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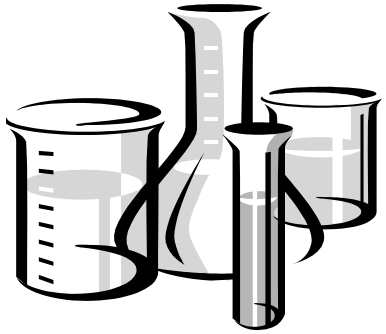
Draft Product Performance Test Guidelines

OCSPP 810.3500: Premises Treatments



NOTICE

This guideline is one of a series of test guidelines established by the Office of Chemical Safety and Pollution Prevention (OCSPP) [formerly the Office of Prevention, Pesticides and Toxic Substances (OPPTS) prior to April 22, 2010], United States Environmental Protection Agency (US EPA) for use in testing pesticides and chemical substances to develop data for submission to the agency under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601, *et seq.*), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*), and section 408 of the Federal Food, Drug and Cosmetic (FFDCA) (21 U.S.C. 346a), referred to hereinafter as the harmonized test guidelines.

The OCSPP test guidelines serve as a compendium of accepted scientific methodologies for research intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. This document provides guidance for conducting appropriate tests, and is also used by EPA, the public, and the companies that are required to submit data under FIFRA. These guidelines are not binding on either EPA or any outside parties, and the EPA may depart from them where circumstances warrant and without prior notice. The methods described in these guidelines are strongly recommended for generating the data that are the subject of the guidelines, but EPA recognizes that departures may sometimes be appropriate. You may propose alternatives to the methods described in these guidelines, with supporting rationale. The agency will assess them for appropriateness on a case-by-case basis.

For additional information about the harmonized test guidelines and to access the guidelines electronically, please go to *https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances*. You may also access the guidelines in *http://www.regulations.gov* grouped by Series under Docket ID #s: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159, EPA-HQ-OPPT-2009-0576, and EPA-HQ-OPP-2011-1017. ***EPA-HQ-OPP-2017-0693*** is the docket number for the FIFRA SAP peer review record containing this draft guideline.

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(a) Introduction

a. Scope. This guideline provides recommendations for the design and execution of laboratory and field studies to evaluate the performance of pesticide products applied in or around premises in connection with registration of pesticide products under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*). This guidance applies to products in any formulation, such as a liquid, aerosol, fog, or bait, if intended to be applied in or around premises. It applies, but is not limited to, invertebrate pests such as cockroaches, filth flies, biting flies, mosquitoes, fleas, ticks, spiders, centipedes, scorpions, and stinging hymenopterans. This guideline does not apply to those products exempt from FIFRA Registration under 40 CFR § 152.25 or to product performance testing described in other agency guidelines.

b. Purpose. This guideline provides laboratory and field study methods to evaluate product performance of pesticides in or around premises against pests and includes statistical analysis and reporting recommendations.

(b) Organization of the Guideline.

a. (a) Introduction;

(b) Organization of the Guideline;

(c) Definitions;

(d) Development of protocols for efficacy studies;

(e) Review of protocols for efficacy studies;

(f) Execution of efficacy studies;

(g) Reporting of completed efficacy studies to the agency;

(h) Retention of records;

(i) Specific guidance for laboratory studies for direct application testing of pesticide products;

(j) Specific guidance for laboratory studies for forced exposure (no-choice) indoor residual applications;

(k) Specific guidance for studies on forced exposure (no-choice) outdoor residual applications;

(l) Specific guidance for laboratory studies for testing indoor pesticide fogger, total release aerosol; space spray, and insecticide vapor strip products;

(m) Specific guidance for laboratory studies for testing ovicidal products;

(n) Specific guidance for laboratory studies for fumigant products;

(o) Specific guidance for laboratory studies of insect growth regulator (IGR) products;

(p) Specific guidance for field studies of outdoor pesticide fogger products, applied directly to pests;

(q) Specific guidance for field studies of outdoor pesticide misting system products;

(r) Specific guidance for laboratory studies of cockroach bait products;

(s) Specific guidance for laboratory studies of flushing products;

(t) Specific guidance for laboratory studies of fly bait products;

(u) Specific guidance for laboratory studies for testing ant bait products;

(v) Specific guidance for field studies for direct treatment of the nest/hive/colony of flying, stinging Hymenoptera, except ants;

(w) Specific guidance for field studies of bait products for flying, stinging Hymenoptera, except ants;

(x) Specific guidance for laboratory studies for resistance ratio determination and characterization of pest population strain susceptibility;

(y) References.

b. General Considerations. Any protocol and/or study developed using this guidance must meet the provisions set forth in several statutes and regulations, including, but not limited to, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 7 U.S.C. 136, *et seq.*) under which EPA regulates pesticides. This guideline does not supersede or overrule the regulations governing research conducted with human subjects such as those contained in 40 CFR Part 26, or any other agency regulations. To the extent there are any unintended conflicts between this guideline and any EPA regulation, the regulation at issue governs.

i. Good Laboratory Practice Standards. Good Laboratory Practice (GLP) Standards set forth in 40 CFR § 160 apply to laboratory studies evaluating pesticide product performance. Part 158 specifies that "applicants must adhere to the good laboratory practice (GLP) standards described in 40 CFR § 160 when conducting studies" [40 CFR § 158.70(b)]. However, studies that do not comply with GLP standards may nonetheless be considered if, in the agency's judgment, the design and conduct of the study provide results that are scientifically reliable. 40 CFR §160.12(b) states that with any submitted research data "[a] statement describing in detail all differences between the practices used in the study and those required by this part" must be submitted to aid in making that determination.

ii. State requirements. Investigators and Sponsors should ensure research is conducted in compliance with any applicable state laws or regulations, which are independent of and additional to those cited in this guideline.

c. Resistance Management Considerations for Registrants and Professional Applicators. Registrants are strongly encouraged to adhere to Insecticide Resistance Action Committee (IRAC) and EPA labeling guidance on resistance management when compiling final product packaging. Similarly, all professional applicators should be trained in and encouraged to alter modes of action as necessary when applying chemical control against pests. A resistance management section and IRAC codes on the label are recommended, especially if label claims suggesting product efficacy against resistant pests are sought.

(c) Definitions

a. **A*pplication rate*** refers to the amount of product applied per pest or unit area or volume (e.g oz/ft^2 or fl. oz. /ft^3). It can also be expressed in seconds of spray per unit area or volume.

b. ***Directions for Use*** refers to the section of a pesticide label that describes how the product can legally be used and how the product must not be used.

c. ***Field study*** refers to a scientific investigation that occurs in a real-world setting, specifically not in a laboratory.

d. ***Flushing*** refers to the rapid exit of an arthropods (or group of arthropods) from a harborage in response to a stimulus.

e. ***Forced exposure*** refers to an experimental design that employs a period of constant, unavoidable contact between a study organism and a pesticidal treatment. Typically, a forced exposure time of no more than one hour for flying pests and no more than four hours for crawling pests is recommended for product performance testing.

f. ***Harborage*** is a sheltered area or refuge for a test organism.

g. ***Knockdown*** refers to a state in which a pest is rendered incapable of coordinated movement or unable to right itself following exposure to a pesticide product.

h. LD_{50} is a measure of lethality of a given toxicant calculated as the dose of toxicant needed to kill 50% of individuals in a test population.

i. LD_{90} is a measure of lethality of a given toxicant calculated as the dose of toxicant needed to kill 90% of individuals in a test population.

j. ***Method of application*** refers to the way a pesticide can be delivered (applied) to a pest or surface. Examples of application methods include aerial application, aerosol spray, dust, liquid sprays, fogging, and bait.

k. ***Moribund*** refers to pests that are on their backs with only a single appendage twitching. Pests exhibiting this behavior should not be considered dead.

l. ***Mortality*** refers to test/study organism death. A ***dead test/study organism*** is one that does not move, even when poked or probed.

m. ***Negative control*** refers to the group of specimens in an experiment that receive no treatment or a treatment with the diluent only, where no response is expected.

n. ***Non-porous surface*** refers to a surface that is not expected to absorb an applied pesticide product, such as commercial linoleum or glazed ceramic tile. Non-porous surfaces are used in studies to determine the amount of time pesticide residues remain active against the intended pest after application.

o. ***Ovicidal product*** refers to a pesticide product that kills pest eggs/egg masses.

p. ***Pest exposure period*** refers to the amount of time a pest comes in contact with a pesticide.

q. ***Pesticide resistance*** refers to a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a pesticide product to achieve the expected level of control (IRAC).

r. ***Photoperiod*** refers to the relative amount of time during the day in which it is light or dark (Borror and Delong, 7th edition)

s. ***Placebo*** refers to a test substance minus active ingredient(s).

t. ***Porous surface*** refers to a surface that is expected to absorb pesticide, such as unpainted/unfinished ¼" thick plywood or concrete. These surfaces are used to determine the amount of time pesticide residues remain active against the intended pest after application.

u. ***Positive control*** refers to the group of specimens in an experiment that receive a pesticide treatment with known efficacy, where a response is expected.

v. ***Premises*** refers to the spaces within structures, their walls, (both inside and outside), and the immediate adjacent surrounding grounds. Such structures include households (e.g. houses, apartments); commercial, industrial and institutional buildings; agricultural structures (e.g. barns); and food-handling establishments.

w. ***Product performance testing*** refers to scientific studies designed to test the effectiveness of a pesticide product against the specific target pest.

x. ***Public health pest*** refers to a species which poses a risk to human health and may cause disease, harm, allergic reactions and/or life threatening situations.

y. ***Quick kill and/or kills on contact*** occurs when pests are exposed to a pesticide and the following conditions are met: 1. at least 90% of pests exhibit knockdown within 10 seconds for stinging Hymenoptera (including fire ants) or within 30 seconds for all other arthropods, 2. following transfer to clean containers within 1 hour (flying insects) or 4 hours (crawling insects) following pesticide exposure, at least 90% mortality is observed by 96 hours after initial pesticide exposure.

z. ***Residual efficacy*** refers to the effect of a pesticide product continuing to provide the intended pesticidal effect at an acceptable level for an extended length of time after application. The product's residues should be effective for at least 24 hours post application.

aa. ***Resistance ratio (RR)*** is a quantitative expression of the resistance of a pest strain to a specific active ingredient or product formulation. A resistance ratio (e.g., RR_{50}) is calculated by dividing a quantitative measure of the lethality of an insecticide (e.g., LD_{50} value) for a pest strain of unknown level of resistance by the corresponding measure of lethality for a strain known to be susceptible to the insecticide.

(d) Development of protocols for efficacy studies. Testing pesticides for efficacy against arthropods of public health importance (herein referred to as pests) begins with development of a study protocol. General considerations in developing a study protocol for efficacy studies include scientific design of the study, data collection, data analysis, and reporting. Each of these topics is discussed in more detail in the sub-sections below. Additional study-specific considerations can be found in Sections (i) through (w).

a. Scientific design of study. The experimental methods should be likely to provide a definitive answer to the research question and include a detailed description of the experimental design, addressing topics (i) through (viii), given directly below.

i. Objectives. For products that kill and/or knockdown pests, the objective of product performance testing is to determine the lowest proposed label rate that kills or knocks down the pest. For products that control pests, the objective is to determine that the pesticide

application has residual efficacy. For products that cause flushing, the objective is to determine that the application induces egress from harborages. In all cases, the scientific objective should be stated clearly and all treated pests should be compared to pests that have received no treatment or a placebo/negative control.

ii. **Test materials and treatments.** End-use formulations should be tested using the lowest labeled application rates for use on the target pest. Test materials should be stored at ambient temperature and humidity for at least one day before use.

1. **Products that target immatures and adults**. Testing should be conducted with adults unless immatures are specifically targeted.
2. **Products that target pest development.** Testing should be conducted with appropriate immature stages or eggs.
3. **Products that target reproductive success of adults.** Testing should be conducted with adults and continue long enough to assess egg production, hatching success of eggs deposited after adults are exposed, and developmental success and survivorship of emergent nymphs/larvae.
4. **Products that target eggs (ovicidal products).** Testing should be conducted with the egg life stage only.

iii. **Application rate determination.** The application rate used in product performance studies should correspond to the lowest application rate from a product label, typically expressed as amount of product per unit area for surface area treatments (e.g. oz/ft^2) or volumetrically for space spray, total release aerosols, and fumigant treatments (e.g. fl. oz. /ft^3). Rates may also be expressed as seconds of spray per unit area or volume. The amount of active ingredient applied per unit area or time should also be given. If the product label directs the user to spray a pest directly, the amount of product applied or seconds sprayed per pest should be provided. A metered bench top sprayer is an example of a spray device that can be used to ensure consistent application volume and even distribution of spray particles. When utilizing such application devices, ensure the deposition of the product mimics a real world product application (e.g. formulation type should not change between an aerosol and a liquid). While rates should be reported in units according to the US traditional systems of weights and measures, units may also be reported using metric system measurements. The method to measure application rate can vary among studies, however the following are common methods of measurement:

1. Weigh the container holding the pesticide before and after application, and divide the difference by the unit area treated.
2. Measure the amount of liquid or bait (and alternate food source) in a container before and after application, and divide the difference by the unit area treated.
3. To determine the quantity sprayed per second, spray five panels for three seconds each. The product container should be weighed before and after each spray and the difference recorded. The mean value of the five replicates should be determined and that result divided by three to determine the average amount of product applied per second of spraying. The same procedure should be conducted to evaluate dust

product formulations except that application should be made from a height of six inches or as directed by the product label.

iv. **Testing conditions.** During product performance testing in the laboratory, temperature should be kept at 25 ± 1 °C (unless otherwise indicated), with a relative humidity of 50-80%, and a photoperiod appropriate for the target pest. The temperature during the test should be kept as constant as possible because changes can affect the performance of the product treatments. Field studies should be conducted in weather that is realistic for use. Extreme weather (temperature, wind and/or precipitation) should be avoided. A food and water source should be provided for all test organisms throughout the study. The specific type of food will vary depending on the species/life stage being tested.

v. **Choice of endpoints.** Study endpoints should be appropriate for the specific objectives of the proposed research and likely to provide a robust answer to the research question. Endpoints, such as knockdown or kill, should be evaluated at the lowest labeled application rate for the target pest. The endpoint selected should be claimed on the proposed label. The following are examples of commonly used endpoints; see specific study sections (i) though (w) for additional information or variations.

1. **Mortality.** Observations should be reported throughout the study but no later than 96 hours after the onset of application initial exposure. Observations of mortality occurring after 96 hours should be justified based on the mode of action and application type. Survival of pests beyond 96 hours in any treatment or control group does not justify making observations after 96 hours. Control mortality should remain equal to or less than 10% throughout the study. The number of dead, knocked down, moribund, and live pests in each replicate should be recorded separately at each time point tested, as practically possible. At a minimum, for mortality calculation purposes, dead and moribund individuals should be recorded separately at the last time point tested or at 96 hours after the onset of application/initial exposure, whichever comes first. A mortality count should include only dead, but not moribund or knocked down, arthropods.

2. **Knockdown.** For knockdown evaluation, observations should be made for up to 10 seconds post-treatment exposure for stinging hymenopterans and 30 seconds post-treatment exposure for all other pests, with confirmed mortality no later than 96 hours after treatment. Control mortality should remain equal to or less than 10% throughout the study. The number of dead, knocked down, moribund, and live pests in each replicate should be recorded separately at each time point tested, as practically possible. For knockdown, a count could include knocked down, moribund, and dead arthropods, but a mortality count should include only dead, but not moribund or knocked down, arthropods.

vi. **Test organisms.** Testing should be conducted with adult pests unless the product is intended to target pest development or immature and/or egg stages of the pest. All sources of pests should be listed in the study methods along with species (strain/race when applicable), sex, and approximate age.

vii. **Representative sampling**.

1. **Replication.** Depending on the results of a power vs. sample size analysis, a minimum of five replicates of ten pests each and balanced (equal number of treated and control replicates) experimental designs are recommended for most studies. Exceptions are noted in the guidance that follows in this document. Other factors that may affect sample size and replication are the number of treatments, the experimental design, and the heterogeneity in the sample pest population (e.g., developmental stage, gender, insecticide susceptibility) and the environment (e.g., different habitat population densities). The protocol should fully describe how sample size and replication were determined.

2. **Rearing, handling, and maintenance of pests.** When applicable, a description of the laboratory colony rearing practices should be included. Details on the collection of the arthropod (how, when, where) and maintenance procedures for field-collected pests should be described.

3. **Untreated control.** In most studies, a negative control should be included, and the number of untreated control replicates should equal the number of replicates for each treatment. When appropriate, a negative control can be treated with diluent only or receives no treatment at all.

4. **Placebo controls.** A placebo control is recommended only when evaluating product performance of flushing products.

5. **Positive controls.** A positive control is recommended only when determining a resistance ratio.

viii. **Quality assurance/Quality control plan.** Protocols should provide for periodic quality assurance inspections that are adequate to ensure the integrity of the study and consistency with the provisions of EPA's GLP regulations (40 CFR §160).

b. Data collection and reporting. Study protocols should provide for collection and reporting of data covering all aspects of the research including those discussed in section (g) of this guideline. GLP regulations specify that each study protocol should provide for collecting and reporting all elements provisioned by the GLP regulation at 40 CFR §160.120.

c. Data analysis. Protocols should include a full description and explanation for the statistical methods proposed to analyze both resistance ratio determinations (if applicable) and product performance test results, taking into account the specific study objectives and variables. If needed, a statistician should be consulted regarding the sample size vs. power of the study design and the statistical methods for data analysis when developing test protocols. Analysis of data is recommended to determine if the mortality rate of the group treated with the product differs from the negative control mortality and if any within treatment effects were significant. Ninety-five percent confidence intervals should be reported. Protocols should explicitly describe the model to be used and demonstrate whether or not assumptions underlying the model can be met for all proposed analyses. Restrictions on randomization of any testing components should be documented clearly and should be accounted for correctly in the statistical analyses. Generally, generalized linear models (GLMs) are recommended to fit models directly to non-normal (e.g., binomial, which describe much of the collected product performance data sets) data using an appropriate link function. GLMs do not involve transforming the response variable, thereby

allowing data to remain on the original scale of measurement. Generalized linear mixed-models (GLMM) may also be appropriate for correlated data. Software for analysis using GLMs or GLMMs is available in many widely sold statistical analysis packages. If survival analyses, such as the Kaplan-Meier Estimator, are used, provide justification for use of the median value to characterize product performance and demonstrate that the underlying assumptions of these analyses have been met. Other analyses including continuous and normality assumptions, such as one-way ANOVA or mixed-effects models, should be described and justified.

(e) Review of protocols for efficacy studies. Protocols proposing novel testing methods should be submitted to EPA for review before the study begins. Proposed data collection sheets may also be included in the protocol submission.

(f) Execution of efficacy studies.

a. Execution of protocol. In cases where a protocol has been submitted to EPA for review, testing should be initiated when the EPA review is complete and if applicable, EPA comments should be incorporated into the revised protocol.

b. Quality Assurance (QA) oversight. Product performance testing is subject to GLP regulations at 40 CFR §160. GLP regulations state that each testing facility should include an independent QA unit. The QA unit monitors and documents execution of each protocol in accordance with the GLP regulations (40 CFR §160.35). The QA unit should inspect each study at intervals adequate to ensure the integrity of the study and maintain written and properly signed records of each periodic inspection. Please see (b)(b)(i) above for the discussion of the use of GLP laboratory methods when conducting product performance studies.

c. Protocol amendments. Amendments are planned changes to the protocol and should be made before the study is executed. All amendments to the protocol should be noted in the written report to the agency.

d. Deviations from protocol. Even when executing the best-designed and most comprehensive protocols, unanticipated deviations from the protocol may occur. All such deviations from the protocol and their impact on the research should be fully reported in the study report submitted to EPA (40 CFR §160.185).

(g) Reporting of completed efficacy studies to the agency.

a. Study identification. Title, identifying study number(s), sponsor, study director, investigators, name and location of the testing facility, and dates of the study should be reported. If tests are conducted outside the U.S., the relevance of the study for U.S. regulatory purposes should be justified in the study report.

b. Study objective(s). The purpose of the study should be stated.

c. Testing conditions. Information on temperature, relative humidity, ambient light and photoperiod, and air flow (where applicable) should be reported.

d. Testing system. Testing system information, including but not limited to the following, should be reported:

i. Pest species tested, including identification of strains of susceptible and field populations if applicable; where pest strains were collected/obtained; and development stage, age, and sex of pests.

ii. Methods for preparation of pests (feeding/starving), including rearing, handling, and maintenance should be identified.

iii. Description of test substance (i.e., product, % active ingredient, and formulation to be tested). Negative control should also be described.

iv. Description of the experimental unit.

v. Treatment application rate and method of application (rate should be consistent with label instructions).

vi. Number of product treatments.

vii. Number of negative control replicates.

viii. Number of replicates per treatment.

ix. Number of individuals per replicate for each treatment including controls.

x. Length of time for pest exposure period to each treatment.

xi. Endpoints and time intervals of endpoint recordings.

e. Data/Results reporting

i. **Raw data.** Include copies of all raw data.

ii. **Results summary.** Report summary test results on all aspects of research. For example, the number of dead, knocked down, moribund, and live pests in each replicate should be reported separately at each time point tested, as practically possible. Also, the percentage of pests killed and knocked down, exclusively, for each treatment at each test interval should be recorded. Statistical variation around the reported mean or median values should be reported. Specifically, 95% confidence intervals for each endpoint of each treatment group should be reported. At a minimum, for mortality calculation purposes, dead and moribund individuals should be reported separately at the last time point tested or at 96 hours after the onset of application/initial exposure, whichever comes first. For knockdown, a count could include both moribund/knocked down and dead arthropods, but a mortality count should include only dead, but not moribund/knocked down, arthropods. Knockdown and/or mortality values may be corrected, as appropriate, for untreated control knockdown and/or mortality with Abbott's Formula or the equivalent. In addition, the amount of product applied and active ingredient delivered per replicate should be reported.

iii. **Data analysis**. Provide a copy of the statistical analysis plan and results from statistical analysis. Refer to Section (d)(c) for recommendations on data analyses, unless otherwise indicated in a study-specific Data Analysis and Reporting Results section. If a product is intended for use on lawns or turf (e.g. flea powders), grass/turf plugs should be included as a test surface. Similarly, if a product is intended for use on trees, shrubs, hedges, etc., recently collected leaves should be included as a test surface. Surfaces should be pre-cut to

4" x 4" or other appropriate sized panels. In some instances, collected leaves are the tested surfaces (for studies examining how pesticide residues perform on leaves). If collected leaves are utilized, they should be contained in a Petri dish.

iv. **Study conclusions.** The report should include a discussion of the study results and conclusions based on treatment endpoints. Conclusions should state why and how the study results do or do not support the tested hypothesis.

v. **Protocol with amendments and study deviations from the protocol**. A copy of the study protocol should be included with amendments and deviations. Deviations should be justified and described together with their impact on the validity of the study. The study should align with the protocol.

(h) Retention of records. The record-keeping provisions of 40 CFR §160.190 and §160.195 apply to records of any study conducted under the GLP rule.

(i) Specific guidance for laboratory studies for direct application testing of pesticide products

a. Study objective: To determine the product performance of an application made directly to pests.

b. Materials and methods

i. **Experimental units.** The test should be conducted with caged arthropods. Typically, a test cage unit is a 16-ounce squat plastic cup with a screened bottom that has the inside upper margin lined with a lubricant to prevent pest escape. Other cage designs are acceptable provided the spray does not pool in the cage after spraying. Tests involving flying insects should use cups with a screened lid to prevent escape but allow spray applications to reach the insects. Smaller pests should be contained collectively (e.g. fleas), while other larger and/or aggressive pests (e.g. scorpions) may require individual cages.

ii. **Number of treatments and replication.** Tests should include a product treatment at the lowest labeled rate and an untreated control. Depending on the results of power vs. sample size analysis, each test should be replicated at least 5 times for each treatment, and each replicate should have a minimum of 10 individuals of the arthropod species being tested, unless pest biology dictates a different allotment to test cages. The number of untreated control replicates should equate to the number of treated replicates in the study. Therefore, for each treatment group there should a total of 50 arthropods, and for each control group there should be a total of 50 arthropods. A minimum of one hundred arthropods should be utilized.

iii. **Application method.** Applications should be made directly to the pests in the cages at the lowest labeled rate for the target pest and should be made from a distance which corresponds to the Directions for Use on the product label. If a range of application distances is to be included on the product label (e.g. apply from a distance of 8-12 inches), the greater distance should be used. See Section (d)(a)(iii) for information on application rate determination.

iv. **Pest exposure to product treatments.** Pests should be removed and placed into clean containers as soon as practical but no more than 4 hours after onset of exposure to pesticide application for crawling pests and 1 hour after onset of exposure to pesticide application for flying pests. Containers should be stored under ambient test site conditions.

v. **Data collection and endpoints.** See Section (d)(a)(v) for more information on choice of endpoints.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results.

d. **Study conclusions.** Summarize study outcomes for direct application testing against pests and discuss their implications for product labeling.

(j) Specific guidance for laboratory studies for forced exposure (no-choice) indoor residual applications

a. **Study objective:** To determine the indoor residual product performance of an application made to different surfaces in a forced exposure (no-choice) test.

b. **Materials and methods**

i. **Experimental unit.** The test should be conducted on a porous surface (unpainted/unfinished ¼" thick plywood) and a non-porous surface (commercial linoleum or glazed ceramic tile). If a product is intended for use on other substrates (e.g. carpeting for flea powders), samples of that substrate should be included as a test surface. Surfaces should be pre-cut to 4" x 4" or other appropriate sized panels.

ii. **Number of treatments and replication.** Depending on the results of power vs. sample size analysis, the test should include at least 5 treated panels and with an equal number of untreated panels for each surface type. Each panel is considered a replicate and each replicate should consist of a minimum of 10 individuals confined to the panel, unless pest biology dictates a different allotment for each surface. At least one-hundred individual pests should be tested for each time point tested per species tested per surface type (including the untreated control specimens). Therefore, a study investigating the residual efficacy of a test substance on a porous surface, non-porous surface, and carpeting would call for a minimum of 300 individuals at each time point.

iii. **Application method.** Applications should be made to each panel at the lowest labeled rate and should be made from a distance which corresponds to the Directions for Use on the product label. If a range of application distances is to be included on the product label (e.g. apply from a distance of 8-12 inches), the greater distance should be used. See Section (d)(a)(iii) for information on application rate determination. Panels should be stored and exposed to ambient indoor conditions to age residues. Panels undergoing aging should be exposed to light and should not be wrapped together.

iv. **Pest exposure to product treatments.** Panels should be aged for a minimum of 24 hours and fully dried before the test arthropods are exposed to them. Crawling pests should be exposed to treated panels for no more than 4 hours. Flying pests should be exposed to treated panels for no more than 1 hour. Flying pests should be confined to a treated surface with a Petri dish or standard World Health Organization (WHO) cone (WHOPES 2013). Cones should be ~9 cm in diameter and should have a small central opening through which flying insects can be introduced. After the exposure period, the pests should be transferred to clean, untreated containers for further observation and evaluation. Containers should be stored under ambient test site conditions. Treated panels may be retested, though individual

pests from any treatments, including the untreated control, should not be reused even if no toxic effect is observed.

v. **Data collection and endpoints.** After an initial challenge 24 hours post application, the pesticide residues on aged surfaces should be tested regularly until the end of the study. One may consult with the agency for a determination on an appropriate testing interval. See Section (d)(a)(v) for more information on choice of endpoints.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results. Results should be reported separately for each surface type tested.

d. **Study conclusions.** Summarize study outcomes for indoor residual control of pests and discuss their implications for product labeling.

(k) Specific guidance for studies on forced exposure (no-choice) outdoor residual applications

a. **Study objective:** To determine the outdoor residual product performance of an application made to different surfaces in a forced exposure (no-choice) test.

b. **Materials and methods**

i. **Experimental units.** If a product is intended for use on buildings or as an outdoor residual perimeter treatment, a non-porous surface (e.g. vinyl siding or tile) and a porous surface (e.g. unpainted concrete) should be used in product performance testing. If a product is intended for use only on pavement, a porous surface (e.g. unpainted concrete) should be used. If a product is intended for use on lawns or turf (e.g. flea powders), grass/turf plugs should be included as a test surface. Similarly, if a product is intended for use on trees, shrubs, hedges, etc., recently collected leaves should be included as a test surface. Surfaces should be pre-cut to 4" x 4" or other appropriate sized panels. Collected leaves, if applicable, should be contained in a Petri dish.

ii. **Number of treatments and replication.** Depending on the results of power vs. sample size analysis, each test should include at least 5 treated panels and 5 untreated panels for each surface type. Each panel is considered a replicate. Each replicate should consist of a minimum of 10 pests confined to a treated or untreated panel/surface sample. At least 100 individual arthropods should be tested for each time point tested per species tested per surface type (including the untreated control specimens). Therefore, a study investigating the residual efficacy of a test substance on a porous surface, non-porous surface, and grass plugs would call for a minimum of 300 individuals. An equal number of negative control replicates should be included.

iii. **Application method.** The lowest labeled application rate for the target pest should be applied to each panel/surface sample and should be made from a distance which corresponds to the Directions for Use on the product label. If a range of application distances is to be included on the product label (e.g. apply from a distance of 8-12 inches), the greater distance should be used. See Section (d)(a)(iii) for information on application rate determination. Treated surfaces should be stored outdoors such that they are exposed to direct sunlight and precipitation. Indoor aging with simulated outdoor conditions may be acceptable on a case-by-case basis and should be supported with justification.

iv. **Pest exposure to product treatments.** Treated surfaces should be aged for a minimum of 24 hours and fully dried before the test arthropods are exposed to them. Crawling pests should be exposed to treated panels for no more than 4 hours. Flying pests should be exposed to treated panels for no more than 1 hour. Flying pests should be confined to a treated surface with a Petri dish or standard WHO cone (WHOPES 2013). Cones should be ~9 cm in diameter and should have a small central opening through which flying insects can be introduced. After the exposure period, the pests should be transferred to a clean, untreated container for further observation and evaluation. Containers should be stored under ambient test site conditions. Treated surfaces may be retested (provided the integrity of the treated surface is maintained (e.g. no disintegrated leaves), though pests from any treatments, including the untreated control, should not be reused even if no toxic effect is observed.

v. **Data collection and endpoints.** After an initial challenge 24 hours post application, the pesticide residues on aged surfaces should be tested for efficacy against the intended pest regularly until the end of the study. One may consult with the agency for a determination on an appropriate testing interval. See Section (d)(a)(v) for more information on choice of endpoints

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results. Results should be reported separately for each surface type tested.

d. **Study conclusions.** Summarize study outcomes for outdoor residual control of pests and discuss their implications for product labeling.

(l) Specific guidance for laboratory studies for testing indoor pesticide fogger, total release aerosols, space spray, and insecticide vapor strip products.

a. **Study objective:** To determine the product performance of indoor fogger, total release aerosols, space sprays, and vapor strip treatments when applied directly to pests or to surfaces.

b. **Materials and methods**

i. **Experimental units.**

1. **Direct application.** The treatment should be applied in a Peet-Grady chamber with a volume of 216 cubic feet or greater (WHOPES 2009). The chamber should have a window for observation. The wall, ceiling, and floor of the room may be lined with plastic or other suitable materials to facilitate cleaning. Six cages of test arthropods should be placed in specific locations within the chamber: place one cage of test arthropods in each corner one foot above the floor, and place two cages where the wall and ceiling meet at the middle of two opposite walls. The inclusion of harborage and type of harborage within each cage is species-dependent. Examples of suitable harborage includes containers covered with mesh or stacked egg cartons. If needed, consult with the agency for guidance.

2. **Residual surface application.** The treatment should be applied in a Peet-Grady chamber with a volume of 216 cubic feet or greater (WHOPES 2009). The chamber should have a window for observation. The wall, ceiling, and floor of the room may

be lined with plastic or other suitable materials to facilitate cleaning. In the chamber for each surface type as described in (j)(b)(i), place one panel in each corner one foot above the floor. Place the last two panels where the wall and ceiling meet at the middle of two opposite walls.

ii. Number of treatments and replication.

1. **Direct application.** The test should include a product treatment at the lowest labeled rate and the lowest labeled exposure period for the target pest and an untreated control. Depending on the results of power vs. sample size analysis, one run of the chamber (and untreated controls) should be replicated at least 5 times. Each chamber should hold six cages. Each cage should contain a minimum of 10 individuals of the arthropod species being tested. An equal number of untreated control replicates, cages, and specimens should also be included. Therefore, for each replicate (i.e. one run of the chamber and untreated controls), 12 cages of each pest species should be used: allot six cages of each species to the product treatment for placement in the chamber and six to the negative control for placement outside the chamber. Therefore, for each exposure period there should be 60 arthropods in the treatment chamber (divided between 6 cages), and 60 arthropods outside of the chamber in the 6 untreated control cages. One hundred and twenty arthropods should be used for each replicate of the test.

2. **Contact with residual surface application.** The test should include a product treatment at the lowest labeled rate at the lowest labeled exposure period for the target pest and an untreated control. Depending on the result of power vs sample size analysis, the treatment (i.e. run of the chamber) should be replicated at least 5 times. An equal number of untreated control replicates and specimens should also be included. For each replicate (i.e. run of the chamber), twelve panels of each surface type for each pest species should be used; allot six panels of each surface type for each species to the product treatment and place in the chamber, while the other six panels should be kept outside the chamber as a negative control for each exposure period. After the application, the product and panels should be held in the sealed container for the amount of time a room or area should be sealed per the label directions. As exhausting the chamber is not instantaneous, ensure that the panels are only exposed to the product for the minimum amount of time as directed on the label. After the chamber has been exhausted, each treated panel for each surface type should be allotted a minimum of 10 individuals of the arthropod species being tested. Therefore, for each surface type there should be 60 arthropods (divided between 6 treated surfaces), and 60 arthropods (divided between 6 untreated surfaces). One hundred and twenty arthropods should be used for each replicate of the test. Vaporizing strips should be assessed in a similar manner.

iii. Application method. The lowest labeled application rate and shortest exposure period for each pest species should be tested. The chamber should be sealed before the product application is made. Applications of indoor fogger and space spray products should be delivered by an automatic dispenser calibrated for the proper droplet size and rate. Vaporizing strips should be hung from the ceiling in the center of the room and total release foggers can be applied as directed within the chamber. At the end of each exposure, the air

in the chamber should be exhausted and any surface residues washed off. Surfaces of the chamber should be clean and dry before the next test. Alternative product application methods may be considered, but should be described and justified. See Section (d)(a)(iii) for information on application rate determination. For residual/aged performance studies, treated panels should be stored and exposed to ambient indoor conditions to age residues. Panels undergoing aging should be exposed to light and should not be wrapped together.

iv. **Pests exposure to product treatments.**

1. **Direct application.** After the application, the product and pests should be held in the sealed container for the amount of time a room or area should be sealed per the label directions. As exhausting the chamber is not instantaneous, ensure that the pests are only exposed to the product for the minimum amount of time as directed on the label. Once the label prescribed exposure period has elapsed, pests should be removed and placed into clean containers as soon as practical but no more than 4 hours after onset of exposure to pesticide application for crawling pests and 1 hour after onset of exposure to pesticide application for flying pests. If the label specifies a longer seal time, a longer interval may be used which corresponds to the label. Containers should be stored under ambient test site conditions.

2. **Contact with residual surface application.** After the application, the product and pests should be held in the sealed container for the amount of time a room or area should be sealed per the label directions. As exhausting the chamber is not instantaneous, ensure that the pests are only exposed to the product for the minimum amount of time as directed on the label. See Section (j)(b)(iv) for further pest exposure methods.

v. **Data collection and endpoints.** See Section (d)(a)(v) for more information on choice of endpoints. For contact with residual surface application specifically, after an initial challenge at 24 hours post application, the pesticide residues on aged surfaces should be tested regularly until the end of the study. One may consult with the agency for a determination on an appropriate testing interval.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results. Report estimated mortality rate (and knockdown rate, if applicable) for each species in each treatment (and for residual treatments, for each surface type) at each height level and all heights combined as corrected rate values. The statistical analysis should consider the random effect of the chamber/replicate/panel and the effect of the treatment cage/panel height on product performance. Generalized linear mixed effects models can be considered for use.

d. **Study conclusions.** Summarize study outcomes for indoor pesticide fogger, total release aerosol, space spray, and insecticide vapor strip testing against pests and discuss their implications for product labeling.

(m) Specific guidance for laboratory studies for testing ovicidal products

a. **Study objectives:** To determine the product performance of pesticide products intended for use as ovicides.

b. Materials and methods. The methods below are for a direct or surface spray application. If an indoor fogger, total release fogger, space spray, or vaporizing strip is being tested, a modified version of the methods in Section (l) should be used. For fumigants, see Section (n).

i. Experimental unit

1. **Direct application.** Treatment should be applied to cohorts of eggs/egg masses of approximately the same age. Laying females should be approximately the same age, as well. Eggs/egg masses should be collected and placed in an arena where they can receive a direct pesticide application. The arena can be a Petri dish or other container as appropriate for the test species or tested product.

2. **Residual surface application.** Treatment should be applied to a porous surface as well as a non-porous surface (see Section (j)(b)(i) for indoor surfaces and Section (k)(b)(i) for outdoor surfaces). Eggs/egg masses of approximately the same age, laid from females of approximately the same age, should be placed on the treated surface intended for testing.

ii. Number of treatments and replication.

1. **Direct application.** The test should include a product treatment at the lowest labeled rate for the target pest and an untreated control. Depending on the results of the power vs. sample size analysis, the test should be replicated at least 10 times for each treatment. An equal number of untreated control replicates and specimens should also be included. Each replicate (arena) should have a minimum of 20 eggs or egg masses of the arthropod species being tested. Therefore, for each treatment group there should a total of 200 eggs/egg masses arthropods, and for each control group there should be a total of 200 eggs/egg masses. A minimum of four hundred eggs/egg masses should be utilized.

2. **Contact with residual surface application.** Depending on the results of power vs. sample size analysis, the test should include at least 10 treated panels and 10 untreated panels for each surface type. Each panel is considered a replicate. Each replicate should consist of a minimum of 20 eggs/egg masses placed on the panel. For each surface tested, the test should include a product treatment at the lowest labeled rate for the target pest and an untreated control. An equal number of untreated control replicates and specimens should also be included. Therefore, for each panel tested, 400 eggs/egg masses should be tested (200 untreated control eggs/egg masses on 10 different panels, and 200 treated eggs/egg masses on 10 different panels).

iii. Application method.

1. **Direct application.** Applications should be made directly to the eggs in the arenas at the lowest labeled rate for the target pest and should be made from a distance which corresponds to the Directions for Use on the product label. If a range of application distances is to be included on the product label (e.g. apply from a distance of 8-12 inches), the greater distance should be used. See Section (d)(a)(iii) for information on application rate determination.

2. **Contact with residual surface application.** Applications should be made to each panel at the lowest labeled rate and should be made from a distance which corresponds to the Directions for Use on the product label. If a range of application distances is to be included on the product label (e.g. apply from a distance of 8-12 inches), the greater distance should be used. A metered bench top sprayer is an example of a spray device that can be used to ensure consistent application volume and even distribution of spray particles. When utilizing such application devices, ensure the deposition of the product mimics a real world product application (e.g., formulation type should not change between an aerosol and a liquid). See Section (d)(a)(iii) for information on application rate determination. Panels should be stored and exposed to ambient indoor conditions to age residues. Panels undergoing aging should be exposed to light and should not be wrapped together.

iv. Pest exposure to product treatments.

1. **Direct application.** After being sprayed directly, eggs should be exposed continuously (no transfer of eggs/egg masses to an untreated container is necessary).

2. **Contact with residual surface application.** Eggs/egg masses should be placed on the treated surface intended for testing. Eggs should be exposed continuously (no transfer of eggs/egg masses to an untreated container is necessary). Containers should be stored under ambient test site conditions. Treated panels may be retested though pests from any treatments, including the untreated control, should not be reused even if no toxic effect is observed. Panels should be aged for a minimum of 24 hours and fully dried before the test arthropods are exposed to them.

v. Data collection and endpoints. As the development of eggs will vary from species to species, provide justification for an appropriate length of time and frequency of observations.

1. **Egg mortality.** The number of un-hatched and hatched eggs should be recorded for the treated and untreated control groups at each observation interval. Eggs/egg masses may be examined microscopically, if needed, to determine if egg hatch has taken place.

c. Data analysis and reporting. Refer to Section (d)(c) of this guideline for guidance on data analysis. In addition, the percentage and cumulative percentage of unhatched eggs/egg masses for each treatment (and for residual studies, for each surface type) at each observation interval should be reported. Report the 95% confidence interval of the estimated egg hatch rate for each treatment. Egg hatch data from the treated group may be corrected for untreated control mortality with Abbott's Formula or the equivalent.

d. Study conclusions. Summarize study outcomes for ovicidal testing against pests and discuss their implications for product labeling. Treatments where nymphs hatch from treated eggs but do not develop into adults will not be considered ovicidal.

(n) Specific guidance for laboratory studies for fumigant products

a. Study objective: To determine the product performance of a fumigant in the laboratory.

b. Materials and Methods.

i. Experimental unit The treatment should be applied to jars containing vials which contain arthropods. Vials should be closed with fine netted cloth and then be placed in airtight jars in controlled environmental chambers. The lids of each jar should have a site for injection of the fumigant (Phillips et al. 2014).

ii. Number of treatments and replication. The test should include a product treatment at the lowest labeled rate and an untreated control. A replicate consists of a single jar containing a minimum of 5 vials. Depending on the results of the power vs. sample size analysis, the test should be replicated at least 5 times for each treatment and each vial should have a minimum of 10 adults of the arthropod species being tested, depending on the targeted life stage. Therefore, for each treatment group there should a total of 250 arthropods, and for each control group there should be a total of 250 arthropods. A minimum of 500 arthropods should be utilized. For testing eggs, see section (m). An equal number of untreated control replicates and specimens should also be included. Pests in the control group should be held in untreated glass containers outside the fumigation chamber for the same period of time as the treatment group.

iii. Application method. Applications should be made at the lowest labeled rate for the target pest and tested at both 59° F (15° C) and 77° F (25° C). The fumigant can be injected into the jars via a calibrated, gas-tight syringe. The rate should be monitored by chemical detection to ensure the target rate was achieved (Phillips et al. 2014).

iv. Pest exposure to product treatments. As soon as possible after the exposure period, transfer the pests out of the vials and place in clean, untreated containers for further observation and evaluation. Containers should be stored under ambient test site conditions.

v. Data collection and endpoints.

1. **Adult mortality.** See Section (d)(a)(v) for more information.

2. **Egg mortality.** As the development of eggs will vary from species to species, provide justification for an appropriate length of time and frequency of observations. The number of un-hatched and hatched eggs should be recorded for the treated and untreated control groups at each observation interval. Eggs/egg masses may be examined microscopically, if needed, to determine if egg hatch has taken place.

c. Data analysis and reporting. Refer to Sections (d)(c) of this guideline for guidance on data analysis. Generalized linear mixed effects models can be considered for use to account for the random effect of jar. In addition, the amount of product should be reported for each replicate, and a copy of the chemical analysis should be included. Refer to Section (g)(e) of this guideline for guidance on reporting results for adults. If eggs are being tested, the percentage and cumulative percentage of unhatched eggs/egg masses for each treatment at each observation interval should be reported. Report the 95% confidence interval of the estimated mortality rate for each treatment. Egg hatch data from the treated group may be corrected for untreated control mortality with Abbott's Formula or the equivalent.

d. **Study conclusions.** Summarize study outcomes for fumigant testing against pests and discuss their implications for product labeling.

(o) Specific guidance for laboratory studies of insect growth regulator (IGR) products

a. **Study objective:** To determine the product performance of an IGR indoors and/or outdoors against pests.

b. **Materials and Methods**

i. **Experimental unit.** The test should be conducted with caged arthropods. Life stages should be evaluated separately and individuals in the population should be approximately the same age. These products may be tested against eggs, nymphs/larvae (all stages/instars), pupae, and/or mixed-sex adults. For more experimental unit information specific to the type of product being tested (e.g. bait, residual spray, etc.), see other experimental design sections in this guideline.

ii. **Number of treatments and replication.** Replication for studies testing products that contain IGRs is dependent on the application type (e.g. direct spray, fogger, bait), the life stage (e.g. eggs, adults), and the treatment type (e.g. contact, residual). See the other number of treatments and replication sections in this guideline for more information.

iii. **Application method.** Replication for studies testing products that contain IGRs is dependent on the application type (e.g. direct spray, fogger, bait) and the treatment type (e.g. contact, residual). See the other number of treatments and replication sections in this guideline for more information.

iv. **Pest exposure to product treatments.** The IGR should be tested alone. Testing should not be performed with mixtures containing multiple IGRs (unless a product contains multiple IGRs) or insecticide products containing IGRs formulated with other active ingredients. The pest exposure recommendations are based on the type of product being tested and should be evaluated as described in other sections of this guideline. Testing on eggs should be conducted as described in Section (m), except that within 24 hours after the majority of hatching occurs, all juvenile arthropods should be moved to clean containers (one container per replicate) (Tunaz and Uygun 2004; Bellinato et al. 2009). Pests should be transferred to clean containers as soon as practical but no more than 4 hours after the onset of the exposure to the pesticide application for crawling pests and after 1 hour after the onset of the exposure to the pesticide application for flying pests. Containers should be stored under ambient conditions.

v. **Data collection and endpoints.** It is recommended that evaluation occur through 30 days post-treatment. Specific evaluation intervals may be product-dependent and may vary based on desired label claims. Some arthropods may have life cycles that takes shorter or longer than 30 days to complete. In those cases, testing should be compressed or extended to a length of time consistent with the time it takes for untreated controls to reach adulthood. If a longer period is necessary for evaluation of IGR effects, justification should be provided. Endpoints may include the following:

1. **Adult mortality.** See Section (d)(a)(v) for more information.

2. **Egg hatch and survival of emerging immatures.** Record any abnormalities in development including deformities. Record egg hatch success and development of hatching nymphs/larvae, if applicable.

3. **Reproductive success.** If claims regarding reproductive success of treated adults are desired, assess and record egg production, hatching success of eggs deposited after adults are exposed, and developmental success and mortality of emergent nymphs/larvae.

c. Data analysis and reporting. Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results. Generalized linear mixed effects models could be considered for use to account for the random effect of replicate. In addition, describe any abnormalities in development including deformities. The estimated percent of each endpoint and its 95% confidence interval should be reported as applicable, and could include mortality rate and developmental success for each applicable life stage, egg production, and egg hatch success.

d. Study conclusions. Summarize study outcomes for IGR testing against pests and discuss their implications for product labeling.

(p) Specific guidance for field studies of outdoor pesticide fogger products, applied directly to pests. See Section (k) for guidance for residual outdoor pesticide foggers.

a. Study objective: To determine the performance of outdoor fogging pesticide products intended for use directly against pests.

b. Materials and methods.

i. Experimental unit.

1. **Direct application.** Treatment should be applied outdoors to a row of five cages containing arthropods. Suggested cages are cylindrical, sized 0.1 x 0.6 m with mesh screens (Alimi et al. 2013), but other appropriate cages can be utilized as appropriate. Cages should be placed parallel to the spray/fog line at the distance from the spray/fog line as prescribed by the label. Cages should also be mounted on poles, 10 feet apart, 3 feet off of the ground.

2. **Contact with residual surface application.** The test should be conducted on panels of various surface types. Outdoor surfaces that should be tested can be found in Section (k)(b)(i). Panels should be placed parallel to the spray/fog line at the distance from the target as prescribed by the label. Surfaces should be 10 feet apart, elevated 3 feet off of the ground (Mani et al. 2005).

ii. Number of treatments and replication.

1. **Direct application.** The test should include a product treatment at the lowest labeled rate for the target pest and an untreated control. Depending on the results of the power vs. sample size analysis, the test should be replicated at least 3 times for each tested arthropod species, and each replicate should have at least 5 cages tested from the spray line with a minimum of 10 individuals per cage. The same numbers of replicates, cages/replicate and individuals/cage for the untreated control should also be included for each test. Therefore, for each treatment group there should a

total of 150 arthropods, and for each control group there should be a total of 150 arthropods. A minimum of 300 arthropods should be utilized Negative control cages should be placed upwind from the treatment site close enough to experience the same abiotic conditions.

2. **Contact with residual surface application.** The test should include a product treatment at the lowest labeled rate for the target pest and an untreated control for each surface type tested. Depending on the results of the power vs. sample size analysis, the test should be replicated at least 3 times for each tested arthropod species, and each replicate should have at least 5 panels per surface type tested. After application, a minimum of 10 individuals should be confined to each panel. The same numbers of replicates and individuals for the untreated control should also be included for each test. Therefore, for each surface type, there should be 15 treated panels and 15 untreated panels with a total of 300 individuals. Negative control panels should be placed upwind from the treatment site close enough to experience the same abiotic conditions.

iii. **Application method.** The lowest labeled application rate, for each pest species to be tested, should be made from a distance which corresponds to the Directions for Use on the product label. Unless other application methods are specified on the label for the pest species, the applicator should walk up the spray line at a fixed pace (e.g. 2km/hr (Alimi et al. 2013)), delivering the minimum amount of pesticide as labeled. See Section (d)(a)(iii) for information on application rate determination.

iv. **Pest exposure to product treatments.**

1. **Direct application.** Pests should be removed and placed into clean containers as soon as practical but no more than 4 hours after onset of exposure to pesticide application for crawling pests and 1 hour after onset of exposure to pesticide application for flying pests. Containers should be stored under ambient test site conditions.

2. **Contact with residual surface application.** See Section (k)(b)(iv) for pest exposure methods.

v. **Data collection and endpoints.** For contact with residual surface application, after an initial challenge 24 hours post application, the pesticide residues on aged surfaces should be tested regularly until the end of the study. The study duration should correspond to the amount of time the product is claimed to be efficacious on the label. One may consult with the agency for a determination on an appropriate testing interval. See Section (d)(a)(v) for more information on choice of endpoints.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results. The data analysis should consider characteristics of cages nested into replicate. Generalized linear mixed effects models can be considered for use.

d. **Study conclusions.** Summarize study outcomes for outdoor pesticide fogger testing against pests and discuss their implications for product labeling.

(q) Specific guidance for field studies of outdoor pesticide misting system products

a. **Study objective:** To determine the performance of outdoor pesticide misting systems as direct contact sprays.

b. **Materials and methods.**

i. **Experimental unit.** This test should be conducted with arthropods in cages outside. Five cages should be placed inside the test site. Cages should be large enough and made of mesh or other material so that the application of the pesticide to the caged insects is not hindered by the cage. A cage with a 12 cm diameter should suffice. Cages should be placed at a distance that corresponds to label claims/directions. The nozzle system should be placed at least 5 feet above the ground (Cilek et. al 2008) or as instructed per label directions. Slide impingers should be utilized to determine droplet size at varying distances from the nozzle.

ii. **Number of treatments and replication.** The test should include a product treatment at the lowest labeled rate for the target pest and an untreated control. Depending on the results of the power vs. sample size analysis, the test should be replicated at least 5 times for each treatment with at least 5 cages in the treatment site, and each cage should have a minimum of 10 individuals of the arthropod species being tested. At least three untreated control cages per replicate each with a minimum of 10 individuals should be placed nearby and upwind from the application for each replicate. Therefore, for each treatment group there should a total of 250 arthropods, and for each control group there should be a total of 150 arthropods. A minimum of 400 arthropods should be utilized

iii. **Application method.** The product should be applied via the intended pesticide misting system and the lowest labeled rate for the target pest that corresponds to the Directions for Use on the product label. See Section (d)(a)(iii) for information on application rate determination.

iv. **Pest exposure to product treatments.** Pests should be removed and placed into clean containers as soon as practical but no more than 4 hours after onset of exposure to pesticide application for crawling pests and 1 hour after onset of exposure to pesticide application for flying pests. Containers should be stored under ambient test site conditions.

v. **Data collection and endpoints.** See Section (d)(a)(v) for more information on choice of endpoints. In addition, record the droplet size recorded at each impinger.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results. In addition, report the droplet size recorded at each impinger. For each pest species, report the estimated percentage killed/knocked down and its 95% confidence interval in each treatment. The analysis should consider characteristics of cages nested into replicate on product performance by using generalized linear mixed effects models.

d. **Study conclusions**. Summarize study outcomes for outdoor pesticide misting system testing against pests and discuss their implications for product labeling.

(r) Specific guidance for laboratory studies of cockroach bait products.

a. **Study objective:** To determine the performance of insecticidal baits intended for use against cockroaches.

b. **Materials and methods**

i. **Experimental unit.** The test should be conducted with arthropods in dual-chamber Ebeling boxes (Ebeling et al. 1966; OECD 2013). Each chamber should have a 1 m^2 footprint and can be either circular or rectangular. One chamber should serve as the harborage chamber and should include a standardized harborage for test cockroaches consisting of either corrugated cardboard or egg cartons. The other chamber should serve as the feeding chamber and should include the bait formulation being tested, a water source, and an alternate food source. The chamber should be connected by a translucent Plexiglas™ tube (500 mm long, 50 mm in diameter) to allow cockroach movement between chambers. The upper lip of each chamber should be treated with Fluon™, petroleum jelly or some suitable substance which prevents cockroach escape. All inside surfaces of each chamber should be covered with kraft paper or a similar type of absorbent paper to prevent contamination from insecticide residues. The kraft paper should be tightly and completely taped such that cockroaches cannot crawl under it. Kraft paper should be replaced after each test. Cockroaches should be 3-14 days old (as adults) when they are added to the harborage chamber and females should be non-gravid. They should be allowed to habituate to the test arena for 3 days, during which time food and water but no insecticidal bait should be present.

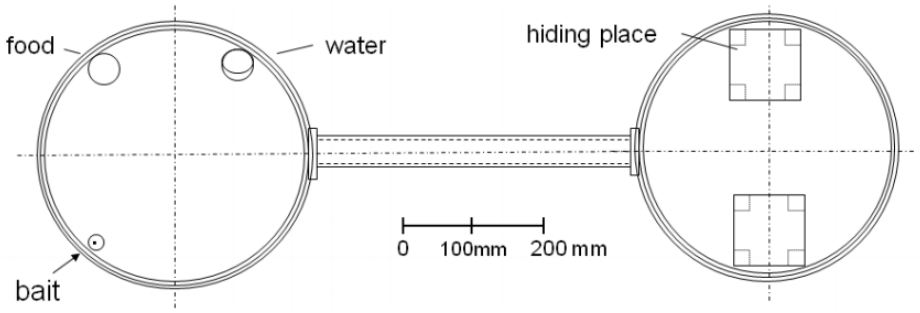
ii. **Number of treatments and replication.** A replicate should consist of 1 dual-chamber Ebeling box (OECD 2013). Depending on the results of the power vs. sample size analysis, a minimum of 10 adults of the species being tested should be used per replicate. A minimum of 5 replicates should be used for each treatment group, and the study method should be balanced to include a minimum of 5 untreated control replicates. Therefore, for each treatment group there should a total of 50 arthropods, and for each control group there should be a total of 50 arthropods. A minimum of one hundred arthropods should be utilized.

iii. **Application method.** After the 3-day habituation period, the bait formulation being tested should be placed in a Petri dish at the lowest labeled rate and added to the feeding chamber (OECD 2013). Efficacy testing of cockroach baits should be product-specific. Because the non-active ingredient components of a bait play an important role in palatability, use a test formulation that matches the subject product in terms of active ingredient and ‘inert’ ingredient profile and concentration. Because bait station efficacy is a function of both the bait formulation and the design of the bait station itself, bait station products should be tested as they would be deployed by a consumer (Appel 1990). This will demonstrate that cockroaches can physically access the bait formulation within a bait station, which is especially important for larger cockroach species. In these instances, a single bait station should replace the bait-containing Petri dish in the feeding chamber.

    1. **Bait placement.** If the feeding chamber is divided into 4 quadrants from a bird’s eye view, the Petri dish containing the test formulation should be added to one of the two quadrants furthest from the connecting tube. The other far quadrant should contain the alternate food source. The water source should be added to one of the quadrants closest to the connecting tube (Figure 1, OECD 2013). Both the alternate food source and water should be provided at the beginning of the 3-day habituation period and should be available to the cockroaches for the duration of the test. A separate Petri dish should contain the same amount of bait, but should be covered

with mesh to prevent cockroaches from accessing it and placed in the last remaining quadrant in the feeding chamber closest to the connecting tube. Alternatively, this Petri dish can be placed immediately outside the test arena such that it is subjected to the same environmental conditions that exist within the test arena. This covered bait should be weighed before and after the test to determine water loss from the bait during the test. The alternate food source, uncovered bait, and protected bait should be weighed before and immediately after the test has concluded to determine the amount consumed by the cockroaches during the test. Water should be replaced as necessary.

Figure 1: Preferred bait/food/water configuration in cockroach bait choice tests (OECD 2013)



2. **Aging of bait.** If desired claims are to be tied to aging (e.g. kills cockroaches for up to x months, or if label DFU imply bait is palatable for x months), the bait used in efficacy tests should be aged for the corresponding amount of time before being presented to test cockroaches in efficacy tests. Bait should be aged at a constant temperature (25 ± 1°C), relative humidity (50-80%), and photoperiod (ranging from 12 hours of light to 12 hours of darkness to 16 hours of light to 8 hours of darkness). Bait should be exposed to light during aging. If a cockroach bait is intended to be used outdoors, aging of bait should be done outdoors at or above the temperature and humidity ranges specified above, with exposure to direct sunlight. Bait should be exposed to rainfall if aged outdoors. Bait intended for outdoor use may be aged indoors if outdoor conditions prohibit outdoor aging (i.e. winter months in cold climates), but rainfall should be simulated.

3. **Choice testing vs. no-choice testing.** Product-specific data to support efficacy claims should be generated with an alternate food source present to ensure a bait formulation is sufficiently palatable (Koehler et al. 1991). No-choice tests with only a bait formulation and no alternate food source can be conducted as part of an initial screening process. Data from these no-choice tests can be submitted as supplemental data. Mortality from cockroach bait studies using choice testing is the endpoint of concern and will confirm palatability.

iv. **Pest exposure to product treatments.** Cockroaches should be exposed to the test formulation for up to 14 days. Any dead individuals should be removed as soon as they are

detected unless product labelling dictates otherwise (Buczkowski and Schal 2001). If a chemical is particularly slow-acting and the test is to extend beyond 14 days, justification should be provided.

v. **Data collection and endpoints.**

1. **Mortality.** The number of knocked down, moribund, dead, and live cockroaches in each treatment group should be recorded separately at 24 hours, 48 hours, 96 hours, 7 days, and 14 days after exposure to the test formulation. Control mortality should remain equal to or less than 10% throughout the study.

2. **Knockdown** (if a knockdown label claim is desired). Record the number of knocked down/moribund cockroaches in each group at the corresponding interval specified on the product label (e.g. a product label claiming knockdown within 30 minutes should be assessed 30 minutes after exposure). Dead individuals at each assessment interval can be considered to have already been knocked down and can be included in the total number of knocked down individuals. For knockdown evaluation, mortality should be confirmed no later than 96 hours after treatment.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results**.**

d. **Study conclusions.** Summarize study outcomes for control of cockroaches and discuss their implications for product labeling.

(s) Specific guidance for laboratory studies of flushing products.

a. **Study objective:** To determine the performance of products intended for use against crawling arthropod pests to induce egress from harborages.

b. **Materials and methods**

i. **Experimental unit.** The test should be conducted with test arenas constructed to simulate a harborage (Hostetler 2014). A cardboard tube measuring 1.5 inches in diameter and 6.75 inches in length may serve as the flushing tower. A 1 x 1.75-inch hole should be cut at the midpoint of each tube, and both ends should be tightly covered with screen/mesh to prevent escape. Each tube should be oriented vertically in a plastic or cardboard secondary containment arena. The upper lip of this secondary containment arena should be treated with Fluon^{TM}, petroleum jelly, or another suitable substance which prevents pest escape. Flushing towers should not be re-used. After each replicate is tested, the secondary containment arena should be replaced entirely so as to prevent confounding results in subsequent tests due to insecticide contamination.

ii. **Number of treatments and replication.** Each replicate should consist of one flushing tower placed into its own secondary containment arena. Depending on the results of the power vs. sample size analysis, at least 10 adults should be used per replicate, and a minimum of 5 replicates should be used for each treatment group. This design should be repeated for each species tested. For aerosol products, one treatment group should receive the test substance, one should receive an aerosol treatment minus active ingredient(s) (i.e. placebo control), and one should receive a non-aerosol water-only treatment. Therefore, for each treatment group (the test substance and the placebo control) there should a total of 50

arthropods, and for each control group (non-aerosol water only treatment) there should be a total of 50 arthropods. A minimum of 150 arthropods should be utilized. Placebo control replicates should be treated with the aerosol propellant minus active ingredient(s) at the same rate as the treatment replicates. The inclusion of a placebo control for aerosols ensures flushing activity is attributable to the product and not the disturbance caused by the aerosol propellant. Flushing tests for non-aerosol products should include a treatment group that receives the test substance, along with a water-only control group.

iii. **Application method and pest exposure to the treatments.** Prior to the start of each test, pests to be used in all treatment groups may be anesthetized with CO_2 or by using a chilling table. Ten individuals should be added to each flushing tower via the hole in the midpoint of the tower and allowed to acclimate for at least 30 minutes. The hole should be stoppered once the pests have been introduced to the tower. Each tower which contains 10 individual pests should then be oriented vertically in its own secondary containment arena (Hostetler 2014).

Immediately prior to treatment, the stopper covering the hole in each tower should be removed to allow pest egress. Aerosol and other non-aerosol products should be applied directly above each flushing tower at the lowest labeled rate from a distance which corresponds to the Directions for Use on the product label. If a range of application distances is to be included on the product label (e.g. apply from a distance of 8-12 inches), the greater distance should be used. The placebo control replicates should be handled in the same manner and should receive only water (for non-aerosols) or propellant minus active ingredients (for aerosols). See Section (d)(a)(iii) for information on application rate determination.

iv. **Data collection and endpoints.** Once the treatment is applied to a replicate, the exit hole should be monitored for 15 minutes. Each time an individual exits the flushing tower, the time should be noted in seconds from the start of the test. The test can be concluded after 15 minutes or when all pests have escaped through the exit hole, whichever occurs first. The following three endpoints should be recorded (Schreck, 1977; Ross 1992):

1. The number of pests which exit the flushing tower within 15 minutes.
2. The time to exit for all individuals in each replicate.
3. The time to exit for only those individuals that escaped from the flushing tower.

c. Data analysis and reporting results.

i. **The number of pests exiting the flushing tower.** Report the number of pests which escape through the exit hole during the 15-minute test period. Calculate the proportion of flushed individuals and its 95% confidence interval.

ii. **Time to exit for all individuals.** Report the time to exit (0-900 seconds) for each pest that escapes through the exit hole. Report the estimated median and its 95% confidence interval of calculate the average time to exit using Kaplan-Meier Estimator for all individuals, and calculate standard error. Individuals that do not exit the tower should be given a time of 900 seconds and coded as right censored. If all individuals exit before 900 seconds and the data

follow normal distribution, mixed-effects models can be used to estimate mean time to exit and its 95% confidence interval.

iii. **Time to exit for individuals that escaped flushing tower.** Report the estimated mean (if data follow normal distribution) or median (if data do not follow normal distribution) and its 95% confidence interval of time to exit for only those individuals that escaped from the flushing tower.

d. **Study conclusions.** Summarize study outcomes for flush testing against pests and discuss their implications for product labeling.

(t) Specific guidance for laboratory studies of fly bait products.

a. **Study objective**: To determine the performance of insecticidal baits intended for use against flies.

b. **Materials and methods**

i. **Experimental unit.** The test should be conducted with arthropods in mesh cages measuring 24 inches x 14 inches x 14 inches. Each cage should be provisioned with a water source and Petri dish containing cotton balls soaked with 10% sucrose solution (or an appropriate alternate food source), both of which should be replaced throughout the study as necessary. Cages should either be washed after each test or replaced so that confounding results from insecticide contamination are avoided. Flies may be lab-reared, and the source of the population used should be described. Water and 10% sucrose should be available as soon as the flies are introduced to the bait to allow ad libitum consumption of both (Hunter White et al. 2007).

ii. **Number of treatments and replication.** A replicate should consist of one cage. For each replicate, a minimum of 50 mixed-sex adults of the fly species being tested should be used. Depending on the results of the power vs. sample size analysis, a minimum of 5 replicates should be used for each treatment group, and the study method should be balanced to include a minimum of 5 untreated control replicates. Therefore, each study should include a minimum total of 10 cages and 500 flies.

iii. **Application method.** Flies should be placed into the cage one day before the bait is introduced, at which point the test will begin. If a pre-test starvation period is utilized, it should not exceed 4 hours, and flies should still have access to water throughout the starvation period (Hogsette et al. 2002). After the 1-day habituation period, the bait formulation being tested should be placed in a Petri dish at the lowest labeled rate and added to the cage. Efficacy testing of fly baits should be product-specific. Because the non-active ingredient components of a bait play an important role in palatability, use a test formulation that matches the subject product in terms of active ingredient and inert ingredient profile and concentration. Because bait station efficacy is a function of both the bait formulation and the design of the bait station itself, bait station products should be tested as they would be deployed by a consumer. This will demonstrate that flies can physically access the bait formulation within a bait station (Morgan et al. 1974). In these instances, a single bait station should replace the bait-containing Petri dish in the bottom of the cage.

1. **Bait placement.** The Petri dish containing the test bait formulation should be placed on the bottom of each cage. A separate Petri dish should contain the same amount of bait, but should be placed immediately outside the cage such that it is subjected to the same environmental conditions that exist within the test arena. This bait should be used to determine water loss from the bait during the test. The uncovered bait and protected bait should be weighed before and immediately after the test has concluded to determine the amount consumed by the flies during the test. Water and sucrose/alternate food should be replaced as necessary.

2. **Aging of bait.** If desired claims are to be tied to aging (e.g. kills flies for up to x months, or if label Directions For Use imply bait is palatable for x months), the bait used in efficacy tests should be aged for the corresponding amount of time before being presented to test flies in efficacy tests. Bait should be aged at a constant temperature (25 ± 1°C), relative humidity (50-80%), and photoperiod (ranging from 12 hours of light to 12 hours of darkness to 16 hours of light to 8 hours of darkness). Bait should be exposed to light during aging. If a fly bait is intended to be used outdoors, aging of bait should be done outdoors at or above the temperature range specified above, with exposure to direct sunlight. Bait should be exposed to rainfall if aged outdoors. Bait intended for outdoor use may be aged indoors if outdoor conditions prohibit outdoor aging (i.e. winter months in cold climates), but rainfall should be simulated.

3. **Choice testing vs. no-choice testing.** Product specific data to support efficacy claims should be generated with an alternate food source present to ensure a bait formulation is attractive and efficacious even if other food options are present (Hogsette et al. 2002). No-choice tests with only a bait formulation and no alternate food source can be conducted as part of an initial screening process. Data from these no-choice tests can be submitted as supplemental data. Mortality from fly bait studies using choice testing is the endpoint of concern and will confirm palatability.

iv. **Pest exposure to the treatments.** Flies should be exposed to the test formulation for up to 7 days. If a chemical is particularly slow-acting and the test is to extend beyond 7 days, justification should be provided.

v. **Data collection and endpoints.**

1. **Mortality.** Record the number of moribund, dead, and live flies in each treatment group 24 hours, 48 hours, 96 hours, and 7 days after exposure to the test formulation.

2. **Knockdown** (if a knockdown label claim is desired)**.** Record the number of knocked down/moribund flies in each group at the corresponding interval specified on the product label (e.g. a product label claiming knockdown within 30 minutes should be assessed 30 minutes after exposure). Dead individuals at each assessment interval can be considered to have already been knocked down and can be included in the total number of knocked down individuals. For knockdown evaluation, mortality should be confirmed no later than 96 hours after treatment.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g) of this guideline for guidance on data analysis and reporting results.

d. **Study conclusions.** Summarize study outcomes for control of flies and discuss implications for product labeling.

(u) Specific guidance for laboratory studies for testing ant bait products.

a. **Study objective:** The laboratory studies described in this section are designed to determine product performance of bait products against foraging ants.

b. **Materials and methods**

i. **Experimental units.** An experimental unit consists of an individual nest arena containing ant workers and connected to two foraging-arenas via plastic tubing (e.g., Tygon® tubing). The nest arena should contain a water source and harborage for the ants. One foraging arena should contain the test material (treated bait) and the other should contain an alternate food source. The length of plastic tubing connecting foraging arenas to the nest arena should be equal. Arenas should consist of an open-top box where interior vertical surfaces are coated with a non-stick material to prevent escape of ants. All arenas used in this study should have the same dimensions, and should be held under the same environmental conditions: 25°-30° C; photoperiod of 16:8 (L:D), and between 70 – 90% RH (Porter & Tschinkel 1987).

ii. **Number of replicates per treatment.** Depending on the results of a power vs sample size analysis, a minimum of five replicates per treatment, with a minimum of 100 workers per replicate arena, should be tested. Design should be balanced with an equal number of treated and control arenas. Therefore, for each treatment group there should a total of 500 ants, and for each control group there should be a total of 500 ants. A minimum of one 1000 ants should be utilized.

iii. **Application method.** Treatment should be product specific and applied at the lowest labeled rate and in the manner and duration as directed by the product label. For bait station evaluation, a single bait station should be deployed in the treated arena. This study design should consist of a choice test to demonstrate acceptability of the bait product and the alternate food source should be palatable, established in the literature, and consistent across trials. The alternate food source should be available *ad libitum* to the ants throughout duration of the study. Control arenas should receive the alternate food source only.

iv. **Ant exposure to product treatments.** Ants should be acclimated to the arenas for a minimum of 24 hours, during which time they are provided with the alternative food source and water. Uneaten food and dead individuals (not to exceed 10% of the original number) should be removed prior to treatment. Tests should be conducted for a maximum of 14 days post-treatment.

v. **Data collection and endpoints.** The total amount of treated bait used, expressed as weight of product per unit area, should be documented for each replicate. If re-baiting occurs, how re-baiting is conducted and thresholds for re-baiting should be recorded. The number of workers per replicate should be reported. Mortality counts should be conducted at intervals $\leq$ 48 hours through the duration of the study. Dead individuals should be removed and counted. Control groups should be assessed in the same manner as those receiving treatment. Following the final assessment, all nest arenas should be frozen to determine the number of surviving individuals and for calculation of the total number of workers.

c. **Data analysis and reporting.** Refer to Sections (d)(c) and (g)(e) of this guideline for guidance on data analysis and data/results reporting. Control mortality should not exceed 25%. In addition, the following information should be reported:

i. **Reduction in worker numbers.** Mortality counts of workers should be reported and generalized linear mixed effect models for Poisson distribution should be used to analyze the number of survival workers (number of survival workers = total workers – mortality counts of workers). Survival rate per treatment group and survival rate ratio between treatment and control groups should be calculated with 95% confidence limits per assessment.

d. **Study conclusions.** Summarize study outcomes for bait products against foraging ants and discuss their implications for product labeling.

(v) Specific guidance for field studies for direct treatment of the nest/hive/colony of flying, stinging Hymenoptera, except ants.

a. **Study objective:** To determine the product performance of direct treatment against all flying, stinging Hymenoptera life stages (except ants) in a nest/hive/colony.

b. **Materials and methods.**

i. **Experimental unit**. Treatment should be applied directly to the nest/hive/colony. Nests should be far enough apart to ensure independence of treatment applications.

ii. **Number of treatments and replication**. The test should include a product treatment at the lowest labeled rate and an untreated control. A replicate should consist of one nest/hive/colony per species tested. A minimum of six nests per species per product treatment (i.e. six replicates) and a minimum of three nests per species for an untreated control (i.e. three replicates) should be tested; nine nests total. Untreated control nests should be far enough from treated nests as to not be affected by nest treatments but close enough to experience the same abiotic conditions (e.g., temperature, weather, etc.). Negative controls should be assessed in the same manner as those receiving pesticide treatment.

iii. **Application method.** Unless other application methods are specified on the label for the pest species, the applicator should stand at the farthest labeled distance and deliver the minimum amount of pesticide. See Section (d)(a)(iii) for information on application rate determination.

iv. **Pest exposure to treatments.** The nests/pests should be exposed for the duration of the study.

v. **Data collection and endpoints.**

1. **Nest activity**. At least two pretreatment assessments should be made within seven days prior to application of treatments. Any assessment of activity at closed nests should be conducted during peak activity as determined by species, such as when ambient temperature is at least 15 °C (59 °F) and between dawn and dusk hours since the combination of light intensity and ambient temperature directly influences foraging activity (Spradbery 1973, Kasper et al. 2008). Assessment of numbers of individuals on a nest should be conducted during times of inactivity. Assessments

should be conducted at approximately the same time and in the same manner and duration at each time point. Assessments may consist of traffic rates at nest entrances if nest is closed or the number of adults on the nest if nest is open. Each assessment for traffic at a nest should consist of the number of individuals entering the nest during five one-minute periods and the number of individuals leaving the nest in five one-minute periods (Kasper et al. 2008). Nests should only be assessed, for the purpose of the study, once per day. Assessments should continue at a nest until zero activity at nest entrances or no activity/live adults on nests is recorded for two consecutive assessments. Data from negative controls serve to confirm colony health during the study.

2. **Nest dissection.** Nest retrieval should be conducted within 24 hours of the final assessment and immediately followed by in-field, nest dissection. A minimum of four out of six treated nests per species should be dissected.

c. Data analysis and reporting.

i. Nest information. Species identification per nest should be documented along with distances between nests/hives.

ii. Nest activity. Pre- and post- treatment mean traffic counts at the nest entrance for closed nests or the number of adults on the nest for open nests should be reported. All ten 1-minute observations per assessment should be averaged and the mean traffic rate for each assessment per nest should be reported (Kasper et al. 2008). For each assessment per nest, report the date, time, temperature, and weather.

iii. Nest dissection. Report the date of the retrieval and dissection along with observations of dead larvae and/or adults on or within the nest and any live individuals.

iv. Data analysis. The analysis should consider the effect of the treatment on product performance. The intent of direct treatment of a nest/hive/colony is to destroy completely the nest/hive/colony.

d. Study conclusions. Summarize study outcomes for the direct treatment of nests/hives/colonies of flying, stinging Hymenoptera (except ants) and discuss implications for product labeling.

(w) Specific guidance for field studies of bait products for flying, stinging Hymenoptera, except ants.

a. Study objective: To determine the performance of insecticidal baits intended for use against flying, stinging Hymenoptera (except ants).

b. Materials and methods

i. Experimental unit. Treatment should be applied to the area surrounding each nest/hive/colony. Study areas should be far enough apart to ensure independence of treatment applications.

ii. Number of treatments and replication. The test should include a product treatment at the lowest labeled rate and an untreated control. A replicate should consist of a study area containing a single nest/hive/colony per species tested. A minimum of six areas per species per product treatment and a minimum of three areas per species for an untreated control should be tested. Therefore, a total of 9 areas (9 nests) should be utilized. . Untreated

control areas should be far enough from treated areas as to not be affected by treatments but close enough to experience the same abiotic conditions. Nests in negative controls should be assessed in the same manner as those receiving pesticide treatment.

iii. **Application method.** Treatment application of baits should be product specific and at the lowest rate directed by the product label. Because the non-active ingredient components of a bait play an important role in palatability, a test formulation should match the subject product in terms of active ingredient and 'inert' ingredient profile and concentration. Also, because bait station efficacy is a function of both the bait formulation and the design of the bait station itself, bait station products should be tested as they would be deployed by a consumer. This will demonstrate that the pest species can physically access the bait formulation within a bait station, which is especially important for larger Vespinae species.

The lowest labeled number of bait stations per unit area should be used and placed at the greatest labeled distances. A separate bait station in each test area should have the entrance covered with mesh to prevent access to it. This mesh-blocked bait station should be used to determine water loss from the bait during the test.

iv. **Pest exposure to treatments.** Pests should be exposed for the duration of the study or as directed on the product label.

v. **Data collection and endpoints.**

1. **Nest activity**. At least two pretreatment assessments should be made within seven days prior to application of treatments. Any assessment of activity at closed nests should be conducted during peak activity as determined by species, such as when ambient temperature is at least 15 $^{\circ}$C (59 $^{\circ}$F) and between dawn and dusk hours since the combination of light intensity and ambient temperature directly influences foraging activity (Spradbery 1973, Kasper et al. 2008). Assessment of numbers of individuals on a nest should be conducted during times of inactivity.

   Assessments should be conducted at approximately the same time and in the same manner and duration at each time point. Assessments may consist of traffic rates at nest entrances if nest is closed or the number of adults on the nest if nest is open. Each assessment for nest traffic should consist of the number of individuals entering the nest during five one-minute periods, and the number of individuals leaving the nest in five one-minute periods (Kasper et al. 2008). Nests should only be assessed, for the purpose of the study, once per day. Assessments should continue at a nest until zero activity at nest entrances or no activity/live adults on nests is recorded for two consecutive assessments. Data from negative controls serve to confirm colony health during the study.

2. **Nest dissection**. Nest retrieval should be conducted within 24 hours of the final assessment and immediately followed by in-field, nest dissection. A minimum of four out of six nests per species on treated areas should be dissected.

c. **Data analysis and reporting results.**

i. **Nest information.** Species identification per nest should be documented along with distances between study areas.

ii. **Bait application.** The amount of product applied, expressed as weight of product per unit area, should be reported for each replicate. If rebaiting occurs, specify how rebaiting is conducted and thresholds for rebaiting. Report the number of bait stations per unit area and location of bait stations, including distance from the nest/hive/colony and other bait stations. Document bait removal by the test species as the difference in pre- and post-weights and accounting for water loss.

iii. **Nest activity.** Pre- and post- treatment mean traffic counts at the nest entrance for closed nests or the number of adults on the nest for open nests should be reported. All ten 1-minute observations per assessment should be averaged and the mean traffic rate for each assessment per nest should be reported (Kasper et al. 2008). For each assessment per nest, report the date, time, temperature, and weather.

iv. **Nest dissection.** Report the date of the retrieval and dissection along with observations of dead larvae and/or adults on or within the nest and any live individuals.

v. **Data analysis.** The analysis should consider the effect of the treatment on product performance. The intent of treatment of an area with bait product is to kill the nest/hive/colony. Report the mean amount of bait applied per unit area and the duration of baiting at which product performance was tested for each species.

d. **Study conclusions.** Summarize study outcomes for the direct treatment of nests/hives/colonies of flying, stinging Hymenoptera (except ants) and discuss implications for product labeling.

(x) Specific guidance for laboratory studies for resistance ratio determination and characterization of pest population strain susceptibility.

a. **Study objective:** To estimate the susceptibility and magnitude of resistance of pest strains to pesticides used in product testing. This study should be used when product performance is measured against resistant strains. When a resistance claim is proposed on a label, the level of resistance of the tested pest should be determined and included in the final study report.

b. **Materials and methods**

i. **Experimental units**. Each replicate should consist of a piece of white filter paper placed on the bottom of a 6 cm glass Petri dish with a screen secured over the top of the Petri dish.

ii. **Number of treatments and replication**. Five concentrations of the active ingredient should be prepared with the appropriate diluent. Depending on the results of the power vs. sample size analysis, the test should be replicated at least 10 times for each treatment. Each replicate should have a minimum of 10 adults of the arthropod species being tested. Therefore, for each treated group there should a total of 100 arthropods, and for each control group there should be a total of 100 arthropods. A minimum of 200 arthropods should be utilized

The negative control should be treated with the diluent for insecticide solution preparation. An appropriate positive control should be used. For the testing of pyrethroid insecticides, deltamethrin is recommended as a positive control. For testing neonicotinoid insecticides, imidacloprid is recommended as the positive control. For all other classes, a standard active ingredient may be proposed to the agency prior to testing.

iii. **Application method.** Active ingredient concentrations should be prepared based on a logarithmic scale, i.e., 0.0001%, 0.001%, 0.01%, 0.1%, and 1.0%. Other concentrations may be used based on previous knowledge of pest susceptibility to the insecticide being tested but a justification should be provided. If a product contains a synergist, use only the insecticide component with a solution concentration based on the active ingredient, not the synergist. An active ingredient concentration should be applied to filter paper in each replicate at a volume that saturates the paper, generally at least 200 µl (200 µl = 0.2 ml) for the 6 cm Petri dish. An equal number of negative control dishes should be prepared with paper treated with the diluent only. Paper should be allowed to dry before exposing the pests.

iv. **Pest exposure to treatments**. Pests should be exposed to the treated filter paper in each treatment for 24 hours.

v. **Data collection and endpoints.**

1. **Mortality.** Record mortality for each treatment at 24 hours post-exposure.

c. **Data analysis and reporting results**. Refer to (d)(c) of this guideline for guidance in data analysis. An analysis using generalized linear mixed models or non-linear mixed models is recommended to determine the LD values for each pest strain tested. Justifications for selecting a model for use should be provided. Report the resistance ratio values and their 95% confidence intervals for each strain for each insecticide tested and the associated data analysis. Resistance ratios should be calculated and reported as follows: LD (lab or field strain)/LD (susceptible strain) = RR. For example: LD_{50} (lab or field strain)/LD_{50} (susceptible strain) = RR_{50}; LD_{90} (lab or field strain)/LD_{90} (susceptible strain) = RR_{90}. A resistance ratio equal to or greater than 100 is characteristic of a resistant strain. The 95% confidence interval of estimated RR can be obtained by conducting a bootstrap simulation**.**

d. **Study conclusions.** Summarize study outcomes for control of tested pests and discuss implications for product labeling.

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