justify subject generalizability.

Charge to the Board

Ethical Considerations

Does available information support a determination that these studies were conducted in substantial compliance with subparts K and L of EPA regulations at 40 CFR part 26?

Brief Overview of the Study

This protocol for these two studies was initially reviewed at the June 2006 meeting of the Human Studies Review Board, 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). At that time, the study failed to comport with the applicable requirements of 40 CFR Part 26, subpart K. The Board also raised questions about: 1) equitable study subject selection and recruitment; 2) description and minimization of risks to study participants; and 3) whether or not the documentation and process of study subject enrollment was sufficient to meet prevailing standards of voluntary informed consent. A revised, Institutional Review Board (IRB)-approved protocol was submitted and reviewed at the October 2006 meeting of the Human Studies Review Board, at which the Board concluded that revised research protocol, as submitted to the EPA, was compliant with the applicable ethical requirements of 40 CFR Part 26, subparts K and L.

Subsequent to the aforementioned October meeting of the HSRB, two dosimetry and efficacy studies for mosquito repellents containing IR-3535 were conducted from October 23 through November 8, 2006 (Carroll 2006c; Carroll 2006d). The studies were performed at a laboratory site in Davis, California, and at two field sites in Butte and Glenn Counties, California, by researchers at Carroll-Loye Biological Research. The studies were sponsored by EMD Chemicals, Inc., Gibbstown, New Jersey; EMD Chemicals is the North American subsidiary of Merck KGaA, Darmstadt, Germany. The documents provided by Carroll-Loye specifically state that each study was conducted in compliance with the requirements of the U.S. EPA Good Laboratory Practice Regulations for Pesticide Programs, as promulgated at 40 CFR Part 160 (Carroll 2006c, 3; Carroll 2006d, 3). Each study was also reviewed and approved by a commercial human subjects review committee, Independent Investigational Review Board (IIRB), Inc., Plantation, FL. Documentation provided to the EPA by IIRB indicates that it reviewed these studies pursuant to the standards of the Common Rule (45 C.F.R. Part 46, Subpart A) and determined them to be in compliance with that Rule.

As submitted to the EPA, each completed study consists of two interdependent analyses: 1) a dosimetry study designed to determine the amount of a formulation

(lotion or pump spray) containing an insect-repelling compound, known as IR-3535, that users would typically apply when provided with one of two compound formulations (lotion or pump spray); and 2) efficacy studies designed to measure the efficacy of IR-3535 as a mosquito repellent for each formulation. Dosimetry was determined either by passive dosimetry using self-adhesive roll-gauze (pump spray formulation) or by direct measurement of compound application (lotion formulation). The efficacy of IR-3535 as a mosquito repellent was 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 were aspirated mechanically prior to biting; prior to initiation of the efficacy study, all volunteers were trained both to recognize a mosquito landing with the intent to bite and to remove such mosquitoes with an aspirator using laboratory-raised, pathogen-free mosquitoes in a controlled laboratory setting. During the field studies, subjects worked in pairs to facilitate identification and aspiration of LIBing mosquitoes during brief exposure periods. The strengths and weaknesses of each study design are described above. The scientific strengths and weaknesses of each study design are described above.

The dosimetry study enrolled a total of 12 individuals, seven women and five men, each of whom tested both the lotion and pump spray formulations. The field-based efficacy study for each formulation enrolled 10 subjects: seven women and three men tested both the lotion and pump spray formulation over two days at a "forest" site in Butte County, and four women and six men tested the pump spray formulation both the lotion and pump spray formulation over two days at a "marsh/pasture" site in Glenn County. One subject enrolled in the dosimetry study participated in "forest" efficacy study, three additional subjects participated in both the "forest" and "marsh/pasture" studies, and a fifth subject participated in the dosimetry, "forest", and "marsh/pasture" studies. All remaining subjects participated in only one of the analytic phases of EMD-004.1 and EMD-004.2. Two control subjects, described as "experienced personnel" (Carroll 2006c, 9; Carroll 2006d, 9) and who were untreated with either repellent formulation, also participated to determine ambient LIBe pressure, giving a total of 26 subjects enrolled. In addition, three alternate subjects were enrolled to: 1) replace any subject who withdrew; and 2) protect the confidentiality of any subject excluded from the study as a result of pregnancy or other potentially stigmatizing condition, as described below. Study documents, however, also include limb measurement information for additional subjects who were not enrolled in either the dosimetry or the efficacy studies. These subjects appear to be enrolled in two additional studies submitted to the EPA by Carroll-Loye Biological Research, EMD-003.1 (Completed Efficacy Studies for Tick Repellents Containing IR-3535 – Lotion) and EMD-003.2 (Completed Efficacy Studies for Tick Repellents Containing IR-3535 – Pump Spray) (Carroll 2006a; Carroll 2006b).

Critique of Study

The Board concurred with the factual observations of the ethical strengths and weaknesses of the study, as detailed in the EPA's Science and Ethics Review (Carley 2006b). In general, the research described in EMD-004.1 and EMD-004.2 comports with

the applicable requirements of 40 CFR Part 26, subparts K and L. The risks to study participants were minimal and were justified by the likely societal benefits, including data on the efficacy of IR3535 as a mosquito repellent. IR3535 is commercially available and has been used as a repellent in Europe for years with no evidence of toxic effects, so the subjects enrolled in this study were unlikely to be at increased risk of experiencing adverse side effects upon exposure. Reactions to mosquito bites are usually mild and easily treated with over-the-counter steroidal creams. The study also excluded subjects who have a history of such severe skin reactions to further minimize the risk of a subject experiencing a severe physical reaction to a mosquito bite. In addition, the study protocol was designed specifically to minimize the likelihood that a mosquito will bite, through the use of clear stopping rules, limited exposure periods, and paired observation; no side effects or adverse events were reported. To minimize the risk that study subjects would be exposed to disease causal agents like WNV, the study protocol called for field tests of repellent efficacy to 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. Although it would have been ideal if the mosquitoes collected during the field studies were subjected to serologic or molecular analyses to confirm that they were free of known pathogens, it is unlikely that failure to do so compromised participant safety in any significant way. Finally, the study protocol also included several mechanisms designed to minimize coercive subject recruitment and enrollment, compensation was not considered to be so high as to unduly influence participation, and minors and pregnant or lactating women were explicitly excluded from volunteering (pregnancy being confirmed by requiring all female volunteers to undergo a self-administered over-the-counter pregnancy test on the day of the study). The potential stigmatization resulting from study exclusion was minimized by the use of so-called “alternate” subjects, allowing for volunteers to withdraw or be excluded from participating without unduly compromising their confidentiality.

As with the two tick repellent studies (EMD-003.1 and EMD-003.2), the revised protocol and informed consent documents used for these mosquito repellent studies were reviewed and approved by IIRB, several days after study subject enrollment began; some subject participating in these studies were re-consented using IIRB-approved documents, but not all were. Although it is unlikely that these changes knowingly and/or seriously impaired the informed consent process, enrollment of subjects using unapproved protocols and consent forms represents a significant and serious departure from accepted review and approval practices. The failure of Carroll-Loye Biological Research to 1) obtain IRB approval of the revised protocol and consent forms prior to enrollment of study subjects, and 2) report these deviations to IIRB, are serious regulatory breaches. The Board thus recommended Carroll-Loye Biological Research report these deviations to the IIRB as soon as possible and work with that organization to develop and implement a corrective course of action.

Second, the IIRB-approved protocol and consent documents specifically stated that they are to 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 (see, for example, Carroll 2006c, 75). One sentinel poultry flock in the area, however, did test positive for WNV during the month prior to conduct of the field studies (Carroll 2006c, 7). Sentinel flocks closer to the two study sites did not test positive for arboviruses during this period, and a leading vector control ecologist consulted by Carroll-Loye reported that "WNV activity in Northern Calfornia [was] effectively concluded for 2006" (Carroll 2006c, 7), so it is unlikely that participant safety was compromised in any significant way. Nevertheless, initiation of field studies following the detection of WNV in a sentinel chicken flock represents a deviation from the approved protocol and should be reported to the IIRB as soon as possible.

Finally, even though two IR-3535-untreated control subjects were enrolled in the study, the IIRB-approved consent documents provided for review do not list the unique risks that these two volunteers faced. These control subjects were "experienced" personnel who were likely aware of these risks, but nonetheless should have been consented using documents that listed these dangers.

HSRB Consensus and Rationale

The Board concurred with the initial assessment of the Agency that studies EMD 004.1 and EMD 004.2 submitted for review by the Board met the applicable requirements of §40CFR26, subparts K and L.

The Board also noted that there were a series of deviations from Subpart K that while not adversely affecting the rights and welfare of human subjects of the study, reflected a lack of familiarity with IRB procedures and protocol requirements described in Subpart K. The HSRB advised the Agency that it recommend investigators perform human research projection training and include completion of such training as part of their submission of protocols or completion studies to the Agency. Examples of such training could include the on-line training program offering by NIH/NCI or development of such a program by EPA.