UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

March 13, 2007

MEMORANDUM:

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Science and Ethics Review of Protocol for Human Study of Mosquito Repellent Performance

FROM: John M. Carley
Ethics Reviewer

Clara Fuentes, Ph.D.
Science Reviewer

TO: Linda Hollis, Chief
Biochemical Pesticides Branch, BPPD

REF: Carroll, S. (2007) Test of Personal Insect Repellents: Efficacy Test Protocol WPC-001, dated January 16, 2007. Unpublished document prepared by Carroll-Loye Biological Research. 75 p.

We have reviewed the referenced protocol for a field test of mosquito repellency from both scientific and ethics perspectives. This review assesses the scientific aspects of the proposed research in terms of the recommendations of the draft EPA Guidelines 810.3700 and of the EPA Human Studies Review Board, and the ethical aspects of the proposed research in terms of the standards defined by 40 CFR 26 subparts K and L and the recommendations of the EPA Human Studies Review Board.

A. Completeness of Protocol Submission

The submitted protocol was reviewed for completeness against the required elements listed in 40 CFR §26.1125. EPA's checklist is appended to this review as Attachment 5. No required elements are missing, and all were provided in the initial submission.

In addition to the protocol itself and the associated informed consent document, the following supporting documents were considered in this review:

- Subject training materials for dosimetry and aspiration (pp. 53-58)
- Labels and MSDSs for test product (pp. 59-62)
- Study Set-up Form and Site Questionnaire: WPC-001 (pp. 63-67)
- Email correspondence between Carroll-Loye Biological Research and Independent Investigational Review Board (pp. 68-71)
- Email from R Roogow of IIRB to John M. Carley of EPA transmitting IIRB minutes, a current IIRB membership roster, and asserting that IIRB procedures were unchanged from previous report (p. 72)
- Minutes of IIRB consideration of WPC-001 at their meeting on 1/23/07 (p. 73)
- IIRB Membership roster dated 1/2/07 (pp. 74-75)

B. Summary Assessment of Ethical Aspects of the Proposed Research

Here is a summary of our observations about the ethical aspects of the proposed protocol. Supporting details are in the attachment.

1. **Societal Value of Proposed Research:** This study will test the field efficacy against mosquitoes of a conditionally registered formulation of the active ingredient Oil of Lemon Eucalyptus (OLE). Efficacy testing is required using this specific formulation to support continued registration of the product. Direct testing of the duration of efficacy is important because consumers, who rely on repellents to avoid insect bites, cannot readily assess the efficacy of a product independent of EPA's approval. There is potential benefit to society in demonstrating field effectiveness of OLE repellents at this reduced concentration, which users may prefer to other repellent products because of their cosmetic or other qualities.

2. **Subject Selection:** Subjects are to be recruited from among "communities of friends, neighbors and scientists" near the laboratory, excluding, however, any who are students or employees of the investigators. Explicit factors exclude as subjects children, pregnant or lactating women, those in poor health or physical condition, or those unable to speak and read English. The sample will thus not be fully representative of the population of potential repellent users. There is no indication that any subjects will be from vulnerable populations.

   Two "experienced" subjects will serve as untreated controls to verify ambient biting pressure from mosquitoes in the field. Although it includes additional inclusion criteria for these experienced subjects, the protocol does not describe how they will be recruited, or how the process of informing them and obtaining their consent to this special role in the research will differ from the process used for the treated subjects. This deficiency must be corrected before the research goes forward.

3. **Risks to Subjects:** Risks of three kinds are identified: risks from exposure to the test material, risks of exposure to biting arthropods, and risks of exposure to arthropod-borne disease. The test material is accurately characterized in the Informed Consent Form as an eye irritant, harmful if swallowed—consistent with the required hazard statements on the registered product label.

   All practical steps to minimize subject risks have been taken:

   - Risks from exposure to the test materials are minimized by excluding candidates with known sensitivity to product ingredients, by monitoring the dosimetry phase of the research, and by applying materials in the repellency phase by technicians.

   - Risks from arthropod bites are minimized by excluding candidates with known sensitivity, by training candidates to remove landing mosquitoes before they have time to bite, and by minimizing exposure of skin.

   - Risks of contracting disease are minimized by conducting the research in areas where mosquito-borne viruses have not been detected for at least a month, and by the same steps that minimize mosquito bites.

   Because of the generally low acute and chronic hazard profile of the material, the design of the research to minimize exposure, and the training of subjects to aspirate landing mosquitoes before they have time to probe or bite, the probability of the identified risks is accurately described as “extremely small”.

4. **Benefits:** There are no direct benefits to subjects. If the testing shows good field efficacy the direct beneficiary of the research is likely to be the sponsor. Assuming eventual regulatory approval, indirect beneficiaries may also include repellent users who prefer these products to other repellents.

5. **Risk/Benefit Balance:** No opportunities to further reduce risk to subjects while maintaining the robustness of the scientific design have been overlooked. The residual risk to subjects is very low, and reasonable in light of the potential benefits to repellent users, which are likely to be realized.

6. **Independent Ethics Review:** The Independent Investigational Review Board, Inc. of Plantation FL has reviewed and approved the protocol and informed consent materials. The IIRB is independent of the investigators and sponsors. Documentation of IIRB procedures was not provided, but a statement was made by the IIRB that this information had not changed since it was previously submitted to EPA.

7. **Informed Consent:** The protocol contains a complete and satisfactory description of the process by which potential treated subjects will be recruited and

informed in California, and for seeking their written consent to participate. A copy of the Informed Consent Form showing approval by the IIRB is included in the protocol.

The protocol description of the process by which potential treated subjects will be recruited and informed in Florida is inadequate, and must be expanded to make it comparable in detail to that provided for California.

The Informed Consent materials are appropriate for both the untreated control subjects and treated subjects.

8. **Respect for Subjects**: Methods proposed for managing information about prospective and enrolled subjects will generally protect their privacy from compromise. Greater assurance could be provided, however, if data collection forms referred to subjects only by coded number rather than by name. Subjects will be free to withdraw at any time, and will be reminded of this at several points. Subjects who withdraw will be compensated for time spent up to the point of withdrawal. Medical care for research-related injuries will be provided at no cost to the subjects.

C. Compliance with Applicable Ethical Standards

This is a protocol 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. Thus the primary ethical standards applicable to this proposal are 40 CFR 26, Subparts K and L. In addition, the requirements of FIFRA §12(a)(2)(P) for fully informed, fully voluntary consent of subjects apply. If the test is conducted in California as proposed, the provisions of the California Code of Regulations, Title 3, §6710 would apply as well. A point-by-point evaluation of how the requirements of 40 CFR 26 Subparts K and L and the criteria recommended by the HSRB are addressed is appended as Attachment 1.

These specific deficiencies should be corrected before the research is initiated:

- Although additional inclusion factors are defined for the "experienced" subjects who will serve as untreated controls, the protocol does not describe how they will be recruited, or how the process of informing them and obtaining their consent to this special role in the research will differ from the process used for the treated subjects.

- The process for recruiting, screening, informing, and consenting subjects in Florida should be described in detail comparable to that of the description of the same process in California, with particular attention to the role played in the process by the mosquito control district administration.

Once these deficiencies have been corrected, the entire proposal must be re-reviewed and approved by the IRB before research can proceed or subjects can be enrolled.

40 CFR 26 Subpart L, at §26.1703, as amended effective August 22, 2006, provides in pertinent part:

> EPA shall not rely on data from any research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.

The protocol requires that subjects be at least 18 years old and exclude female subjects who are pregnant or lactating. Thus §26.1703 would not forbid EPA to rely on a study executed according to this protocol.

D. Summary Assessment of Scientific Aspects of the Proposed Research

The study will test the field efficacy as a mosquito repellent of a conditionally registered repellent product containing Oil of Lemon Eucalyptus, or OLE. The main objective of the study is to quantify the efficacy of the formulation to prevent mosquito landings in the field. A secondary objective of the study is to characterize through dosimetry testing the amount of this pump-spray formulation typically applied by consumers.

Biting pressure will be monitored for one minute every 15 minutes during the test by two untreated subjects, each attended by two technicians. Mosquitoes landing on untreated subjects will be aspirated by the attending technicians to prevent biting and for later identification. Treated subjects will work in pairs to facilitate observations, and will expose treated skin for 1 minute at 15 minute intervals until they experience a confirmed landing with intent to bite (LIBe), or until the end of the test period—whichever comes first.

1. **Study design:** The protocol has two objectives: to test the field repellent efficacy of the conditionally registered OLE formulation, and to establish a typical consumer dose for the product, to be used as the standard dose in the efficacy phase. These objectives can be met by the study as proposed.

2. **Statistical design:** The sample size is larger than is required by EPA guidelines—large enough to ensure robust averages across subjects, but small enough to be economical. Two untreated subjects are proposed to establish and confirm ambient biting pressure; no statistical comparisons to the untreated controls are proposed. No positive control or negative vehicle control is proposed. Because there is only one test material, efficacy testing will not be blinded. Repellency will be reported as “Complete Protection Time”, calculated as the mean time across all treated subjects from application of the repellent to the first confirmed landing with intent to bite (LIBe). Time of LIBes will be reported with a precision of 15-minute intervals, with standard deviation and 95% confidence interval.

3. **How and to what will human subjects be exposed?** In the initial dosimetry phase, subjects' lower arms and legs will be exposed for a few minutes to test material to estimate a "typical consumer dose." In the repellency phase the standardized typical dose, expressed as volume per unit area, is scaled to the measured surface area of each subject's limb and applied by a technician to the subject's forearm or lower leg. The repellent will remain in place for 8 to 14 hours during the field test. In addition, subjects in the field efficacy phase will be exposed to potential bites by wild mosquitoes, and (with very low probability) to arthropod-borne diseases.

4. **Endpoints and Measures:** In the dosimetry phase the applied dose will be expressed as mass per unit area; a "typical consumer dose" will be calculated as the mean of individual mean doses applied. This standard unit dose will be used for each subject in the repellency phase. In the field repellency phase, complete protection time (CPT) will be measured as the mean time from initial application of a typical consumer dose to the first confirmed LIBe, and will be presented with standard deviation and 95% confidence interval. Subjects will be trained in the laboratory to recognize a "LIBe", and to aspirate landing mosquitoes before they have time to bite. In the field subjects will work in pairs, checking each other as well as themselves. All reported LIBes will be verified by a research technician.

E. Compliance with applicable Scientific standards

This protocol adequately addresses the following elements according to applicable scientific standards:

- Scientific objectives
- Experimental design for achieving objectives
- Methods for estimating dose of test material
- Quantification of efficacy of the test materials
- Data collection, compilation and summary of test results
- Discussion of the statistical power of the study
- Justification for sample size in dosimetry and repellency phases
- Rationale for use of two untreated negative control subjects to monitor biting pressure.

This protocol does not adequately address the following elements:

- No explicit hypothesis is stated.
- No explanation is given for employing negative controls as stated on page 10, under section 6.2.2 for the Dosimetry assay.
- Information on diagnostic statistical tests for normality, or information on how to analyze non-normally distributed data is lacking.

- The proportion of males and female subjects that will comprise the sample for testing efficacy is not mentioned.
- The procedure by which limb surface area will be measured is not described in detail. The exact location of the 4 dosimeters should be recorded for later placement at the same limb location, and their length before and after application of the test material should coincide.

Attachments:

1. Summary Review of Carroll-Loye Protocol WPC-001 dated 1/16/2007
2. §26.1111 Criteria for IRB approval of research
3. §26.1116 General requirements for informed consent
4. §26.1117 Documentation of informed consent
5. §26.1125 Criteria for Completeness of Proposals for Human Research

EPA Protocol Review: WPC-001

Title: Efficacy Test Protocol #WPC-001: Test of an Oil of Eucalyptus-based Personal Insect Repellent

Date: 16 January 2007

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1. Societal Value of Proposed Research

(a) What is the stated purpose of the proposed research?

"The objective of this study is to test the mosquito repellent efficacy characteristics of the test material. . . . [E]fficacy will be measured as Complete Protection Time, . . . defined herein as the time between application of test material and the First Confirmed 'Lite with Intent to Bite.'" (p. 4)

(b) What research question does it address? Why is this question important? Would the research fill an important gap in understanding?

"This study will test the efficacy of a new formulation of Oil of Lemon Eucalyptus (OLE) that is intended to improve its value and increase user acceptance. US/EPA requires new repellent formulations to be registered, and some registrants must present new efficacy data as part of the registration review." (p. 4)

(c) How would the study be used by EPA?

EPA will consider the study in defining acceptable label claims for repellent efficacy for the test material.

(d) Could the research question be answered with existing data? If so, how? If not, why not?

The concentration of OLE in this product is lower than that in other registered repellents containing it, so EPA requires product-specific efficacy data to support its registration.

(e) Could the question be answered without newly exposing human subjects? If so, how? If not, why not?

"Human subjects are . . . the target system for the test material, and sufficiently reliable models for repellency testing have not been developed. In addition, subjects will self-administer the test articles during dose determination." (p. 5)

2. Study Design

(a) What is the scientific objective of the study? If there is an explicit hypothesis, what is it?

"The objective of this study is to test the mosquito repellent efficacy of the test material. . . . [E]fficacy will be measured as Complete Protection Time, . . . defined herein as the time between application of test material and the First Confirmed 'Lite with Intent to Bite.'" (p. 4)

"Determining dosage is a main objective of this study. Dosage for repellency testing will be the mean of the subject means determined . . . in the dosimetry portion of this study." (p. 8)

No explicit hypothesis is stated.

(b) Can the study as proposed achieve that objective or test this hypothesis?

The two objectives cited above can be achieved by the study as proposed.

2.1 Statistical Design

(a) What is the rationale for the choice of sample size?

"In efficacy testing, we will use 10 subjects. . . and 2 untreated control subjects per field trial. In the dosimetry potion of the study, 10 subjects will be engaged. . . ." (p. 16) The rationale for this sample size appears on pp. 16-18. A sample size of 10 reflects a compromise between cost and precision; it is larger than the minimum of 6 required by EPA, and promises to provide an acceptably robust measure of average complete protection time at reasonable cost.

(b) What negative and positive controls are proposed? Are proposed controls appropriate for the study design and statistical analysis plan?

"In efficacy testing, we will use . . . 2 untreated control subjects per field trial." (p. 16) "There is no control in which the formulation matrix without the repellent active [ingredient] is tested. There is no a priori basis for anticipating significant repellent activity in the matrices, and the study objective is to examine efficacy of the end products." (p. 11) There are no positive controls. Use of two untreated controls to confirm continued pest pressure throughout the field testing is appropriate for the study design. Omission of matrix and positive controls is appropriate for the study design. No direct comparisons of treated and untreated subjects are contemplated in the statistical analysis plan.

(d) How is the study blinded?

The dosimetry phase is not blinded. In the efficacy phase, "[b]ecause the treated condition will be evident to experimenters and subjects, and a single test material is under study, neither group will be effectively blinded." (p. 13)

(d) What is the plan for allocating individuals to treatment or control groups?

"All subjects that are not untreated controls will be assigned to the treatment group. . . . Negative control subjects will be selected exclusively from among experienced personnel. To be regarded as experienced personnel, a candidate subject must have an undergraduate (or higher) degree in life sciences, or be a vector control professional, or have participated in at least five Carroll-Loye repellent efficacy studies." (p. 13)

(e) Can the data be statistically analyzed?

Yes. The dosimetry phase provides for three applications per limb per subject. These three values per limb will be averaged for each subject, and those individual means will be used to estimate the grand mean across all 10 subjects.

The efficacy phase provides for testing in two distinct locations. At each location ten individual values for CPT will be obtained and averaged. At least some of the subjects are likely to be different in the two field tests.

(f) What is the plan for statistical analysis of the data?

Statistics will be computed with the software 'SAS JMP' Version 5.0.1.2 (SAS Institute, Cary, NC). (p. 30)

The typical consumer dose will be calculated in the dosimetry phase from the average amount of product (in grams) delivered by subjects and captured by 4 evenly-spaced

gauze 'bracelets' on the subject's forearm or lower leg. The surface area of subject's limbs is calculated as the product of the length of the limb by the average of 4 evenly-spaced circumferences. The dosimeter 'bracelets' are 2.5 cm wide, and centered on the points at which the circumferences of the limb were measured. The surface area of the dosimeters is 4 times the average limb circumference times 2.5 cm. The unit dose in g/cm^2 applied to the dosimeters is calculated as the weight difference in the dosimeters before and after application divided by the area of the dosimeters. (Calculation of overall unit dosage in the dosimetry phase is unnecessarily complicated by scaling up from the area of the dosimeters to the full skin area of the treated limb; this does not add any precision to the calculation of the applied dose.) Once calculated, the grand mean unit dose in g/cm^2 is divided by the specific gravity of the formulation to obtain a standard volumetric unit dose in ml/cm^2. This is scaled up to the volumetric dose for the treated limb of each subject in the efficacy phase, based on the surface area of the limb to be treated. (pp. 30-31)

"Subject effects on dosing behavior will be examined with non-parametric tests for *n*-sample independent cases (Kruskal-Wallis tests). In multiple regression analysis, the average amount of test material intercepted by each subject's dosimeters, as well as dosing per unit skin surface area, will be examined in relation to the distance from nozzle to skin, the number of times the pump was actuated, and limb size. The relationship between dosing behavior and dosage will also be examined with Spearman-rank correlation tests." (p. 31)

In the efficacy phase "Complete protection time is measured as the length of time from initial application to the first confirmed LIBe. . . . CPT measured in this way will yield a single time value for each subject. Mean CPT will be calculated across all 10 subjects, and will be presented with standard deviation and 95% confidence interval." (p. 32)

"Mean LIBing pressure will be calculated as the number of LIBes received per untreated control subject and per period and span of exposure." (p. 32)

(g) Are proposed statistical methods appropriate to answer the research question?

The proposed statistical measures for dose determination and duration of repellency are appropriate in the case that the data are normally distributed. The protocol does not address how to analyze non-normally distributed data. Based on efficacy data for other oil of lemon eucalyptus repellents, a distribution effectively close to a normal fit would be expected, but the protocol should incorporate a diagnostic test for normality, and discuss data transformation procedures or alternate non-parametric tests for statistical analyses of data which may not fit a normal distribution.

The protocol should explain the rationale for choosing the Kruskal-Wallis test, and what information it will provide to answer the research question.

(h) Does the proposed design have adequate statistical power to definitively answer the research question?

Gender is known to relate to differences in mosquito attractiveness among human subjects. The protocol should specify the proportion of male versus female subjects that will comprise the sample.

Variability is expected to be high for the dosimetry test.

2.2 How and to what will human subjects be exposed?

(a) What is the rationale for the choice of test material and formulation?

The test material is conditionally registered by EPA as Reg No. 305-62. Product specific efficacy testing of this material was required by EPA as a condition of the product's registration.

(b) What is the rationale for the choice of dose/exposure levels and the staging of dose administration?

A "typical consumer dose" will be estimated in the dosimetry phase as the average quantity of product applied per unit area of treated skin by ten subjects. This standard unit dose will be used for all subjects in the efficacy phase. One limb (forearm or lower leg) of each subject will be treated; exposure to the repellent will be continuous throughout the period of the efficacy test.

Subjects will also be exposed for one of every 15 minutes during the efficacy phase to "all or some of wild *Aedes vexans, Aedes melanimon, Aedes taeniorhynchus, Culex tarsalis* and *Culex pipiens* mosquitoes, and possibly other mosquito species that occur in the same habitats." (p. 11)

(c) What duration of exposure is proposed?

The dosimetry phase will involve 16 brief exposures to the test material over a period of two hours. The repellency phase will last for 8-14 hours, including travel time; the period of exposure is unclear, but the data collection form (p. 52) can accommodate reports over a period of 7.5 hours.

2.3 Endpoints and Measures

(a) What endpoints will be measured? Are they appropriate to the question(s) being asked?

"Variables to be measured" are listed in §10.1. They include "Subject forearm and lower leg surface area, subject self-dosing behaviors, weight of test materials delivered to the surrogate skin (gauze dosimeters), and number of mosquito lites with intent to bite (LIBes) on the treated surface." (p. 24)

These are the appropriate endpoints to measure. Clarity would be improved if the language were made consistent between this listing and the discussion of each measure in sections 10.4.1, 10.4.2, 10.4.3, and 10.4.5. In addition:

- §10.4.1 defines the formula by which subject limb surface area will be calculated, but does not describe the procedure by which it will be measured, or how the location of the centers of the four circumferences will be recorded to enable later placement of the dosimeters at the same location.

- §10.4.3 refers incorrectly to "dosimeters of known surface area." The surface area of the dosimeters is a calculated value, known only as the product of limb circumference and 2.5 cm, the width of the gauze. To ensure complete coverage of the full circumference, the gauze material will have to be cut long and overlapped; thus the area of the dosimeter gauze before application will be greater than the surface area of the dosimeter once it is in place.

(b) What steps are proposed to ensure measurements are accurate and reliable?

- Alternate subjects will be enrolled to ensure adequate sample size
- Subjects will be trained to recognize a "LIBe"
- Subjects will work in pairs, checking each other as well as themselves
- LIBes will be verified by a research technician

(c) What QA methods are proposed?

"Protocol Review and Comments must take place before data collection commences. In-Life Inspection must include observing the measurement and recording of key variables by subjects and researchers. In addition, the final report will be audited for completeness and accuracy. A QAU Statement will address compliance and noncompliance or any omissions in auditing. Findings from the In-Life Inspection and the Final Report, as well as the QAU Statement will be transmitted to both the Study Director and to the Sponsor Monitor." (p. 33)

Reports of QAU findings should be incorporated into the final report.

(d) How will uncertainty be addressed? Will point estimates be accompanied by measures of uncertainty?

"Mean CPT will be calculated across all 10 subjects, and will be presented with standard deviation and 95% confidence interval." (p. 32)

3. Subject Selection

3.1 Representativeness of Sample

(a) What is the population of concern? How was it identified?

The population of ultimate concern consists of people who would purchase and use insect repellents. Little information is available to characterize this population, but it is presumed that users of insect repellents are highly diverse in age, gender, physical size, general health, attractiveness to biting insects, and other characteristics. The population from which subjects are recruited appears to be chosen largely on the basis of convenience, and is not screened for past or likely future use of repellents.

(b) From what populations will subjects be recruited?

If the study is conducted in California: "Participants are recruited by verbal networking through our academic and personal communities of friends, neighbors and scientists in Davis CA. . . . Initial contact is through word-of-mouth and telephone contact with subjects who have participated in previous Carroll-Loye repellent efficacy tests and have agreed to be in our Volunteer Database. . . . Those who will serve as untreated control subjects are limited to experienced technical personnel. . . ." (pp. 18-19)

If the study is conducted in Florida: "For over a decade we have worked with the Florida Keys Mosquito Control District. . . . We contact the administration of the District to inform them of our need for subjects. The administration gives District employees the option of participating in our field studies." (p. 19)

No further information about the demographic makeup of either sampling frame is provided. The meaning of the statement that the administration of the Florida Keys Mosquito Control District "gives District employees the option of participating in our field studies" must be clarified.

(c) Are expected participants representative of the population of concern? If not, why not?

By excluding children, pregnant or lactating women, non-English speakers, and those in poor physical condition, among others, the exclusion criteria will mean that participants will not be representative of at least some segments of the population of concern.

(d) Can the findings from the proposed study be generalized beyond the study sample?

Yes.

3.2 Equitable Selection of Subjects

(a) What are the inclusion/exclusion criteria? Are they complete and appropriate?

Inclusion: age 18-55, written consent, speak and read English. (p. 15)

Additional inclusion criteria specific to the two untreated subjects: "To qualify for candidacy as a subject who exposes untreated skin, an individual must be regarded as competent to do so by the Principal Investigator, must have participated in at least five prior Carroll-Loye repellent efficacy trials, or have participated in at least three such trials and have a least two years of experience as a college life sciences major, or be professionally employed in vector control services." (p. 15)

This statement of criteria for untreated controls paraphrases and differs in detail from other explanations of the same criteria for untreated controls in sections 8.2 and 8.3.1, on p. 13 and quoted above in item 2.1(d). The criteria should be stated only once, preferably in §9.1.2.

Exclusion: hypersensitivity to mosquito bites, sensitivity to any product ingredients, poor physical condition, unwillingness to submit to brief query about personal condition, use of insect repellent within one day before study, unwillingness to abstain from alcohol, smoking, and perfumed products, pregnant or lactating, unable to apply test materials, student or employee of Study Director, unaccustomed to outdoor activity. (pp. 15-16)

In general, these criteria for inclusion and exclusion appear appropriate. More information about how they affect the representativeness of the sample is desirable.

(b) What, if any, is the relationship between the investigator and the subjects?

Subjects are recruited from the investigator's "academic and personal communities of friends, neighbors and scientists in Davis CA." (p. 18)

"Our subjects are mainly University of California—Davis graduate and undergraduate students in life science programs with which the Principal Investigator is associated. Students in his laboratory who depend on him directly for employment or scholastically are not eligible to participate." (p. 19)

(c) If any potential subjects are from a vulnerable population, what is the justification for including them?

No subjects from a vulnerable population are proposed.

(d) What process is proposed for recruiting and informing potential subjects?

The recruiting/informing process to be used if the research is conducted in California is extensively described in the protocol on pp. 18-20 and in the informed consent documents on pp. 39-47.

The process to be used if the research is conducted in Florida is not described beyond the assertion that the "administration [of the Florida Keys Mosquito Control District] gives District employees the option of participating in our field studies." (p. 19) This suggests that information about the research is provided to potential subjects by their employers, who also "give them the option" to participate. This is unacceptable.

(e) If any subjects are potentially subject to coercion or undue influence, what specific safeguards are proposed to protect their rights and welfare?

"Students in [the PI's] laboratory who depend on him directly for employment or scholastically are not eligible to participate." (p. 19)

More information about recruiting in Florida is needed to ensure that the decision by Mosquito Control District employees to participate in this research would be free from coercion or undue influence by their administrators.

3.3 Remuneration of Subjects

(a) What remuneration, if any, is proposed for the subjects?

"[E]ach research study participant will receive a cash payment of $20 per hour. . . . If you are designated as an 'alternate subject' you will be paid for the hours you spent being trained, plus you will receive a payment of $50 to compensate for being inconvenienced." (p. 46)

(b) Is proposed remuneration so high as to be an undue inducement?

Probably not, although the hourly rate has risen by one-third since the last protocol from Carroll-Loye reviewed by the HSRB in January 2007.

(c) Is proposed remuneration so low that it will only be attractive to economically disadvantaged subjects?

No.

(d) How and when would subjects be paid?

"Payment will be made at the end of each visit or whenever you withdraw from the study." (p. 46)

4. Risks to Subjects

4.1 Risk characterization

(a) Have all appropriate prerequisite studies been performed? What do they show about the hazards of the test materials?

An MSDS (p. 62) for Cutter Lemon of Eucalyptus Insect Repellent Pump reports that the product has been tested on animals for potential oral and dermal toxicity. It causes eye irritation. Inert ingredients are considered relatively safe.

"The insect repellent product proposed for testing have all been tested in animals for potential oral and dermal toxicity. The OLE active ingredient has an extensive toxicity data file, has previously be registered by EPA, and has a positive safety record in consumer use." (p. 9)

The test material is conditionally registered by EPA, and is supported by the full range of required toxicity data. It bears hazard labeling in Toxicity Category II with the signal word "Warning" because of its ability to cause "substantial but temporary eye injury." (See sample label on p. 59)

(b) What is the nature of the risks to subjects of the proposed research?

Risks are of three kinds: the risk of reaction to the repellent tested, the risk of reaction to arthropod bites, and the risk of contraction of an arthropod-borne disease.

"The repellent active ingredient has a low acute and chronic risk profile. . . . The concentration of the active ingredient in the product being tested is lower than that of other products currently EPA-registered and marketed in the US." (pp. 5-6) This passage in the protocol fails to mention the potential for eye irritation; this risk is better characterized in the informed consent document: "The repellent will irritate the eyes on contact, and it is harmful if swallowed." (p. 44).

"[E]ven if you have not had a serious skin reaction to a mosquito bite previously, it is possible that such a reaction could occur if you receive any bites during this study. Swelling, redness and itching near the site of the bite are all symptoms of an allergic reaction to a mosquito bite." (p. 44)

"[T]here is a slight possibility that you will contract a disease carried by mosquitoes if you are bitten, such as West Nile virus or equine encephalitis." (p. 44)

(c) What is the probability of each risk associated with the research? How was this probability estimated?

No numerical probability is estimated. Potential subjects are told "you are probably at no more risk than you would experience when engaged in normal outdoor activities in a similar rural area at the same time of year." (p. 44) "Since you will work to quickly remove mosquitoes before they have an opportunity to bite, and few of the mosquitoes present are likely to carry the virus, your chances of getting West Nile fever or another disease from a mosquito bite are probably extremely small." (p. 45)

4.2 Risk minimization

(a) What specific steps are proposed to minimize risks to subjects?

- The risk of a skin reaction to a mosquito bite is reduced by excluding candidate subjects who are aware of having a history of such reaction.
- Candidates with known sensitivity to any product ingredients are excluded.
- Subjects will be trained to quickly remove any mosquitoes that attempt to bite them, before penetration or injection of saliva if possible.
- Mosquitoes used for aspirator training will be lab-reared and disease-free.
- Subjects will be instructed to cover any treated skin immediately if more than one mosquito attempts to bite during any exposure period.
- Subjects will expose small areas of treated skin for only 4 minutes per hour. Other parts of the body will be protected with provided fabric.
- At the end of each one-minute exposure period subjects will move away from the area with mosquito activity. Partners will assist each other to cover the treated area.
- Subjects will be teamed with a partner for joint observation; experienced technical personnel will be present at all times to assist.
- Field tests are conducted in an area where West Nile virus has not been detected by county or state agencies for at least a month.
- Only 2 untreated controls to confirm biting pressure.
- Exposure of untreated controls for no more than 4 min/hour; exposed skin may be covered immediately following the first LIBe.
- Untreated controls will be attended by two assistants with aspirators to remove any mosquitoes that land with intent to bite.
- First Aid materials will be available on-site
- Epi-Pens will be on-site to treat anaphylactic allergic reactions.
- No control with formulation matrix exclusive of active repellent ingredients.
- A physician who has read the protocol and discussed the research with the Study Director will be on call on the day of field testing.

(b) How do proposed dose/exposure levels compare to established NOELs/NOAELs for the test materials?

Actual dose levels will only be established by the results of the first, dosimetry phase of the proposed study. The dosimetry phase is intended to establish a "typical

consumer dose", expected to be far below any NOELs/ NOAELs for the test material, although this relationship is not calculated. Given that animal testing for dermal toxicity is reported to be available for the test material, the ratio of the dose to the animal NOAEL could be included in the study report, and a stopping rule could be added in case the margin between the "typical dose" and the animal NOAEL is insufficient to justify proceeding.

(c) What stopping rules are proposed in the protocol?

"Any subject showing adverse skin reactions will immediately stop further participation." (p. 22)

"Subjects are instructed to immediately cover exposed skin with the protective mesh provided if more than one LIBe occurs in a one-minute exposure period. Similarly, if subjects receive a LIBe and recall receiving another in either of the two previous exposure periods, they are to ask their data recording technician to verify the recollection from the data record. If verified, the subject is instructed to immediately cover the limb as above." (p. 29)

"If more than one mosquito attempts to bite you on your treated skin in one of the one-minute periods, or if one mosquito attempts to bite in two of three consecutive exposure periods (that is, 15 or 30 minutes apart), you should cover the skin and not expose it again." (p. 43)

(d) How does the protocol provide for medical management of potential illness or injury to subjects?

"If you are injured as a result of being in this study, a consulting physician who is aware of the study will be contacted immediately by telephone. Medical treatment will be available from a health care facility." (p. 45)

(e) How does the protocol provide for safety monitoring?

"[T]echnical personnel will monitor, and subjects will self-monitor, for allergic and irritant skin reactions, particularly redness, edema, itching or pain, and report any such reactions to the Study Director. Any subject showing adverse skin reactions will immediately stop further participation. The treated skin will be gently washed with clean water and mild soap to remove the test product, and the area will be gently dried with a clean towel. The subject will be removed from further exposure to mosquitoes.

"On the day of testing, a physician who has read the protocol and discussed the research with the Study Director will be on call. In unlikely event of a Type 1 allergic reaction (anaphylaxis), we will contact 9-1-1 by cellular or satellite telephone and cooperate as instructed with emergency personnel. . . .

"[T] Study Director will assess skin condition of affected subjects should any bites inadvertently occur during efficacy testing."

"As part of Medical Management, the Study Director will record all benign and adverse health observations." (pp. 22-23)

(f) How does the protocol provide for post-exposure monitoring or follow-up? Is it of long enough duration to discover adverse events which might occur?

"All subjects are asked to contact the Study Director and a physician of their own choice at any time should they develop a rash . . . within 48 hours of the conclusion of the test day." (p. 23) While subjects may indeed be asked to do this, the Informed Consent Document is silent on this point.

"If you experience any of the symptoms described above in the month following the field test you should contact a medical practitioner and inform the Principal Investigator." (p. 45)

(g) How and by whom will medical care for research-related injuries to subjects be paid for?

"Carroll-Loye Biological Research will cover the costs of such medical treatment that are not covered by your own insurance or by a third party. If necessary, Carroll-Loye Biological Research will transport you to receive medical attention and pay costs associated with the reasonable and appropriate treatment for any injuries incurred as a result of participation in the study." (p. 45)

5. Benefits

(a) What benefits of the proposed research, if any, would accrue to individual subjects?

"There are no immediate benefits to you from your participation." (p. 46)

(b) What benefits to society are anticipated from the information likely to be gained through the research?

"Against the slight risks are balanced substantial and reasonably likely benefits. Insect-borne disease is of growing significance in the United States and around the world where U.S. citizens are active. Moreover, discomfort associated with nuisance biting restricts many work and pleasure activities. Because EPA-registration required efficacy data, a test such as that proposed here is the only path toward further product development and greater availability of new OLE mosquito repellent to consumers in the United States." (p. 7)

"[B]y serving as a participant you may assist in making new insect repellent products available to consumers." (p. 46)

(c) How would societal benefits be distributed? Who would benefit from the proposed research?

The direct beneficiary of the research is likely to be the sponsor. Assuming eventual regulatory approval, indirect beneficiaries would include those repellent users who prefer the new formulations to previously available repellents.

(d) What is the likelihood that each identified societal benefits would be realized?

The testing is likely to demonstrate that the new formulations are effective, and thus the sponsor is likely to realize a direct benefit from the research. Realization of other societal benefits will depend on consumer acceptance of the new formulations.

6. Risk/Benefit Balance

(a) How do the risks to subjects weigh against the anticipated benefits of the research, to subjects or to society?

The protocol systematically reduces risks to subjects without reducing the robustness of the scientific design. No opportunities to further reduce subject risk have been overlooked. Thus the resulting risk to subjects is very low—as low as or lower than the risk to anyone engaged in outdoor activity where mosquitoes are active. The potential benefits to repellent users from a wider variety of effective repellents with different cosmetic characteristics are likely to be realized, and make the residual risks to subjects in this proposed research reasonable.

7. Independent Ethics Review

(a) What IRB reviewed the proposed research?

Independent Investigational Review Board, Plantation FL

(b) Is this IRB independent of the investigators and sponsors of the research? Yes

(c) Is this IRB registered with OHRP? Yes

(d) Is this IRB accredited? If so, by whom?

Not reported. IIRB is not listed as accredited on the AAHRPP website.

(e) Does this IRB hold a Federal-Wide Assurance from OHRP?

Not reported. IIRB is not listed as holding an FWA on the OHRP website.

(d) Are complete records of the IRB review as required by 40 CFR 26.1125 provided?

Correspondence between the IIRB and the investigator was provided by the Investigator. Documentation of IRB procedures was not provided, but a statement is included from the IIRB (p. 72) that there had been no changes since this information had previously been submitted to EPA.

(e) What standard(s) of ethical conduct would govern the work?

"U.S. EPA Good Laboratory Practice Regulations (40 CFR 160); 40 CFR 26 subparts K and L; FIFRA §12(a)(2)(P); California State EPA Department of Pesticide Regulation study monitoring (California Code of Regulations Title 3, Section 6710)." (p. 8)

8. Informed Consent

(a) Will informed consent be obtained from each prospective subject? Yes.

(b) Will informed consent be appropriately documented, consistent with the requirements of 40 CFR 26.1117? Yes.

(c) Do the informed consent materials meet the requirements of 40 CFR 26.1116, including adequate characterization of the risks and discomforts to subjects from participation in the research, the potential benefits to the subject or others, and the right to withdraw from the research? Yes.

(d) What is the literacy rate in English or other languages among the intended research subjects?

100%. English literacy is a requirement for participation.

(e) What measures are proposed to overcome language differences, if any, between investigators and subjects? n/a

(f) What measures are proposed to ensure subject comprehension of risks and discomforts?

Frequent opportunities to ask questions.

(g) What specific procedure will be followed to inform prospective subjects and to seek and obtain their consent?

See pp. 18-20 and ICF pp. 39-48. The procedure to be used in Florida is inadequately described, and suggests that the administrators of the cooperating Florida Keys Mosquito Control District will provide much of the information to potential subjects.

(h) What measures are proposed to ensure fully voluntary participation and to avoid coercion or undue influence?

Candidates are offered repeated opportunities to decide not to participate; participants are offered repeated opportunities to withdraw. Exclusion factors rule out participation by employees or students of the Study Director. Recruitment of alternate subjects ensures that subjects will not be reluctant to withdraw lest the validity of the investigation be compromised. The statement that "[t]he administration gives District employees the option of participating" (p. 19) suggests the possibility of inappropriate influence by supervisors over the choice of District employees to participate.

9. Respect for Subjects

(a) How will information about prospective and enrolled subjects be managed to ensure their privacy?

Subjects are identified by name and by number. Only the number is used on data collection forms for the repellency phase, but the full name as well as the subject number appears unnecessarily on all other sample forms provided (pp. 49-51.) It would be better to use only the subject number on all but one master directory that linked names to numbers. Recruitment of alternate subjects provides an opportunity for discrete withdrawal without explanation. Subjects are told they "may access [their] own records by contacting the Study Director," and that they will not be identified in any published reports of the study. (p. 46)

(b) How will subjects be informed of their freedom to withdraw from the research at any time without penalty?

Subjects are so informed in the recruitment process (pp. 18-20) and in the Informed Consent Form (pp. 46-47).

(c) How will subjects who decline to participate or who withdraw from the research be dealt with?

Subjects who decide not to participate will simply go their way. Subjects identified as alternates, and any who withdraw from the research, will be paid for their time (p. 46). How soon after they withdraw subjects in the efficacy would be able to leave the field study site would depend on how they got there; this is not explained.

§ 26.1111 Criteria for IRB approval of research
Protocol SCI-001

Criterion	Y/N	Comment/Page Reference
(a)(1)(i) Risks to subjects are minimized by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk.	Y	
(a)(1)(ii) Risks to subjects are minimized, whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.	N/A	
(a)(2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (for example, the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.	Y	
(a)(3) Selection of subjects is equitable, taking into account the purposes of the research and the setting in which it will be conducted, and being particularly cognizant of the special problems of research involving vulnerable populations, such as prisoners, mentally disabled persons, or economically or educationally disadvantaged persons.	Y	
(a)(4) Informed consent will be sought from each prospective subject or the subject's legally authorized representative, in accordance with, and to the extent required by §26.1116.	Y	Expand p. 19 description of consent process in Florida; clarify role of district administration
(a)(5) Informed consent will be appropriately documented, in accordance with, and to the extent required by §26.1117.	Y	
(a)(6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.	Y	
(a)(7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.	Y	
(b) When some or all of the subjects are likely to be vulnerable to coercion or undue influence, additional safeguards have been included in the study to protect the rights and welfare of these subjects.	N/A	

§26.1116 General requirements for informed consent
Protocol SCI-001

Criterion		Y/N	Comment/Page Reference
No investigator may involve a human being as a subject in research covered by this subpart unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative		OK	All subjects will provide legally effective informed consent.
An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence		OK	The procedure described in §9.1.4.2 provides sufficient opportunity to consider. . . and minimizes the possibility of coercion or undue influence. The procedure described in §9.1.5.2 must be expanded.
The information that is given to the subject or the representative shall be in language understandable to the subject or the representative		OK	Information is clearly presented in plain English
No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence		OK	The IC contains no exculpatory language
(a) In seeking informed consent the following information shall be provided to each subject	(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental	OK	p. 39
	(2) A description of any reasonably foreseeable risks or discomforts to the subject	OK	pp. 44-45
	(3) A description of any benefits to the subject or to others which may reasonably be expected from the research	OK	p. 46
	(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject	OK	p. 45
	(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained	OK	p. 46
	(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained	OK	Compensation p. 46 Treatment p. 45
	(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject	OK	p. 46
	(8) A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled	OK	p. 46-47
(b) When appropriate, one or more of the following elements of information shall also be	(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject may become pregnant) which are currently unforeseeable	OK	p. 45
	(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent	OK	p. 47
	(3) Any additional costs to the subject that may result from participation in the research	OK	p. 46
	(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject	N/A	
	(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject	OK	p. 45
	(6) The approximate number of subjects involved in the study	OK	p. 40
(e) If the research involves intentional exposure of subjects to a pesticide, the subjects of the research must be informed of the identity of the pesticide and the nature of its pesticidal function.		OK	p. 39

§26.1117 Documentation of informed consent
Protocol SCI-001

Criterion	Y/N	Comment/Page Reference
(a) Informed consent shall be documented by the use of a written consent form approved by the IRB and signed by the subject or the subject's legally authorized representative. A copy shall be given to the person signing the form.	OK	Form pp. 39-47 Procedures pp. 18-20
(b)(1) The consent form may be a written consent document that embodies the elements of informed consent required by §26.1116. This form may be read to the subject or the subject's legally authorized representative, but in any event, the investigator shall give either the subject or the representative adequate opportunity to read it before it is signed; or	OK	Proposed IC form meets requirements of §26.1116; procedure described in protocol §9.1.4.2 provides adequate opportunity to read it before it is signed.
(b)(2) The consent form may be a short form written consent document stating that the elements of informed consent required by §26.1116 have been presented orally to the subject or the subject's legally authorized representative. When this method is used, there shall be a witness to the oral presentation. Also, the IRB shall approve a written summary of what is to be said to the subject or the representative. Only the short form itself is to be signed by the subject or the representative. However, the witness shall sign both the short form and a copy of the summary, and the person actually obtaining consent shall sign a copy of the summary. A copy of the summary shall be given to the subject or the representative, in addition to a copy of the short form.	N/A	

40 CFR 26.1125 Submission of proposed human research for EPA review
Carroll-Loye SCI-001 (Version of 11/2/2006)

Any person or institution who intends to conduct or sponsor human research covered by §26.1101(a) shall, after receiving approval from all appropriate IRBs, submit to EPA prior to initiating such research all information relevant to the proposed research specified by §26.1115(a), and the following additional information, to the extent not already included:

	Requirement		Y/N	Comments/Page Refs
The following Information, to the Extent not already included:	§1125(a) a discussion of:	(1) The potential risks to human subjects	Y	pp. 5-7, 44-45
		(2) The measures proposed to minimize risks to the human subjects;	Y	pp. 6, 7, 11, 13, 15, 22-24, 27-29
		(3) The nature and magnitude of all expected benefits of such research, and to whom they would accrue	Y	pp. 7, 46
		(4) Alternative means of obtaining information comparable to what would be collected through the proposed research; and	Y	p. 5
		(5) The balance of risks and benefits of the proposed research.	Y	pp. 7
	§1125(b): All information for subjects and written informed consent agreements as originally provided to the IRB, and as approved by the IRB.		Y	pp. 39-48.
	§1125(c): Information about how subjects will be recruited, including any advertisements proposed to be used.		Y	pp. 18-20. No advertisements used
	§1125(d): A description of the circumstances and methods proposed for presenting information to potential human subjects for the purpose of obtaining their informed consent.		Y	pp. 18-20
	§1125(e): All correspondence between the IRB and the investigators or sponsors.		Y	pp. 63-75
	§1125(f): Official notification to the sponsor or investigator. . . that research involving human subjects has been reviewed and approved by an IRB.		Y	p. 37-38
all information relevant to the proposed research specified by § 26.1115(a)	(1) Copies of • all research proposals reviewed by the IRB, • scientific evaluations, if any, that accompanied the proposals reviewed by the IRB, • approved sample consent documents, • progress reports submitted by investigators, and reports of injuries to subjects.		Y n/a Y n/a	pp. 1-36, 39-68 None accompanied the proposal p. 39-48 Initial review of new proposal
	(2) Minutes of IRB meetings . . . in sufficient detail to show • attendance at the meetings; • actions taken by the IRB; • the vote on these actions including the number of members voting for, against, and abstaining; • the basis for requiring changes in or disapproving research; • a written summary of the discussion of controverted issues and their resolution.		Y Y Y n/a n/a	IRB minutes p. 73 No changes required by IRB No controverted issues
	(3) Records of continuing review activities.		n/a	n/a for protocols
	(4) Copies of all correspondence between the IRB and the investigators.		Y	Provided by investigator; pp. 63-75
	(5) • A list of IRB members identified by name; earned degrees; representative capacity; indications of experience such as board certifications, licenses, etc., sufficient to describe each member's chief anticipated contributions to IRB deliberations; • any employment or other relationship between each member and the institution, for example, full-time employee, a member of governing panel or board, stockholder, paid or unpaid consultant.		Y Y	pp. 74-75
	(6) Written procedures for the IRB in the same detail as described in §26.1108(a) and §26.1108(b).		Y	On file with EPA (Claimed CBI)
	(7) Statements of significant new findings provided to subjects, as required by §26.1116(b)(5).		n/a	n/a for protocols