# Product Performance Protocol Review
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# Registration Division

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**Summary:** Two proposed protocols for research on the efficacy of IR-3535 formulations were submitted to EPA for review. A "Template Protocol" describing universal procedures, instructions, and the experimental designs for insect repellent testing was also included. I commented on the scientific soundness of these procedures and how they compare to the current draft of the Insect Repellent Testing Guideline OPPTS 810.3700, referred top as "EPA Guideline" in this review. An ethics review of these protocols will be prepared by another reviewer at EPA.

The submitted protocols differ significantly from the current draft of the EPA guideline although they are closer to the version published in 2000. Of significance in the mosquito tests is the use of only one control (untreated) subject in field and laboratory studies compared to an equal number of treated vs. untreated replicates recommended in the EPA guideline. The EPA guideline also recommends the use of untreated and treated limbs on the same subject instead of a separate test subject in order to reduce variability and provide a balanced test design. The field study design is unclear as to how treated and untreated replicates can be compared because the evaluation intervals for each differ. For instance, mosquito landings in untreated and treated replicates are recorded at different times during the study. Execution of the study is further complicated because repellency is recorded at very frequent intervals instead of every hour as recommended by the EPA guideline. Generally, more details on how data are recorded in the studyshould be added. Examples of data sheets were not included; the chain of custody of the test substance is not described; and justifications of many of the procedures are not fully explained. More detailed reference to the Common Rule is also advised.

The procedures for tick testing would benefit from greater detail and clarification. How can these data be used to support repellency time claims? How would hours of repellency be calculated?

## Entomologist's Recommendations:

1. Many of the protocol deficiencies can be corrected by including more detailed descriptions of the study design and procedures. I recommend that protocols that involve human subject testing fully conform to Good Laboratory Practices (GLP) as described in 40 CFR Part 160. as mentioned in 40 CFR part 26, which states that the human testing has to be done according to GLP, i.e., including the protocol.
2. The number of untreated (negative) control replicates should be increased to improve the statistical reliability and balance of the study design.
3. Data recording intervals should be increased to 60 minutes to provide hourly repellency values and decreased exposure of untreated subjects to mosquito bites.
4. Product specific repellent testing should be done with the repellent formulation as it is intended for registration, use and application to the skin. For instance, an aerosol formulation should be sprayed on the skin as directed by the proposed label, not "liquefied" and applied by pipette onto the skin. This procedure is consistent with the current draft of the EPA guideline and the product specific data requirements described in 40CFR Part 158.

## Entomologist's Review and Comments:

**These protocols address some of the Standard Operating Procedures described in 40 CFR Part 160. 81 (subpart E–Testing Facilities Operation). Information provided in protocols also addresses GLP protocol and conduct of the study requirements described in 40 CFR Parts 160.120 and 130. However, more detail is required to fully comply with the requirements of both parts.**

**Protocol C-L-001 (Template Protocol) dated March 26, 2006**
**Title: Test of Personal Insect Repellents**

1. The protocol acknowledges California Department of pesticide Regulations (CDPR) regulations, and cites EPA's 1982 Subpart G guidelines and draft 810.3700 guidelines. It also cites ASTM method E-939-94. It should also cite FIFRA §12(a)(2)(P) and 40 CFR 26 subparts K and L.

2. In section 4(B), the only endpoint described is "time to first confirmed failure". This is the equivalent of "First Confirmed Bite". However, this standard is not applicable to tick testing, nor does it address calculation of relative protection for other test arthropods.

3. In section 4(C)(2) the number of subjects used is 6 treated subjects, 2 positive controls, and 1 negative control. This distribution is attributed to 1999 draft of the EPA guideline, but is inconsistent with the current draft guideline, which call for equal numbers of treated and untreated subjects.

4. The procedures described in section 4(F) appear intended to apply to both laboratory and field tests, for repellency of many kinds of arthropod pests. This degree of generality is not consistent with meaningful precision. Several points in section 4(F) merit comment:

    a. The reference to confirming biting pressure with 1 minute exposure of the untreated control every 15 minutes is inconsistent with the procedure in either of the specific protocols provided for review.

    b. The reference to testing the carrier of the topical product as an "appropriate control article" is not explained. This would be neither an untreated control nor a positive control, and appears inconsistent with section 4(C)(2). In essence, this type of treatment could be used to test the repellent nature of the carrier.

    c. The practice of "distribution of multiple repellents among forearms and lower legs" in a single test could result in unreliable data. The "basic approach" of applying repellent to only one limb of a test subject is preferable. Use of an untreated limb of a treated subject as an untreated control is also a sound practice as described in the current draft of the EPA 810.3700 guideline.

    d. EPA's draft guidelines recommend dosing at the typical consumer dose, and explaining how this dose was determined. In the subject protocol, the reference to a "commonly used, industry standard" three-fold range is inconsistent with this recommendation. The products should be applied from packaging and in the form intended for sale and registration.

    e. The procedures (a) through (f) at the top of p. 8 and applying to subjects are appropriate.

    f. The procedures (a) through (d) at the bottom of p. 8 raise some questions.

        i. Paragraph (a) says "sample test arthropods are collected." Samples aren't needed in laboratory testing. In field testing, they should be collected, and then identified and described in the report. When and how to collect these specimens should be described.

        ii. It is important to establish and confirm biting pressure throughout the period of the test. More detail is needed to determine the acceptability of the procedure described here: "a minimum estimated biting rate of 30 bites/hour must be observed on the control participant(s) before the initiation of a trial, and maintained during the entire period." In a field test involving exposure of untreated controls continuously for 8 hours, as is proposed in

protocol CL-004, the untreated control subject would thus be expected to receive at least 240 mosquito bites in the course of the test. This is far more than is needed to confirm biting pressure.

g. Only sites in California are mentioned in paragraph 4(F)(3) at the top of page 9. Specific protocol CL-004 also provides for an alternative site in the Florida Keys, where populations of arthropods, including some which vector diseases, differ from those in central California in ways which could affect the risks to subjects.

5. Section 4(G) lists 18 ways in which risks are minimized. Some merit comment:

a. Item 4 notes that threshold efficacy is "normally" demonstrated in the laboratory before field testing, so that subjects in field tests can be expected to receive few or no bites. Preliminary laboratory testing has not usually been required by EPA, and may or may not be conducted. However, registrants and researchers determine a dose range through laboratory studies to evaluate in field tests before conducting them.

b. Items 8-10 note that subjects are provided with an aspirator to remove landing mosquitoes, taught how to tell when a mosquito has landed, and paired with a "buddy" to monitor mosquito landings. But this procedure means that the landing of a mosquito, rather than a probe or bite, becomes the event that determines failure of repellency. This in contrary to the draft guidelines.

c. Item 11(a) states that "in lab tests with mosquitoes, individuals expose treated arms for no more than one minute at intervals of fifteen minutes." This differs from current EPA guideline recommendations where a five minute exposure time is recommended at one hour intervals.

d. Item 12 states that untreated controls "are always Study Directors or experienced management personnel." This is inconsistent the statement in section 4(F) that untreated limbs of treated subjects may be used as controls, and with the statement in the specific protocols CL-03 and CL-04 that subjects are distributed randomly to treatment and control groups, in a way that is blinded to the experimenter.

**Protocol EMD-003 Laboratory Tick Repellency dated April 13, 2006**

1. The statement of objective in section 5.1 should mention that it is a laboratory study of tick repellency.

2. I do not understand Section 5.2.

3. Section 5.3 should also cite FIFRA §12(a)(2)(P), 40 CFR 26 subparts K and L, and, since the laboratory is in California, the California Code of Regulations Title 3 Section 6710.

4. Section 6.1.1 should clarify that the three formulations to be tested are not registered products. Are these pending products or research formulations?

5. Sections 6.1.2 and 9.3 describe applying all formulations as a liquid by pipette, notwithstanding that the three variant formulations are described as a lotion, a pump spray, and an aerosol. This does not meet the recommendation in EPA draft guidelines that the repellent product be applied and tested as it is proposed to be registered, and weakens the justification for the test.

6. In sections 6.1.3 and 6.2.3 the references to lower legs should be deleted. These are not applicable to tests of tick repellency using only arms.

7. In section 6.4 the developmental stage, age, rearing/feeding practices, methods for ensuring freedom from disease, etc. should all be specified in addition to the species of test organism. Only one species of test organism is mentioned compared to the EPA guideline where species from three tick genera are recommended.

8. In section 7.2 the reference to travel to a field site should be deleted because this is a laboratory study.

9. Section 8.3.1 states that assignment to treatment or control groups will be random. This is not consistent with the assertion in section 4(G)(12) of the template protocol CL-001 that untreated controls will always be Study Directors or experienced management personnel. Also in section 8.3.1 delete the inappropriate reference to legs.

10. In section 8.3.2 consider adequacy of the number of controls.

11. Section 8.4.1 refers to pre-screening ticks for activity “in locomotion and travel”. The protocol should specify when this will be done, by whom, and how screened ticks will be handled between this pre-screening test and their use on a treated subject. In addition, the relation of this pre-screening to the untreated control should be explained.

12. It is not clear why the number of subjects is described in section 9.1.3 in approximate terms. It is stated precisely in section 8.3.2.

13. In section 10.1:

a. EPA's draft guideline defines a failure of repellency as crossing at least 2 cm into the treated area within 5 minutes. This protocol allows only 3 minutes for observation—a less demanding standard.

b. Lines 8-9 include the contradictory statements "ticks are observed one at a time" and "each subject selects one or two ticks".

c. The "pool of unused pre-screened, qualified ticks" is not described. This is the same issue noted above in commenting on section 8.4.1.

d. It is unclear whether the subject's forearm is vertical or horizontal during the test. EPA's draft guideline recommends vertical, since it is known that questing ticks tend to move upward.

e. The protocol states simply "used ticks are retired from the study." It should state how, especially since it is the subjects who remove them and must "retire" them.

f. This passage should specify how and by whom time will be kept, both for the 15-minute cycles and for the start and end of the 3-minute periods for testing repellency.

14. In section 10.1.1 there is no mention of how controls will be employed.

15. Section 10.1.2 refers to a "data form", a sample of which should be incorporated into the protocol. The protocol should also explain who will fill it out and sign it.

16. In section 11.2 the passage should be corrected to read ". . . subjects treated with each of the three test repellents, . . ."

17. In the discussion of statistical methods in section 11.3:

    a. How will the passage of time be addressed in assessing repellency?

    b. Given the statement that "percent repellency is calculated as the total number of challenges in which ticks did not cross the barrier divided by the total number of challenges made, with the quotient multiplied by 100", what does it mean to say "mean percent repellency is calculated for each subject?" Is the grand mean determined by adding subject means together and dividing that value by the number of subjects? Chi-square is then applied to verify the statistical reliability of the means? This section requires more explanation.

18. Section 14 should acknowledge the obligation to notify both the IRB and CDPR of amendments and/or adverse events.

**Protocol EMD-004: Field Mosquito Repellency dated April 13, 2006.**

1. Section 4.1 and 12 should specify the location of the test.

2. Section 5.1 should mention mosquitoes in the "objective of research"

3. I do not understand Section 5.2.

4. Section 5.3 should also cite FIFRA §12(a)(2)(P), 40 CFR 26 subparts K and L, and, if the research is conducted in California, the California Code of Regulations Title 3 Section 6710.

5. Section 6.1.1 should clarify that the three formulations to be tested are not registered products. Are these pending products or research formulations?

6. Sections 6.1.2 and 9.3 describe applying all formulations as a liquid by pipette, notwithstanding that the three variant formulations are described as a lotion, a pump spray, and an aerosol. This does not meet the recommendation in EPA draft guidelines that the repellent be applied and tested as it is proposed to be registered, and weakens the justification for the test.

7. Section 6.4 should describe how the presence of these genera of mosquito will be established, and should provide for identification by species and, if possible, subspecies or strain of mosquitoes in the test area. Procedures should be described.

8. Section 8.3.1 states that assignment to treatment or control groups will be random. This is not consistent with the assertion in section 4(G)(12) of the template protocol CL-001 that untreated controls will always be Study Directors or experienced management personnel.

9. It isn't clear that a single untreated control as specified in section 8.3 is sufficient to support calculation of relative protection as a measure of repellency. The current EPA guideline recommends the same number of treated and untreated control replicates.

10. Section 8.4.1 describes continuous exposure of an untreated control to confirm biting pressure throughout the period of the test. EPA's draft guidelines recommend exposing an untreated control for up to five minutes per hour to confirm continued biting pressure, and provide for exposing the untreated limb only long enough to receive the 5 probes or bites required. This results in far less exposure of the untreated control subject(s). Section 8.4.1 also mentions the possibility that untreated control exposure might be intermittent, but makes no mention of intermittent exposure of treated subjects. This could invalidate the calculation of relative protection described in section 11.3. Section 8.4.1 also refers

to “mean liting rate”, calculated as the number of lites per period of exposure. It is not clear what this rate is a mean of.

11. Section 9.1.2 should include as an addition exclusion factor recent repellent use, as is mentioned in the informed consent material.

12. It is not clear why the number of subjects is described in section 9.1.3 in approximate terms. It is stated precisely in section 8.3.2.

13. Section 10.1 describes mosquito landings as the events to be measured, and predicts that landing mosquitos will be aspirated before biting. In periods of heavy biting pressure this might not be possible. EPA’s draft guideline recommends that probes/bites be the events measured, not landings.

14. Section 10.1.1 provides for continuous exposure of treated subjects throughout the test, with landings recorded in 5 minute blocks. EPA’s draft guideline recommends either continuous exposure or intermittent exposure at fixed intervals. The latter would reduce both exposure and risk to subjects with no loss of validity of information.

15. A sample of the data form mentioned in section 10.1.2 should be incorporated into the protocol.

16. The passage in section 11.2 should be corrected to read “. . . subjects treated with each of the three test repellents, . . .”

17. The first paragraph in section 11.3 describes calculation of Relative Protection. It is not clear that a single untreated control is sufficient to support this calculation; EPA’s draft guideline recommends equal numbers of treated and untreated subjects/replicates. If control exposure is intermittent, as provided for in section 8.4.1, this calculation would be invalid.

18. The second paragraph of section 11.3 defines Complete Protection time CPT) as time to first confirmed failure. Because of the treatment of a “lite” or landing as the measured event, this treats a confirmed landing—i.e., two landings within 30 minutes—as a failure of efficacy. EPA’s draft guideline considers repellency to fail only when a landing mosquito probes or bites.