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 These prior guidelines are superseded by this guideline.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:

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

The term *bite* refers to an insect penetrating skin with its mouthparts and ingesting blood, with resulting abdomen swelling and color change.

The term *first bite* refers to the first bite received.

The term *land* refers to an insect that lands, but does not probe or bite.

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

The term *protection time* (PT) refers to the time from application of the repellent to the time until the first bite (FB) or until the repellent no longer reduces bites by 95%. 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.

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

The term *repellent* refers to a pesticide product that causes insects to be driven or kept away from an identified area.

(c) Overview.

(1) Purpose..

(A) This guideline concerns the product performance testing for evaluation of all registered 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 40 CFR Part 160.1 to 160.195. According to 40 CFR Part 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." Non-GLP practices are addressed in , 40 CFR Part 160.12(b) which states that "[a] statement describing in detail all differences between the practices used in the study and those required by 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.

(B) 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 (I) 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 (ii) 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 less than five hours (e.g., one to four hours) of repellency, use at least five treated adult test subjects. For a label claim of five or more hours of repellency, use at least ten treated test subjects. Equal numbers of adult male and female test subjects are preferred. Test subjects should be between the ages of 18 and 55 and in good physical health to decrease possible variation among subjects. Subjects should avoid alcohol and fragrance products (e.g., perfume, cologne, hair spray, lotion, etc.) for at least twelve hours before, and during, the test. Each test subject's other limbs may be used for a test replicate by applying the identical active ingredient at the same or varying concentrations. If varying concentrations are used, it is

particularly important that the test subject not rub or contaminate the 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 at least 50% ethanol or isopropyl base rubbing alcohol and 50% 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 three to four 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 colored clothing. Hands may be covered with latex gloves. A test subject should receive no more than one treatment per test, replicated up to four 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 one 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. If the repellent is measured in volume rather than weight, it should be converted to weight based on the density of the product for the report submitted 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 (e.g., a test subject) 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 (e.g., a test subject) and between means as required. Explain the reasons and assumptions for each statistical analysis used.

(d) Mosquitoes and biting flies.

(1) General considerations for mosquito and stable fly LABORATORY tests.

(I) Species. Conduct mosquito tests with at least three genera of human biters; *Aedes aegypti*, an *Anopheles* sp., and a *Culex* sp. Conduct stable fly tests with one 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 five to ten days old. Stable flies should be three 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 to 24 hours immediately before the test. Use test insects for only one test and destroy them after the trial. Appropriate alternative rearing methods may also be acceptable (e.g., Gerberg and Barnard (1998)).

(iv) Mosquito and stable fly density. There should be at least one mosquito for each 100 cm^3 and at least 200 mosquitoes in each test cage. There should be at least one 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 56,640 cm^3, square or rectangular, with one sleeved opening for the subject's arm. Use each cage for only one test subject and treatment at a time. Keep the temperature during the test at 22°C to 27°C, relative humidity 50% to 80%. Testing conditions should attempt to duplicate the preferred feeding time of day for the test species. For species that feed during the day lights should be turned on (e.g., *Aedes aegypti*) and light should be off or dimmed for night feeders (e.g., *Anopheles* spp. and *Culex* spp.). Avoid exhaling into the test cage because the introduction of CO_2 could bias mosquitoes (or other flies) towards biting.

(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) Controls. A negative (untreated) control is recommended to verify biting pressure. When the repellent is applied to a forearm of a test subject, 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 ten mosquito landings or probes within 30 seconds or five stable fly landings or probes

in 60 seconds as thresholds for a subject to qualify as a participant. Prior to exposing a treated forearm, an untreated 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 (e.g, treated with 30% DEET) 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 five minutes. Record the number of bites or probes in each exposure period. Expose test subject's treated arm for five minutes every thirty minutes while biting pressure lasts - that is, until the controls no longer receive ten mosquito landings in 30 seconds or five stable flies landings 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) Data reporting. 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 mosquito, black fly (gnats, southern buffalo gnats), ceratopogonid (no-see-ums, punkies, biting midges), sand fly, tabanid, and stable fly FIELD tests.

(I) Species. Test with species that occur in the United States. Tests may be conducted in other countries or territories (e.g., Canada or Puerto Rico) with species that are found in the United States. EPA may consider data collected with foreign species. Collect 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 subsequent 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 at least every hour during the test. Although a total body count would obtain a realistic biting pressure, an exposed forearm or lower leg is acceptable. The preferred method 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 for five minutes or until the recommended number of bites occurs. The Agency recommends five bites or probes in five minutes on one exposed limb for mosquitoes, black flies, ceratopogonids, and tabanids, and one bite or probe in five minutes for stable flies. Aspirate insects landing during this time into a vial for subsequent identification. A subject receiving the recommended number of bites or probes in less than five minutes may cover his or her untreated limb.

(iii) Test sites and testing conditions. Conduct at least two field tests. For mosquitoes, conduct tests in environmentally distinctive habitats (forest, grassland, salt marsh, wetland, beach, barns, urban environments) containing different species. They need not be in different states. Organisms that typically occur in one habitat (e.g., black flies, stable flies) should be tested in two separate areas or in the same area on two separate days. Data from areas where biting pressure is below the levels listed in paragraph (d)(2)(ii) are unlikely to provide reliable and reproducible results. Report the time of day (beginning and ending) and 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 ten miles per hour.

(iv) Treated test subjects. In addition to the requirements of paragraph (d)(3)(I), for field tests, test subjects should be at least three meters apart during the test. Subjects should engage in a variety of normal outdoor activities during experiments such as walking, standing, squatting, sitting, and raising and lowering arms during experiments. Test subjects should not use any form of tobacco at anytime following treatment with the repellent until the end of 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) Controls. A negative (untreated) control is recommended to determine biting pressure. The preferred negative control is the untreated forearm or lower leg of the treated test 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 five minutes every hour is the recommended minimum. A positive control (e.g, treated with 30% DEET) is optional.

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

(ix) Reporting. The investigator or an associate (e.g., buddy system or another test subject) 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.

(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 or probing the protected subjects, and biting or probing the unprotected subjects in a standard exposure period.

(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 section (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, distant 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, black fly (gnats, southern buffalo gnats), ceratopogonid (no-see-ums, punkies, biting midges), sand fly, tabanid, and stable fly FIELD tests for candles, coils, and vaporizing mats.

(I) Species. Test with species that occur in the United States. Tests may be conducted in other countries or territories (e.g., Canada or Puerto Rico) with species that are found in the United States. EPA may consider data collected with 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.

(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)). If more than one test subject are exposed to the same candle, coil, or mat, the number of bites among these subjects should be averaged.

(v) Test area size and preparation. See paragraph (c)(3)(ii) 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) Controls. A negative (untreated) control of the same size as the test area is desirable to determine biting pressure. The preferred negative control is the forearm or lower leg of the test subject, but an untreated test subject or individual is also acceptable. Untreated subjects should be in the same vicinity as treated subjects but "control" subjects should not be close enough to be protected by the area-wide repellent. There should be a minimum of one control subject for every five 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 five minutes every hour is the recommended minimum. A positive control (e.g, treated with 30% DEET) 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) Data reporting. The number of bites and probes should be recorded by a study director or partner, not the test subject. The percent reduction in bites and/or probes when compared to the negative control should be reported. Report the duration of repellent protection and the mean time to 50% or 95% 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 five to ten 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 50±80%, and photoperiod 16:8 (light:dark). Adults should not be blood fed. Use fleas for only one 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 one flea per 9 cm^3 and at least 100 fleas in each test cage.

(v) Test cage and testing conditions. Cages should be at least 28,000 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 one 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).

(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) Controls. A negative (untreated) control is desirable to verify biting pressure. When the repellent is applied to a forearm of a test subject, 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 ten landings or probes within 30 seconds for the subject to qualify as a test participant. Prior to exposing a treated forearm, an untreated 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 ten landings have occurred the control forearm may be removed from the test cage. If ten landings do not occur, additional fleas should be added to the cage until ten landings or probes occur. A positive control (e.g, treated with 30% DEET) is optional.

(x) Exposure period for treated subjects. Within thirty minutes after treatment the subject's forearm should be inserted through the sleeve into the cage of fleas for five minutes. Record the number of landings for each exposure period. Subjects should expose their

arms to the fleas for five minutes at a time at intervals of thirty minutes or less until the control arm no longer receives 10 landings 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) Data reporting. 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 flea 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 chiggers.

(1) General considerations for ticks and chiggers 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, and American dog ticks. Chiggers 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 chiggers. 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 chiggers for only one test and destroy them after the trial. Justify any alternative rearing techniques in the study report.

(iv) Number of ticks or chiggers. Expose at least five ticks or at least five chiggers 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 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

(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 mouthparts cannot penetrate.

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

(ix) 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 so as not to damage the body or forelegs (e.g., with a soft artist's paint brush, forceps, Q®tips) 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 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 (e.g, treated with 30% DEET) is optional.

(x) Test procedure. Test subjects should place their fingertips on a flat surface with palms raised above the surface and the forearm held perpendicular to the surface to create a vertical test area. The investigator should place ticks or chiggers, one at a time, on the test subject's forearm with a suitable instrument (e.g, artist's paintbrush, forceps, Q®tips) approximately 2 cm from the edge of the treated area of the forearm, near the wrist. The tick or chigger should be guided gently (e.g., with paint brush, forceps, Q®tips) in the direction of the test material. After moving toward the margin of the test material on the test subject's forearm, ticks or chiggers should be allowed five minutes to cross the margin onto the test material. Report ticks or chiggers that cross at least 2 cm onto the test material (toward the elbow) as 'not repelled'. Once a tick or chigger has been recorded as not repelled, replace it by a tick or chigger that was not previously tested. Expose a new group of ticks or chiggers to the test material every 30 minutes.

(xi) Data reporting. 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 chiggers FIELD tests. Although the Agency does not routinely require field tests for tick and chigger 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.

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