UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 16, 2009

MEMORANDUM

SUBJECT: EPA Responses to HSRB Questions Directed to EPA Concerning Space Repellent Testing

FROM: John M. Carley
Human Research Ethics Review Officer
Office of Pesticide Programs (7501P)

TO: Paul I. Lewis, Ph.D.
Designated Federal Official
Human Studies Review Board
Office of Science Advisor (8105R)

Earlier this week you sent us a list of 26 background questions raised by the HSRB concerning efficacy testing of spatial repellents, ten of them identified as for EPA only. You also asked that we address those ten first, so our answers could be provided to the HSRB's consultant, who will also address the remaining questions.

Attached are our responses to the ten EPA-only questions. These responses have been developed in consultation with the OPP entomologists who review repellent efficacy tests. I have appended to the responses a copy of EPA's 1999 draft guideline for efficacy testing of repellents, which includes a section addressing testing of space repellents. No other guidelines applicable to this kind of testing are known to EPA.

We will continue to work on our responses to the other 16 questions, on the fact sheet mentioned in this response, and on the other background materials for the February 17 HSRB teleconference. We hope to be able to release all background materials for that meeting to Hamaad Syed for posting to the ESC portal by the end of next week.

Attachments

General Questions for Proposed February 17, 2009 HSRB Meeting: Session on Space Insect Repellent Studies Involving Human Subjects

Questions from HSRB Working Group:
Celia B. Fisher (HSRB Chair), Jan Chambers and Michael Lebowitz
January 6, 2009

EPA responses in bold italics

Questions for EPA:

Based on the workgroup's recommendations and to assist both the proposed spatial insect consultant to the Board and the Board at the February 2009 HSRB meeting, the HSRB Chair would like to request that EPA prepare a brief overview of the type of spatial repellent studies they anticipate presenting to the Board in the future. Below are some topics the Workgroup thought might be included in this summary:

A fact sheet summarizing the current range of registered spatial repellents, their active ingredients, delivery mechanisms, permitted label claims, and the design of supporting efficacy tests is being prepared separately from this response, and will be distributed with the other background materials for the February 17 meeting..

All we can predict with confidence about the kinds of protocols that will be presented to the Board in future is that they will all involve intentional exposure of human subjects. Our current best guesses about what may come up for spatial repellents are reflected in the answers below.

1. Are protocols expected to test products that repel or destroy insects, or both?

   EPA regulates products that repel insects as repellents and those that kill insects as insect toxicants. The efficacy of products that kill insects is not tested through research involving human subjects. When label claims for a product which has both knockdown and repellent properties are supported by human testing, proposed claims for repellency are generally accepted and claims for knockdown or "control" of insects are generally disallowed. The Board can expect to see testing proposals only for products regulated as repellents.

2. Which insects will be studied?

   We expect most new spatial repellents to claim effectiveness mainly against mosquitoes, but some may also bear claims for repelling other flying insects.

3. What delivery devices will be proposed?

   Recent registration of differing devices intended to emit pyrethroids through volatilization appear to be the most active area of current spatial repellent

development. Specific emitting devices may vary substantially as this technology is refined.

4. What is the nature of the environments to be tested? As an example open field, confined location or other environments? What would be an expected testing area size?

   In the USA, spatial repellents are generally intended for use in outdoor, protected spaces, such as patios or decks. The protocols the Board sees in future will be designed to provide data to support proposed label claims with respect to the size and shape of the area protected. Past tests of space repellents using volatile pyrethroids have involved a test area of 100-700 square feet.

   Ideally, testing of a spatial repellent would generate results that could be used modularly to generate directions for use—i.e., if efficacy testing shows the size and shape of the area of protection relative to the location of an emitting device and the direction and strength of the wind, then an array of similar shapes covering the entirety of a larger area to be protected would show where multiple emitters should be placed.

5. Will both continuous and intermittent exposures be used?

   This will depend on the objective of the test. Testing intended to measure duration of protection might involve intermittent exposure over a longer period of time, as you have seen in tests of topically applied repellents. We expect most spatial repellent testing to be designed to determine relative protection over a relatively brief period of continuous exposure, consistent with the expected pattern of use. The time pattern of release of the active ingredient from a spatial repellent emitter, and the pattern of movement and persistence of the repellent in the environment, can usually be measured without using human subjects.

6. How does EPA evaluate the utility for labeling and public safety of sponsored research assessing the efficacy of spatial repellents versus research on the minimal amount of biocide necessary to be effective?

   The objective of most efficacy testing is to determine whether, when used as intended, the product will perform up to the claims made for it. When in the course of a pesticide risk assessment EPA becomes concerned about the potential margin of exposure in a particular use pattern, we often consider reduced dosage as a potential means of risk management. In some cases dose can be reduced without compromising efficacy—usually through changes in application technology.

   This kind of situation has not arisen for any repellents, but if EPA were concerned that the MOE for a repellent use was too low, we might require testing to determine the minimum effective dose. Generally we do not require such testing.

For topically applied repellents, duration of effectiveness is an important discriminant between products containing the active ingredient at different concentrations. The user of a 30% DEET product is obviously getting more exposure than the user of a 10% DEET product, and the 30% product may be no more effective than the 10% DEET product for the first few hours of use. But the higher concentration product is likely to remain effective much longer, and 30% once or 10% three times at intervals of 4 hours might both be considered the "minimum effective dose" if the requirement were for 12 hours of protection.

In the case of a spatial repellent, the effective dose depends primarily on the rate of release of the repellent material from the emitter—which is constant or nearly constant—and secondarily on the duration of exposure. We assume that at any given relative position and distance from the emitter, relative protection would tend to vary directly with the rate of release of the repellent material from the emitter. Efficacy testing of relatively short duration can show the relative protection provided by the emitter at its constant rate of release, and these results can be extrapolated with confidence to longer durations.

7. What information will be needed for the label (e.g., time of loss of efficacy, protection time)? What endpoints are needed by EPA to determine this label information?

   The endpoints needed in testing depend on the proposed label claims, and more than one kind of testing may be needed to address a single claim. For example, assume a claim that a spatial repellent repels mosquitoes within an area of nnn square feet surrounding and down-wind of the emitter. The repellency claim could be supported by a comparison of mosquito landings on human subjects in an untreated area to those in an area treated with the repellent—i.e., by a test of relative protection. The area-of-protection claim would require a study design permitting observation of the area protected and its spatial relationship to the emitter. A further claim that protection lasted for x hours might be supported by physical data measuring the duration and rate of repellent release from the emitter.

8. If the number of subjects within a test area influences product efficacy assessments, how is that information incorporated into labeling decisions?

   If testing were to show that efficacy varies with the number of people in a test area, this would probably be reflected on the label. But since most testing uses only one number of people—i.e., one sample size for all replicates—this kind of information is unlikely to be available. EPA does not require it.

   Most consumer use of spatial repellents is expected to be in relatively small areas containing at least several people. It is thus appropriate to test them with subjects placed closer together than might be acceptable in a test of a topically applied repellent, for which separation of subjects is needed to minimize interaction.

9. If inhalation effects are a concern with treated subjects, and subjects with inhalation vulnerabilities are excluded from the research, how would the data from the research be used to ensure that safety levels protect such vulnerable populations? Is there a formula (e.g. a 10X factor) that is used?

   EPA's obligation under FIFRA is to register only those products which it judges would not be likely to cause unreasonable adverse effects, either when used as directed on the label or when used consistent with common use practice. EPA would not consider registering a spatial repellent material for which the inhalation toxicity was unknown, or which gave rise to a concern for potential inhalation effects under conditions of use. Any such concerns would be addressed in the risk assessment rather than in the design or review of efficacy studies.

10. Are there EPA or other Federal or industry guidelines for this type of research and if so what are the salient points in these guidelines that sponsors/investigators should consider for both scientific and ethical considerations?

    Performance testing conducted with human subjects would be subject to the rules for protection of human subjects in 40 CFR part 26. A brief discussion of testing of space repellents appears in §(d)(4) of the attached 1999 EPA Guideline 810-3700 for efficacy testing of repellents. No other guidelines applicable to this kind of outdoor spatial repellency testing are known to EPA.

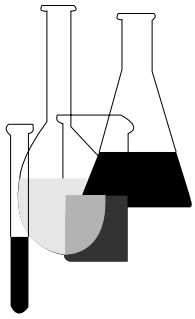
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–99–369
December 1999

Product Performance Test Guidelines

OPPTS 810.3700 Insect Repellents For Human Skin and Outdoor Premises



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/ OPPTS Harmonized Test Guidelines'' or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: opp-docket@epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: oppdocket@epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 810.3700 Insect repellents for human skin and outdoor premises.

(a) **Scope**—(1) **Applicability**. This guideline describes test protocols that EPA believes will meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) **Background**. The source materials used in developing this guideline are OPP test guidelines 95–9 Treatments to control pests of humans and pets and 95–10 Mosquito, black fly, nonbiting midge, and biting midge (Pesticide Assessment Guidelines, Subdivision G: Product Performance, EPA report 540/9–82–026, October 1982) to the extent they address similar issues. These prior guidelines are superseded by this guideline.

(b) **Definitions**. The following definitions are of special importance in understanding this guideline:

95% repellency refers to 95% reduction in bites when compared to controls.

Bite refers to an insect penetrating skin with its mouthparts and ingesting blood, with resulting abdomen swelling and color change.

First bite refers to the first bite received.

Land refers to an insect that lands, but does not probe or bite.

Light or *probe* refers to an insect landing and penetrating the skin with its mouthparts, without ingesting blood.

Protection time (PT) refers to the time from application of the repellent to the time until the first bite (FB). This is the period of time a repellent is expected to remain efficacious. For ticks and chiggers, this refers to the period between the time of application of the repellent to time of a tick or chigger crawling onto human skin.

Repellency refers to a lack of insects probing or biting human skin where repellent has been applied. For ticks and chigger mites, this refers to no ticks or chiggers crawling onto the portion of human skin where repellent has been applied.

(c) **Overview**—(1) **Purpose**—(i) This guideline concerns the product performance testing for evaluation of pesticides used to repel mosquitoes, biting flies, fleas, chiggers and ticks from human skin and outdoor premises. Commercial pesticide formulations used to repel these pests from human skin include, but are not limited to, liquid or pressurized products for spray treatments, material or articles impregnated with the pesticide, lotions, coils, candles, or vaporizing mats. Good Laboratory Practice Standards (GLP) apply to these laboratory and field studies as defined in Title 40 of the Code of Federal Regulations (CFR) at 40 CFR 160.1 to 160.195. According to 40 CFR 160.17 ‘‘EPA may refuse to consider

reliable for purposes of supporting an application for a research or marketing permit any data from a study which was not conducted in accordance with this part.'' All testing must be done with the end-use product formulation or treated article. All study submissions must include all raw data sheets and photographs (e.g., photographs of laboratory test setup and arena, arms in cage, field site with test subjects, repellent application) to document testing in both the laboratory and field.

(ii) This guideline recommends specific methods for conducting product performance testing of insect repellents. As a guideline, it does not impose mandatory requirements. It does, however, reflect the Agency's considered recommendations for minimum steps necessary to develop reliable data on repellent product performance. Deviations from this guideline should, therefore, be fully explained and justified.

(2) **Use of human volunteers**. FIFRA Section 12(a)(2)(P) defines it as unlawful ''for any person to use any pesticide in tests on human beings unless such human beings are fully informed of the nature and purposes of the test and of any physical and mental health consequences which are reasonably foreseeable therefrom, and freely volunteer to participate in the test.'' 40 CFR 26.116 outlines the elements of informed consent. Include protocols and signed consent forms with the submitted study report.

(3) **General considerations**. The following general discussions of test issues and test procedures apply to the testing of each type of insect, tick, or mite addressed by this guideline.

(i) **Treated test subjects**. The number of test subjects per species being tested is dependent upon the repellent hourly claim on the label. For a label claim of 1 to 4 hours of repellency, use at least 5 treated test subjects. For a label claim of 5 or more hours of repellency, use at least 10 treated test subjects. Equal numbers of adult male and female test subjects are preferred. Test subjects should avoid alcohol, caffeine, and fragrance products (e.g., perfume, cologne, hair spray, lotion, etc.) for 12 hours before, and during, the test. Each test subject's other limbs may be used for a test replicate by applying the identical repellent.

(ii) **Test area size and preparation**. Use the test subject's forearm, wrist to elbow, as the test area. Wash the area with unscented (fragrance free) soap, rinse it with water, then with a solution consisting of 70% ethanol or isopropyl base rubbing alcohol and 30% water, and dry it with a towel. Calculate and report the surface area (in cm^2) of each test subject's forearm. You may measure the circumference of the arm at the wrist, the elbow, and 3 to 4 equally spaced points between; then multiply the average of these measures of circumference by the distance from the wrist to elbow (or from the ankle to the knee). Cover areas above and below the forearm with a material a proboscis cannot penetrate. Avoid dark col-

ors. Hands may be covered with latex gloves. A test subject should receive no more than 1 treatment per test, replicated up to 4 times, once with each limb. Test subjects should avoid exertion which might increase perspiration, or abrasion, rubbing, touching, or wetting the treated area.

(iii) **Amount of repellent applied**. Store the test formulation at room temperature and ambient humidity before the test. Report the age of the test formulation; it should be less than 1 year old. Apply 1 g of liquid aerosol or pump spray test material or 1 g to 1.5 g of cream, lotion, or stick per 600 cm^2 of the test area evenly to the forearm or lower leg. If 1 g or 1.5 g seems inappropriate, establish the typical dose applied around a 95% confidence interval and report these data to the Agency.

(iv) **Data reporting**. The reporting of test results should include the following:

(A) **Labeling by protection time (PT)**. Report the duration of repellent protection until the time of first bite (FB) for each test subject. Report the mean PT and standard error for each test species. Statistical testing should examine variability between repetitions and between means as required. Explain the reasons and assumptions for each statistical analysis used.

(B) **Labeling by 95% repellency**. Report the duration of repellent protection based on the period of 95% reduction in bites for each test subject. Report the mean PT and standard error based upon a 95% reduction in bites for each test species should be reported. Statistical testing should examine variability between repetitions and between means as required. Explain the reasons and assumptions for each statistical analysis used.

(d) **Mosquitoes and biting flie**—(1) **General considerations for mosquito and stable fly laboratory tests**—(i) **Species**. Conduct mosquito tests with at least 3 genera of human biters; *Aedes aegypti*, an *Anopheles* sp., and a *Culex* sp. Conduct stable fly tests with 1 species; *Stomoxys calcitrans*. Identify test insects as to genus and species and by subspecies or strain if that information is available.

(ii) **Stage, age, and sex**. Mosquitoes should be adult females 5 to 10 days old. Stable flies should be 3 days old. Report the age or age range of the test insects.

(iii) **Rearing techniques**. Rear larvae under optimal conditions for the species. As a general guide, most species should be reared at 27±3°C, relative humidity 80±10%, and photoperiod 16:8 hours (light:dark). Use other conditions when they are more suitable for a particular species, and justify use of alternative rearing techniques in the study summary. Feed adults 10% sucrose and no blood meal before the test. Starve test insects

for 12 hours immediately before the test. Use test insects for only 1 test and destroy them after the trial.

(iv) **Mosquito and stable fly density**. There should be at least 1 mosquito for each 100 cm^3 and at least 200 mosquitoes in each test cage. There should be at least 1 stable fly for each 500 cm^3 and at least 45 stable flies in each test cage.

(v) **Test cage and testing conditions**. Cages should be at least 20,000 cm^3, square or rectangular, with 1 sleeved opening for the subject's arm. Use each cage for only 1 test subject and treatment at a time. Keep the temperature during the test at 22°C to 27°C, relative humidity 50% to 80%, and lights on.

(vi) **Treated test subjects**. See paragraph (c)(3)(i) of this guideline.

(vii) **Test area size and preparation**. See paragraph (c)(3)(ii) of this guideline.

(viii) **Amount of repellent applied**. See paragraph (c)(3)(iii) of this guideline.

(ix) **Negative controls**. A negative (untreated) control is recommended to verify biting pressure. When the repellent is applied to a forearm, the preferred negative control is the untreated forearm of the test subject, but another untreated subject may be used as a control instead. Wash, rinse, and dry control forearms exactly like treated forearms. Before the test begins subjects should expose their forearms to the mosquitoes or stable flies in the test cage to establish their attractiveness. The Agency recommends a minimum of 10 mosquito lands or probes within 30 seconds or 5 stable fly lands or probes in 60 seconds as thresholds for a subject to qualify as a participant. Every hour, a control forearm should be inserted through the sleeve into the cage and exposed to mosquitoes for up to 30 seconds or to stable flies up to 60 seconds to verify biting pressure. The forearm may be removed from the test cage as soon as it has received the necessary number of probes. A positive control is optional.

(x) **Exposure period**. Thirty minutes after treating with the repellent, test subject's forearm should be inserted through the sleeve into the cage of insects for 5 minutes. Record the number of bites or probes in each exposure period. Expose test subject's treated arm for 5 minutes every 30 minutes while biting pressure lasts—that is, until the controls no longer receive 10 mosquito lands in 30 seconds or 5 stable flies lands in 60 seconds. Subjects may then continue the test using a second cage until the repellent fails. Test subjects should avoid rubbing their arm when putting it into and out of the cage and between exposure periods.

(xi) **Analysis**. See paragraph (c)(3)(iv) of this guideline.

(2) **General considerations for mosquito, blackfly (gnats, southern buffalo gnats), ceratopogonid (no-see-ums, punkies, biting midges), sandfly, tabanid, and stable fly field tests**—(i) **Species**. Test with species that occur in the United States, although EPA may choose to consider data collected with foreign species. Determine species by aspirating insects into a vial before the test, while determining biting pressure, and periodically throughout the test. Take the aspirated insects to the laboratory for identification and describe them by genus and species and by subspecies or strain if that information is available.

(ii) **Biting pressure**. Measure biting pressure before treatment and every hour during the test. The preferred way is to use the untreated forearm or lower leg of the test subject, but an untreated test subject is also acceptable. Allow the target pest to bite or probe (preferably to probe, so insect density is not reduced and the subject experiences as little discomfort as possible) for 5 minutes or until the recommended number of bites occurs. The Agency recommends 5 bites in 5 minutes for mosquitoes, black flies, ceratopogonids, and tabanids, and 1 bite or probe in 5 minutes for stable flies. Aspirate insects landing during this time into a vial for identification. A subject receiving the recommended number of bites or probes in less than 5 minutes may cover his or her untreated limb.

(iii) **Test sites and testing conditions**. Conduct at least 2 field tests in environmentally distinctive habitats (forest, grassland, salt marsh, wetland, beach, barns, urban environments) suitable for the target insect. They need not be in different states. For mosquitoes, habitats with different species are preferred. Data from areas where biting pressure is below the levels listed in paragraph (d)(2)(ii) of this guideline are unlikely to provide reliable and reproducible results. Report at least the following weather during the test: Temperature, relative humidity, cloud cover, precipitation, light intensity, and wind speed during 90 seconds of observation for each exposure period. Wind speed should not exceed 10 miles per hour.

(iv) **Treated test subjects**. In addition to the requirements of paragaph (d)(3)(i) of this guideline, for field tests, test subjects should be at least 3 meters apart during the test and engage in usual outdoor activity including normal movement. Normal movement includes intermittent walking, standing, squatting, sitting, and raising or lowering arms. Usual outdoor activity includes sitting or slow walking. Test subjects should not use any form of tobacco at anytime following treatment or throughout the test.

(v) **Test area size and preparation**. See paragraph (c)(3)(ii) of this guideline.

(vi) **Amount of repellent applied**. See paragraph (c)(3)(iii) of this guideline.

(vii) **Negative controls**. A negative (untreated) control is recommended to determine biting pressure. The preferred negative control is the untreated forearm or lower leg of the subject, but an untreated test subject or individual is also acceptable. Wash, rinse and dry control limbs exactly like treated limbs. It is preferred if the untreated control is continuously exposed; exposing the untreated limb for 5 minutes every hour is the recommended minimum. A positive control is optional.

(viii) **Exposure period for treated subjects**. Continuous exposure for duration of test.

(ix) **Analysis**. The investigator or an associate should record the number of bites and probes, rather than the test subject. Report the duration of repellent protection for each test subject. For each test site, report the mean time and standard error to first bite (FB) or the mean percent reduction in bites and standard error. See also paragraph (d)(3)(iv) of this guideline.

(3) **General considerations for treated articles or clothing**—(i) **Application to the treated article**. Evaluations of repellent impregnated clothing or treated articles should report the repellent used, impregnating formulation, method of impregnation, garment treated, amount of repellent absorbed per unit area of fiber.

(ii) **Application of bed nets, head nets, net jackets, table cloths, and other treated articles**. Repellents may be used to treat materials used for bed nets, head nets, loose jackets, table cloths, clothing, or other treated articles. Reports of field tests with treated netting should include: Type of netting (fibers absorb and retain repellent treatments at differing degrees), mesh size and weight per unit area of netting, impregnating formulation, method of impregnation, amount of repellent absorbed per unit area or weight of netting, construction of the experimental item (e.g., bed net), and method of exposure. Compare the subjects protected by treated articles or clothing to subjects protected by the same article or clothing untreated with a repellent. Determine product performance by comparing the numbers of mosquitoes penetrating the nets, biting the protected subjects, and biting the unprotected subjects in a standard exposure period. Consider it a bite or probe whenever an insect proboscis penetrates the treated material.

(iii) **Laboratory test**. Conduct laboratory tests according to the general design laid out in paragraph (d)(1) of this guideline. Alter the recommended test by fastening a strip of the impregnated material to the test subject's forearm.

(iv) **Field test**. Conduct field tests according to the general design laid out in paragraph (d)(2) of this guideline. Determine biting pressure before the test begins. Expose the area of the body that the label claims to be protected by the treated article. Leave another part of the body, dis-

tant from the treated article, untreated and exposed to determine biting pressure, or use a separate untreated subject as a control.

(4) **General considerations for mosquito, blackfly (gnats, southern buffalo gnats), ceratopogonid (no-see-ums, punkies, biting midges), sandfly, tabanid, and stable fly field tests for candles, coils, and vaporizing mats**—(i) **Species**. Test with species that occur in the United States, although EPA may choose to consider studies using foreign species. Determination of species should be in accordance with paragraph (d)(2)(i) of this guideline.

(ii) **Biting pressure**. The determination of biting pressure should be in accordance with paragraph (d)(2)(ii) of this guideline. In addition, landing rates should be determined on the exposed forearm of a volunteer.

(iii) **Test sites and testing conditions**. Selection of test sites and conditions should be in accordance with paragraph (d)(2)(iii) of this guideline. The test should be replicated at the test site if the biting pressure is less than recommended in paragraph (d)(2)(ii) of this guideline.

(iv) **Treated test subjects**. The number of test subjects should be in accordance with paragraph (c)(3)(i) of this guideline. If more than 1 test subject are exposed to the same candle, coil, or mat, the number of bites should be averaged.

(v) **Test area size and preparation**. The procedures described for determination of the test area size and preparation of the test area should be in accordance with paragraph (d)(2)(v) of this guideline.

(vi) **Number and location of candles, coils, or vaporizing mats**. The number and placement of the candle, coil, or mat should be consistent with the label directions. Test subjects should be located at the maximum distance from the candle, coil, or mat the label recommends. If the label states that the candle, coil, or mat should be placed upwind, then test subjects should remain downwind. Otherwise, test subjects should move around the circumference of the test area periodically. Report this time interval with study results.

(vii) **Negative controls**. A negative (untreated) control of the same size as the test area is desirable to determine biting pressure. When the repellent is applied to a forearm the preferred negative control is the untreated forearm or lower leg of the test subject, but an untreated test subject or individual is also acceptable. There should be a minimum of 1 control subject for every 5 treated test subjects. Control subjects should remain upwind and far enough from the treatment area not to be affected by the repellent. Wash, rinse, and dry control limbs exactly like treated limbs. It is preferred that the untreated control is continuously exposed; exposing the untreated limb for 5 minutes every hour is the recommended minimum. A positive control is optional.

(viii) **Exposure period for test subjects**. Expose subjects for as long as the label says the candle, coil, or mat will burn. Protection time should be the same as burning time.

(ix) **Analysis**. The number of bites and probes should be recorded by a study director or partner, not the test subject. When compared to the negative control, at least 50% of the insects should be repelled. Report the duration of repellent protection and the mean time to 50% reduction in bites and standard error for each test site. See also paragraph (c)(3)(iv) of this guideline.

(e) **Fleas**—(1) **General considerations for flea laboratory tests**—(i) **Species**. All product performance tests should be conducted using the cat flea (*Ctenocephalides felis*).

(ii) **Stage, age, and sex**. Use adult, male and/or female fleas that are 5 to 10 days old. Report the age or age range of the test insects.

(iii) **Rearing techniques**. As a general guide, rear fleas at 27±3°C, relative humidity 80±10%, and photoperiod 16:8 (light:dark). Adults should not be blood fed. Use fleas for only 1 test and destroy them after the trial. Justify using any alternative rearing techniques in the study report.

(iv) **Flea density**. There should be at least 1 flea per 9 cm^3 and at least 100 fleas in each test cage. Twenty five fleas should be added to the test cage after each exposure period.

(v) **Test cage and testing conditions**. Cages should be at least 900 cm^3 in volume; square, circular, or rectangular; plastic or glass; with an opening on the top to insert the test subject's arm. Cages should have a rough floor (such as clean sand). Limit replications to 1 test subject and treatment at a time for each cage. Keep the temperature during the test at 22-27°C, relative humidity at 50–80%, and the lights on.

(vi) **Treated test subjects**. The number of test subjects per species being tested should be in accordance with paragraph (c)(3)(i) of this guideline.

(vii) **Test area size and preparation.** The test subject's forearm, wrist to elbow, should be used as the test area. The procedures described for determination of the test area size and preparation of the test area should be in accordance with paragraph (c)(3)(ii) of this guideline. Areas above and below the forearm should be covered with a material the flea's mouthparts cannot penetrate.

(viii) **Amount of repellent applied**. See paragraph (c)(3)(iii) of this guideline.

(ix) **Negative controls**. A negative (untreated) control is desirable to verify biting pressure. When the repellent is applied to a forearm the preferred negative control is the untreated forearm of the test subject, but an untreated test subject or individual is also acceptable. Wash, rinse, and dry control forearms exactly like treated forearms. Before treatment the subject should expose his or her forearm to the fleas in the test cage to establish the subject's attractiveness. The Agency recommends at least 10 lands or probes within 30 seconds for the subject to qualify as a test participant. Every hour a control forearm should be inserted through the sleeve into the cage and exposed to the fleas for up to 30 seconds to verify biting pressure. As soon as 10 lands have occurred the control forearm may be removed from the test cage. A positive control is not required.

(x) **Exposure period for treated subjects**. Within 30 minutes after treatment the subject's forearm should be inserted through the sleeve into the cage of fleas for 5 minutes. Record the number of lands for each exposure period. Subjects should expose their arms to the fleas for 5 minutes at a time at intervals of 30 minutes or less until the control arm no longer receives 10 lands in 30 seconds. Subjects may then continue the test using a second cage, until the repellent fails. Test subjects should avoid rubbing the repellent when putting their arms into the cage and between exposure periods.

(xi) **Analysis**. Report the duration of repellent protection for each test subject. Report the mean protection time and standard error for each test. See also paragraph (c)(3)(iv) of this guideline.

(2) **General considerations for field tests**. Although the Agency does not routinely require field tests for flea repellents, it may request field test data, especially for candles, coils, and vaporizing mats. Field tests may also be conducted and submitted voluntarily. If an acceptable field test is conducted, reapplication time under the ''Directions for Use'' should reflect its results.

(f) **Ticks and chigger mites**—(1) **General considerations for ticks and chigger mites laboratory tests**—(i) **Species**. Tick species should be disease free and include: The blacklegged tick (deer tick, *Ixodes scapularis*), western blacklegged tick (deer tick, *Ixodes pacificus*), lone star tick (*Amblyomma americanum*), American dog tick (*Dermacentor variabilis*), and relapsing fever tick (softbacked tick, *Ornithodoros turicata*). Test with the species the label claims to repel. If the label claims effectiveness against ''ticks,'' testing should be with deer ticks, lone star ticks, American dog ticks, and softbacked ticks. Chigger mites tested should be from the *Trombiculidae family; Eutrombicula splendens* or *E. cinnabarrs* are preferred species. Identify test animals by genus and species, and by subspecies or strain if that information is available.

(ii) **Stage and age**. Test products that claim to repel ticks that vector disease with both adult and nymphal life stages of the blacklegged, lone star, American dog, and softbacked ticks. Test products that claim to repel ticks but do not mention disease carriers with adult or nymphal life stages of the blacklegged, lone star, American dog, and softbacked ticks. Test immature chigger mites. Report the age or age range of all test animals.

(iii) **Rearing techniques**. Rear test organisms at 22±3°C, relative humidity 50–80%, and photoperiod 16:8 (light:dark). Use ticks or chigger mites for only 1 test and destroy them after the trial. Justify any alternative rearing techniques in the study report.

(iv) **Number of ticks or chigger mites**. Expose 5 ticks or 5 chigger mites to the treated forearm in each exposure period. Do not reuse a test organism which has been recorded as not repelled; use an untested tick or chigger mite instead.

(v) **Testing conditions**. Keep the temperature during the test at 22°C to 27°C, relative humidity 50% to 80%, and the lights on.

(vi) **Treated test subjects**. See paragraph (c)(3)(i) of this guideline.

(vii) **Test area size and preparation**. The procedures for determination of the test area and preparation of the test area should be in accordance with paragraph (c)(3)(ii) of this guideline. The area above and below the test area should be covered with a material that the tick and/or chigger mite mouthparts cannot penetrate.

(viii) **Amount of repellent applied**. See paragraph (c)(3)(iv) of this guideline.

(ix) **Negative controls**. A negative (untreated) control is recommended to verify biting pressure. The negative control should be the untreated forearm of the test subject. Wash it, rinse it, and dry it exactly like the treated forearm. Before treatment subjects should expose their forearms to the test organism to establish their attractiveness. The test organism should be picked up with a soft artist's paintbrush (so as not to damage the body or forelegs) and placed on the test subject approximately 2 cm from the area of the forearm where the repellent has been applied, near the wrist. Place a new tick or chigger mite 2 cm below the test area once it has crossed onto the test area of the forearm. Do not reuse a test organism. A positive control is not required.

(x) **Test procedure**. Test subjects should place their fingertips on a flat surface with palms raised above the surface. The investigator should place ticks or chigger mites, 1 at a time, on the test subject's forearm with an artist's paintbrush approximately 2 cm from the edge of the treated area of the forearm, near the wrist. The tick or chigger mite should be guided gently with paint brush in the direction of the test material. After

moving toward the margin of the test material on the test subject's forearm, ticks or chigger mites should be allowed 5 minutes to cross the margin onto the test material. Report ticks or chigger mites that cross at least 2 cm onto the test material (toward the elbow) as ''not repelled.'' Once a tick or chigger mite has been recorded as not repelled, replace it by a tick or chigger mite that was not previously tested. Expose a new group of ticks or chigger mites to the test material every 30 minutes.

(xi) **Analysis**. Report the duration of repellent protection for each test organism and subject; this may be done as a percent reduction in the number of ticks crossing the repellent. See also paragraph (c)(3)(iv) of this guideline.

(2) **General considerations for ticks and chigger mites field tests**. Although the Agency does not routinely require field tests for tick and chigger mite repellents, it may request field test data, especially for candles, coils, and vaporizing mats. Field tests may also be conducted and submitted voluntarily. If an acceptable field test is conducted, reapplication time under the ''Directions for Use'' should reflect these results.