Extracts Relevant to SCI-001
From HSRB 16 April 2007 Report of January 2007 Meeting

From Summary (p 3):

Insect Repellent Efficacy Protocol SCI-001

Scientific Considerations

- The Board raised several concerns about sample size, population generalizability and statistical analysis in SCI-001 that should be addressed. If the recommendations provided by EPA and those suggested by the Board are followed, protocol SCI-001 appears likely to generate scientifically valid data to assess the efficacy of the test products against mosquitoes.

- The protocol would satisfy the scientific criteria recommended by the HSRB, namely, producing important information that cannot be obtained except by research with human subjects, and having a clear scientific objective and study design that should produce adequate data to test the hypothesis.

Ethical Considerations

- The Board concluded that the protocol should meet the applicable requirements of 40 CFR part 26, subparts K and L if the points raised in the EPA review and in this report are adequately addressed.

From Report (pp. 22-27)

Insect Repellent Efficacy Protocol SCI-001

Background

The objectives of this study will be to test the mosquito repellent efficacy characteristics of three test materials, to compare them to one another, reinforce measurements of time for which they are effective, and to contrast them with the U.S. military issue topical insect repellent. Test Material #1 is LipoDEET, which contained 30% DEET that had lipid spheres and inhibits evaporation, improved field, and reduced plasticizing and odor. Test Material # 2, Coulston's Duranon, is 20% DEET in a controlled-release, low-odor formulation. Test Material #3 is Insect Guard II, which contains as active ingredients 17.5% DEET, 5% N-octyl bicycloheptane dicarboximide (synergist), and 2.5% Di-n-propyl isocinchomerate (fly repellent). Test Material # 4, 3M Ultrathon (military issue repellent), contained 34.34% DEET in a polymer-based lotion to extend efficacy and reduce plasticizing.

This study will be similar to EMD-004 in terms of the dosimetry phase, efficacy measurements (time to "first confirmed landing with intent to bite"), and training of subjects in aspirating mosquitoes before they bite. The field conditions and timing of exposure also will be similar (treated subjects work in pairs, untreated controls work with 2 assistants to aspirate

landing mosquitoes, and both treated and untreated subjects are exposed to the mosquitoes for 1 minute every 15 minutes). The field testing sites will be in the California Central Valley or Florida Keys, with expected wild mosquito populations of *Aedes vexans, Ochlerotatus melanimon, O. taeniorhynchus,* and *Culex pipens*. The test results would be analyzed using unspecified statistics. Measurements would be reported with 95% confidence intervals of the mean and associated standard deviations. The efficacy of each treatment would be compared to that of Ultrathon. The sample size reflects a compromise between financial and ethical concerns, although it was difficult to pre-determine sample size without knowing the distribution of outcome values. EPA guidelines recommend 6 replicates, which is considered sufficient to show statistical significance at $P<0.05$. EPA recommended changes to the protocol to include developing a full description of the statistical analysis plan to compare means and to assess within-treatment variability, and to define a testable hypothesis.

Charge to the Board

Scientific Considerations

If the proposed research described in Protocol SCI-001 from Carroll-Loye Biological Research is revised as suggested by EPA, does the research appear likely to generate scientifically reliable data, useful for assessing the efficacy of the test substances for repelling mosquitoes?

Board Response to the Charge

The proposal intends to test the efficacy of three novel formulations of N,N-diethyl m-toluamide (DEET). Three topical formulations containing DEET will be tested against a positive control, Ultrathon (35% DEET). The objectives, design and methods are adequately described. The plans for statistical analyses of the data, however, require significant revision as detailed below.

Comments and suggestions for revision or clarification:

- *Experimental design*: While this is not identified as a limitation of the study, nowhere is it justified the randomization to left and right limbs. Thus is there any reason to believe that products will be more or less effective on the two limbs? In addition the Board questioned why use right/left as a blocking variable (that is subsequently ignored in the analysis) in the design?
- *Statistical analysis*: The investigator proposed computing the means and confidence intervals around those means for CPT in each treatment group (three test products plus the positive control). But this simple analysis makes comparisons across products more difficult. If comparisons are to be made the following approach should be considered:
    - Let CPT_{ij} denote the CPT measured on the *ith* subject in the *jth* treatment, where $i=1,\ldots,10$ and $j=1,\ldots,4$.
    - Fit a linear model to the 40 measurements, with a fixed effect for treatment. Other fixed effects can also be included if they happen to be of interest.

This approach permits direct comparisons among products. In particular, it is possible to obtain a point estimate and a confidence interval for the difference in CPT in a test product and in the comparison product, one of the objectives of the study.
If the entire study is replicated in two locations *using different subjects*, then an even better approach is to fit a model to the entire set of 80 measurements, but adding an effect for location (and perhaps an interaction between location and treatment) to the model. The investigator can easily fit the model using JMP. Just use the Fit X by Y option in the Analyze menu and then choose ANOVA.

- *Interpretation of results*: Results from this study need to be interpreted judiciously. Given the large variability in individual attractiveness to mosquitoes, the small sample size seriously limits conclusions that the sample is representative of the population of individuals who might eventually be users of these products. While the long list of exclusions is justifiable, one consequence is that the population represented in the sample is different from the population of potential users.
- *Sample size*: Including 10 subjects per treatment is probably sufficient, but the justification provided by investigators is not convincing. First, in order to estimate power an estimate of the within-treatment variance in the response variable is needed. The investigator does not provide such an estimate in the discussion. Thus it is unclear how they can argue that "from the standpoint of statistical power, six treated and one untreated subject are sufficient to demonstrate a significant effect at $P<0.05$". Second, the argument used to justify no more than 10 subjects per treatment states that "adding subjects beyond six increases the precision of the means estimate only slowly". This argument relies on the assumption that the between-person variance in CPT does not change as sample size increases, which in general is not true. The information on inter-individual variability drawn from studies completed by these investigators may be used to guide and justify sample size. Submission of the completed protocol to EPA should include evidence that steps like those described above were taken to justify sample size.
- *Sample Size Considerations for Subject Drop-Outs.* In previous studies, subjects dropped out at different points potentially confounding the quantification of the CPT. Criteria need to be established for how long subjects must remain in the study in order for their data to be used. Criteria for when a new subject must be run as a substitute to meet the sample size requirements must also be determined.
- *Assumption of normality of CPT measurements*: In choosing statistical analyses the investigator must select the appropriate model for the distribution of the data that will be used. The methods for the statistical analysis of the data rely heavily on the assumption that measurements are normal. Because of the small sample size, departures from normality can have important consequences on the validity of the methodology proposed here. In this case, the assumption of normality is probably justifiable and in any case can be easily tested and corrected for. There seems to be some confusion) regarding the *exponential family of distributions* and the *exponential distribution*. The latter is a standard probability model for variables such as time which are strictly positive and tend to exhibit a rounded L shape when plotted. The former has nothing to do with the study at hand. If CPTs can all be expected to be noticeably larger than 0, then approximating the exponential model with a normal model may be justifiable.
- *Measurement variables*: Although it is clear from the rest of the protocol discussion, the investigator might consider adding CPT to the list of variables given in Section 10.1.

- *Dose*: Even though it is suggested that the typical consumer exposure should be far below the dermal toxicity benchmarks, there is no indication of such toxicological data in the MSDS included with this submission. Typical consumer dose and known toxicity benchmarks should be clearly identified.

HSRB Consensus and Rationale

The Board raised several concerns about sample size, population generalizability and statistical analysis in SCI-001 that should be addressed. If the recommendations provided by EPA and those suggested by the Board are followed, protocol SCI-001 appears likely to generate scientifically valid data to assess the efficacy of the test products against mosquitoes. In addition, the protocol would satisfy the scientific criteria recommended by the HSRB, namely, producing important information that cannot be obtained except by research with human subjects, and having a clear scientific objective and study design that should produce adequate data to test the hypothesis.

Charge to the Board

Ethical Considerations

If the proposed research described in Protocol SCI-001 from Carroll-Loye Biological Research is revised as suggested by EPA, does the research appear to meet the applicable requirements of 40 CFR part 26, subparts K and L?

Board Response to the Charge

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 2006c). In general, the research described in Protocol SCI-001comports with the applicable requirements of 40 CFR Part 26, subpart K and L. The risks to study participants are limited and appropriate steps have been taken to minimize these risks. The risks to participants are justified by the likely societal benefits, including data on the efficacy of new topical formulations containing DEET as a mosquito repellent. DEET is commercially available and has been used as a repellent for years with no evidence of substantial toxic effects, so the subjects enrolled in this study are 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. In addition, the study protocol is designed to minimize the likelihood that a mosquito will bite, through the use of clear stopping rules, limited exposure periods, and paired observation. To minimize the risk that study subjects will be exposed to illnesses resulting from WNV, the study protocol calls for field tests of repellent formulations 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.

The Board recommended that the investigator collect mosquitoes during the field studies and that they be subject to serologic or molecular analyses to confirm absence of known pathogens. Finally, the study protocol 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.

The Board concluded that research described in Protocol SCI-001 minimizes risks to subjects and has appropriate stopping rules in place. The safety monitoring proposed seems reasonable and appropriate in light of the level of risk to subjects. Despite this generally favorable assessment, the Board considered several additional matters relevant to subject recruitment and the overall conduct of the study.

First, as noted in the Agency’s review of Protocol SCI-001, the protocol does not describe how untreated controls would be recruited. The protocol implies that controls will be recruited in the same manner as subjects in the “exposure” arm—via “word-of-mouth” and a Volunteer Data Base maintained by the Principal Investigator. The protocol should clarify how untreated controls will be recruited. The Board also found it a bit unusual that the IRB did not ask to review a script of the proposed recruitment phone call as most IRBs regard recruitment as the first step in the IC process and require that all recruitment activities be reviewed. This would include any fliers, emails, letters, or local ads as well, which should be submitted to IIRB for review.

Second, the Board discussed several issues related to subject recruitment and consent. First, the risks associated with DEET exposure during the course of the study are mischaracterized in the submitted informed-consent document, which refers to sprayed applications containing alcohol. Since the study involved the application of lotions to the skin, these risks should be redescribed. In addition, the informed-consent document is structured in a manner that does not apply to unexposed control subjects. Also, the submitted informed-consent document indicates that up to 40 subjects may participate in the study when the correct number should be 48 (10 exposed and 2 controls per arm of the study).

Third, the Board discussed the fact that the proposed sample size is slightly larger than what EPA has historically required (10 exposed subjects vs. the historical norm of 6 exposed subjects). The protocol provides a rationale for this approach (pp. 13-15), which is meant to reduce the probability that the sample over-represents individuals who are “inherently unattractive” to mosquitoes. In light of the limited risks to subjects, this departure from the historical norm was viewed as acceptable by the Board.

Fourth, the Board found it difficult to assess the qualifications of the IIRB based on the materials that were supplied. Although the Board did not have significant concerns about the overall quality of the IRB’s review of the protocol, it would be reassuring to the Board if some type of documentation of the IRB’s qualifications were provided to the Agency for review (e.g., evidence of member training, accreditation by an external professional body, etc.).

HSRB Consensus and Rationale

The Board concluded that the protocol should meet the applicable requirements of 40 CFR part 26, subparts K and L if the points raised in the EPA review and in this report are adequately addressed.