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

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes and Final Report for the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) Meeting Held May 8-9, 2018

TO: Richard Keigwin
Director
Office of Pesticide Programs

FROM: Marquea D. King, Ph.D.,
Designated Federal Official, FIFRA SAP Staff
Office of Science Coordination and Policy

THRU: Steven Knott, M.S.
Executive Secretary, FIFRA SAP Panel
Office of Science Coordination and Policy

Stanley Barone Jr., M.S., Ph.D.
Acting Director
Office of Science Coordination and Policy

Attached, please find the meeting minutes for the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on May 8-9, 2018. This report addresses a set of scientific issues being considered by the Environmental Protection Agency regarding methods for efficacy testing of pesticides used for premise treatments for invertebrate pests and treatment for fire ants.

Attachment

cc:

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OPP Docket

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FIFRA Scientific Advisory Panel
Meeting Minutes and Final Report
No. 2018-05

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Methods for Efficacy Testing of Pesticides Used for Premise Treatments for Invertebrate Pests and Treatments for Fire Ants

May 8-9, 2018
FIFRA Scientific Advisory Panel Meeting,
Held at the EPA Conference Center
One Potomac Yard,
Arlington, Virginia

NOTICE

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the U.S. Environmental Protection Agency (EPA or Agency) Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The SAP serves as a primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. FQPA Science Review Board members serve the FIFRA SAP on an *ad hoc* basis to assist in reviews conducted by the FIFRA SAP. The meeting minutes and final report are provided as part of the activities of the FIFRA SAP.

The FIFRA SAP carefully considered all information provided and presented by the Agency, as well as information presented by the public. The minutes represent the views and recommendations of the FIFRA SAP and do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

The meeting minutes and final report do not create nor confer legal rights nor impose legally binding requirements on the EPA or any other party. The meeting minutes and final report of the May 8-9, 2018 FIFRA SAP meeting represent the SAP's consideration and review of scientific issues associated with "Methods for Efficacy Testing of Pesticides Used for Premise Treatments for Invertebrate Pests and Treatments for Fire Ants." Steven Knott, M.S., FIFRA SAP Executive Secretary, reviewed the minutes and final report. James McManaman, Ph.D., FIFRA SAP Chair, and Marquea D. King, Ph.D., FIFRA SAP Designated Federal Officer, certified the minutes and final report which is publicly available on the SAP website (http://www.epa.gov/sap) under the heading of "Meetings" and in the public e-docket, Docket No. EPA-HQ-OPP-2017-0693, accessible through the docket portal: http://www.regulations.gov. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/sap. Interested persons are invited to contact Dr. Marquea D. King, SAP Designated Federal Officer, via e-mail at king.marquea@epa.gov.

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No. 2018-05

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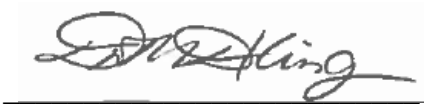
FIFRA Scientific Advisory Panel Meeting,
Held at the EPA Conference Center
One Potomac Yard,
Arlington, Virginia

James McManaman, Ph.D.
Chair
FIFRA SAP

Date: July 17, 2018

Marquea D. King, Ph.D.
Designated Federal Officer
FIFRA SAP Staff



Date: July 17, 2018

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
May 8-9, 2018

Methods for Efficacy Testing of Pesticides Used for Premise Treatments for Invertebrate Pests and Treatments for Fire Ants

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LIST OF ACRONYMS AND ABBREVIATIONS

Agency	United States Environmental Protection Agency
CDC	Center for Disease Control
CO_2	Carbon dioxide
EPA	United States Environmental Protection Agency
FFDCA	Federal Food, Drug and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
GLP	Good Laboratory Practices
IGR	Insect Growth Regulator
IMT	Individual Mound Treatment
IRAC	Insecticide Resistance Action Committee
OCSPP	Office of Chemical Safety and Pollution Prevention
OPPTS	Office of Pollution Prevention and Toxic Substances
OTC	Over The Counter
LC_{50}	Lethal Concentration (that kills 50% of a population)
LD_{50}	Lethal Dose (that kills 50% of a population)
LT_{50}	Lethal Time (required to kill 50% of a population)
RIFA	Red Imported Fire Ant
RH	Relative Humidity
TSCA	Toxic Substances Control Act
TOD	Time of Day
USDA-APHIS	U. S. Department of Agriculture – Animal and Plant Health Inspection Service
UV-A	Ultraviolet A (long-wave)
UV-B	Ultraviolet B (shortwave)

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP completed its review of the set of scientific issues being considered by the Environmental Protection Agency (EPA) regarding the Methods for Efficacy Testing of Pesticides Used for Premise Treatments for Invertebrate Pests and Treatments for Fire Ants. Advance notice of the meeting was published in the Federal Register on January 26, 2018. The review was conducted in an open Panel meeting held in Arlington, Virginia, on May 8-9, 2018. The draft guidelines, supplemental files, and related documents in support of the SAP meeting are posted in the public e-docket at http://regulations.gov (ID: EPA-HQ-OPP-2017-0693). Dr. James McManaman chaired the meeting. Dr. Marquea D. King served as the Designated Federal Officer.

In preparing these meeting minutes and final report, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. The meeting minutes and final report address the information provided and presented at the meeting, especially the Panel response to the Agency charge.

AGENCY PRESENTATIONS

Welcome and Opening Remarks – Daniel Rosenblatt, Deputy Director, Registration Division (RD), Office of Pesticide Programs (OPP), EPA

Product Performance Data Requirements and the Importance of Efficacy Testing Guidance – Daniel Rosenblatt, Deputy Director, RD, OPP, EPA

Background and Introduction to Proposed Methods for Efficacy Testing of Premises and Fire Ant Treatments – Jennifer Saunders, Ph.D., RD, OPP, EPA

Draft Product Performance Test Guidelines 810.3500 Premise Treatments – Jennifer Saunders, Ph.D., and Jacquelyn Herrick, M.S., RD, OPP, EPA

Draft Product Performance Test Guidelines 810.3100 Treatments for Red Imported Fire Ants – Dee Colby, Ph.D., and Matthew Aubuchon, Ph.D., RD, OPP, EPA

PUBLIC COMMENTERS

Oral statements were presented as follows:

Clark "Chuck" Klein, Ph.D., Global Development Manager, BASF, Urban Pest Control, Research Triangle Park, NC

Steven Bennett, Ph.D., Vice President of Scientific Affairs, Household & Commercial Products Association, Floor Care Division Staff Executive Pest Management Products Division Staff Co-Executive, Washington, DC

Written statements were provided as follows:

Jonathan Berger, Senior Project Leader, BASF

Jan Brill, Senior Regulatory Affairs Consultant, Bayer Crop Science

Steven Bennett, Ph.D., Vice-President, Scientific Affairs, Household & Commercial Products Association

Steve Ditto, US Regulatory Affairs Manager, MGK

Aaron Hobbs, President, Responsible Industry for a Sound Environment

Kristen van den Meiracker, Co-Owner, JAK Consulting Services

Janet Kintz-Early, Ph.D., Founder and Co-Owner, JAK Consulting Services

Siavash Taravati, Ph.D., Area Integrated Pest Management Advisor, University of California Cooperative Extension, Los Angeles County

EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (Agency) is updating a number of guidelines intended to assist in the development of appropriate protocols to test product efficacy. 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. These documents provide guidance for conducting appropriate tests, and are also used by EPA, the public, and the companies that are required to submit data under FIFRA. EPA Product Performance Test Guidelines OPPTS 810.3500 Premises Treatments and 810.3100 Soil Treatment for Imported Fire Ants were first published in March 1998. To increase clarity and consistency in efficacy testing and to include current scientific standards, the Agency is revising these product performance guidelines. The FIFRA SAP Panel was charged with providing recommendations to the Agency on these proposed draft guidelines covering the following topics of interest: premise treatments and red imported fire ant treatment guidelines. The Agency's draft guidelines as well as the Agency overview presentations at the May 8-9, 2018 SAP meeting discusses these topics.

Premise treatment guideline (OPPTS 810.3500)

The Agency document contains recommended test methodologies for a wide range of products intended to kill, control, flush, and/or knockdown invertebrate premises pests, such as cockroaches, ticks, mosquitoes, flies, and wasps. The guideline does not cover treatment of livestock or pets, wide area-mosquito control, or bed bug products. In addition to guidance for testing efficacy of direct pesticide application to pests, residual treatments, and cockroach and fly baits in the laboratory, the proposed guideline also includes field testing methods for outdoor misting systems, Hymenoptera nest treatments, and outdoor foggers. Finally, methods for resistance ratio determination and characterization of pest population strain susceptibility are described.

The Panel provided specific recommendations on premise pest controls related to kill, knockdown, residual control and flushing; protocol specifications for sample sizes and replicates. The Panel provided conclusions on the differences between laboratory and in field insecticide applications, details are further explained in the document text. Extensive comments were provided on the experimental design focusing on the details on area test size, dispersal of droplets, insecticide resistance, data collection, and label claims. The Panel provided insights on factors affecting the longevity of products in the environment and consensus was reached on the details of experimental designs of outdoor misting systems. There were differences of opinion regarding the necessity of field tests to prove efficacy in outdoor settings and concurrence with the statistical methodology presented by the Agency.

Fire Ants (Red) guideline (OPPTS 810.3100)

The proposed red imported fire ant treatment guideline contains recommended test methodologies for evaluating the performance of pesticide products for the treatment and control of red imported fire ant colonies/mounds. The updated guideline does not cover premises treatments for red imported fire ant workers/foragers, such as direct application to pests. Field tests for both mound- and area-applied pesticide products are proposed, along with accompanying laboratory studies for baits, barrier treatments, and insect growth regulators.

The Panel encouraged the Agency to better define terminology (i.e., mounds, brood, plot) and clarified the species (red, black, and hybrid) of ant which this guideline should apply to. The Panel highlighted aspects of the guideline which were found to be insufficient, such as amount of bait, use of controls, test

duration, standards regarding number of mounds needed for a successful test. Consensus was reached by the Panel when commenting on the replicates needed in a laboratory test, the lack of biological differences among red, black, and hybrid fire ants thereby making it unnecessary to perform species determination prior to initiating a field trial.

Overall, the Panel provided a very in-depth review of both draft guidelines and was able to offer the Agency cogent and implementable actionable recommendations to move towards the finalization of each document. The responses provided by the Panel addressed a number of the strengths and limitations of the Agency's written draft guidelines. The Agency appreciates the public comments and Panel recommendations which will further enable scientifically credible assessment of impacts of pesticide treatment and efficacy on human health and the environment. The Panel recommendations will also lead to the publishing of final guidelines by early 2019.

DETAILED PANEL DISCUSSION AND RECOMMENDATIONS

TOPIC: Premise treatments

The U.S. Environmental Protection Agency's (Agency) OCSPP 810.3500 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.

Question 1: The draft guidelines describe test methods for evaluating the efficacy of a variety of pesticides to treat premises. Please discuss:

a. Whether, given the objectives and the types of products being evaluated, the test methods are appropriate to evaluate the efficacy of premises products and to support pesticide labeling claims related to kill, knockdown, residual control, and/or flushing.

b. Whether there are additional or alternative test methods beyond those discussed in the draft guidelines for testing the efficacy of premises pesticide products.

Response 1: The Panel concluded that the draft guidelines were a significant improvement to the 1998 guidelines and proposed the following changes or alternatives to further clarify the objectives for products used in premise pest control treatments:

a. The guidelines have clear objectives for products used in premises pest control. The Panel recommended that the test methods, even though some details are to be modified for consistency and practicality, should be appropriately described to support pesticide labeling claims for kill, knockdown, residual control and flushing. The Panel recommended that guidelines should reference EPA's official list of pests of significant public health importance (PRN 2002-1) so registrants and other readers are clear about the scope of the guidelines. The Panel pointed out that products used as spatial repellents were not mentioned in the guidelines.

b. Perhaps the most critical key to these protocol guidelines is the specification for minimal sample sizes and numbers of replicates. For some pests this will be more difficult to achieve than others. For example, a test on scorpions may not be practical if 35 individuals per treatment, for example, are required for replication (see Sample size for premises studies Supporting Document). A test where availability of scorpions only allows for 20 replicates may still provide useful information for registration, especially if the results are statistically significant. Also, significance through use of non-parametric tests may provide adequate evidence to justify registration. The Panel suggested that results from multiple tests with lower sample sizes may provide enough data for meta-analysis to justify registration.

The Panel also recommended the definition of moribund be reconsidered. There should be a reasonable time defined where if an adult test subject has not recovered, it should be determined that it will not

recover. This may be different with larvae where the determination may not be classified as "failed to recover" after a set amount of time, but rather "failed molt" to the next instar.

The Panel recommended that use of the same controls when testing multiple treatments/products on the same day should be allowed rather than requiring an equal number of controls and treatment groups for every product. It is also suggested "replication" be defined to avoid potential pseudo-replication. For example, passing a spray wand over 5 cups containing insects taken from the same cage does not represent 5 true replicates.

The Panel made several recommendations regarding conditions during the test. Having food and water present during the treatment period is not necessary for most pests and could confound results because of ingestion of contaminated food. When testing foggers, total release aerosols, vapor strips and flushing agents, especially with pests that harbor in cracks and crevices, care should be taken to create harborages similar to those used by the pest, e.g. rolled or stacked cardboard harborages for roaches (T. J. Bohnert, B. L. Reid, and G. W. Bennett 1988). For baits, translating label rates of application to small cages is impractical because of the minuscule amounts of bait involved. Because bait depletion by consumption is not a limiting factor for efficacy in the field, baits should be provided *ad libitum* for testing. Also for baits, the committee determined there is little point in measuring the amount consumed by the test insects because the amount consumed may be trivial in small-scale testing. It is also difficult to measure consumption accurately because of water absorption. For flies, deposition of crop contents onto the product during feeding make measuring bait difficult as well. The Panel recommended removing this requirement.

Regarding sections a-h on general test conditions, the Panel recommended rewriting part of these sections to distinguish between field and lab studies. Specific edits are described below:

Page 8; Regarding test conditions the Panel concluded that the requirement for laboratory temperatures at 25 ± 1 °C is too stringent and doesn't represent how the products are applied in the field. The guidelines should provide flexibility in laboratory conditions. A wider range of temperatures or ambient conditions should be permitted that would fall within the range of typical laboratory conditions. The "no later than 96- hour" statement conflicted with guideline language elsewhere in this document for bait products, where mortality was followed for 14 days or longer in some cases.

Page 9; Regarding the QA and QC plan, for a study-based QA plan, all protocols should be provided to the Agency for quality assurance inspections.

Page 10; g, c, Regarding the testing conditions, current wording says, "Information on temperature, relative humidity, ambient light and photoperiod, and air flow (where applicable) should be reported". The statement says should be reported, suggesting that all conditions are required. The "(where applicable)" seems to refer to air flow only. Many of the environmental conditions cannot be recorded for field conditions. This should be rewritten for clarity with the "where applicable" at the end of the statement, indicating that this wording applies to all of the conditions. This wording would be clearer.

Page 13; Regarding the example of linoleum as a non-porous surface, the Panel noted that linoleum is not a truly non-porous substrate but semi-porous. Glazed ceramic tile, glass or stainless steel are preferred substrates for non-porous substrate testing.

Page 14; Regarding residual tests on plants, the Panel agreed that removing leaves from live plants after treatment would be preferable to removing leaves at the time of treatment and storing. Product

breakdown and metabolism on living plant tissues may be quite different from dead leaves that have been harvested and stored. Also, plant species differ in properties such as waxiness, hairiness, and metabolism, making it difficult to identify a single plant that could be used as a typical species. The Panel suggested identifying two to three species (i.e., boxwood and azalea) to be used that vary in these properties.

Page 20; Regarding application method for fumigant products, the Panel recommends test temperatures should be provided with a range, for example, $\pm$ 3^{o}F.

Page 21; Regarding testing products that contain insect growth regulators (IGRs), the Panel suggested clarifying why the IGR must be tested separately for IGR-toxicant combination products. For example, if the hypothesis is that the two components act in a synergistic manner, this can be confirmed by testing each component individually first (Mosqueira et al., 2010, Darriet et al., 2005).

Roaches:

Page 25; Regarding the experimental arena for cockroach bait testing, the Panel noted that test arena sizes ca. 2 sq. (centiare 2 square) meter are too large to be practical for only 10 adults required per replicate. The Ebeling choice boxes (Agency recommended) may not be most practical since they are only about 1 square foot in size. They can cost up to $75 each to construct yet are reusable. Even if most companies and testing laboratories do not have choice boxes, those data from choice boxes should still be acceptable to the agency. The Panel recommended that the testing arena should be flexible and simple enough to allow using disposable materials, or the tests are in a stainless steel and glass arena, so the surfaces could be cleaned between uses. The Panel also note that the guidelines require that alternate food resources be present when testing baits. It would be advisable to identify species-appropriate food resources to avoid bias due to the relative attractiveness and palatability of different "alternative" foods.

Page 27; Regarding test arenas for flushing testing, the Panel recommended the size of arena/harborage should be flexible and simpler (Reid, 1988).

Flies:

Page 29; Regarding fly bait products, the guidelines specify continuous exposure for 7 days. In practice, these products are used to deliver a single acute toxic dose, and potentcy is often compromised by environmental conditions within a day or so of application. The Panel recommended a shorter exposure period, something in the 4 to 8-hour range, and those tests to be conducted with hungry flies, because house flies in the field are generally hungry. The guidelines specify a 4-hour starvation period prior to test initiation. While four hours might suffice for very young flies that have not fed much before testing, older females (3-5 days old) with partially developed eggs (>48 hrs old) require a longer starvation period to achieve avidity. The Panel recommended increasing the starvation period to 12 hours with water provided (no shorter than 8 hours).

The guidelines specify fly cages that the Panel regarded as too small, and therefore, recommended bait testing for flies should be conducted in larger arenas. The Panel suggested cages that are three or four feet per side, to allow more normal expression of fly behavior and food approach and to reduce incidental (non-feeding) contact with baits due to random movement. Since lab tests are not necessarily predictive of performance in the field, field tests are advisable to provide a more realistic assessment of performance. Field test account for resistance (for known toxicants and levels of resistance), competing natural food resources, environmental conditions, and the complexity of house fly behavior. Semi-field tests in large windowless rooms could also appropriately simulate field conditions, often with greater control over variables. For fast-acting toxicants this can be done with the "pizza box" test where products are placed in pizza boxes and the number of dead flies in the box is counted one to four hours after

placement. Slower-acting toxicants pose a greater challenge because the flies may or may not die near the bait and it is impractical to measure a reduction of local groups of adults in a selected area. A possible approach for slower acting toxicants would be to sweep-net flies from a field site and confine them in a cage (see above for cage size recommendations) with the bait and other local food resources from the local environment, albeit this would be interpreted as a cage test. The cages should be left at the field site and dead flies counted at an interval appropriate for the toxicant. At the end of the test period, the dead flies should be counted along with any killed surviving flies to determine percent mortality in the caged population.

Ants:
Page 31; Regarding arena size for ant bait product testing, the Panel noted the size of arena is not specified. Arena size and amount of bait applied in the given area should be considered. Depending on the species, especially with ants housed in nest cells, maintaining a prescribed relative humidity (RH) for the test area may not be important, because the RH may be maintained within the nest cell. The Panel also recommends removing the maximum acceptable pre-test mortality restriction. It is given as 10% observed mortality prior to the introduction of treatments. The Panel agrees this is not needed as long as number of dead insects are recorded before the treatments and subtracted from total of insects tested.

Page 35; Regarding resistance ratio determination, a resistance ratio of 100X to document control of resistant pests is stringent and might require seeking out highly inbred strains obtained from laboratory selection experiments. With flies, product failure is common at 30X resistance ratios. If a product represents new chemistry, the Panel suggests that demonstrating control of pests with 30X resistance levels should be sufficient.

Question 2: In Section (d)(a)(iii), a metered bench top sprayer is given as an example of a spray device that can be used to ensure consistent application volume and even distribution of spray particles. It is also stated that when utilizing such application devices, one should ensure the deposition of the product mimics the proposed product's intended method of application (e.g., formulation type should not change between an aerosol and a liquid). Please discuss:

a. Whether a metered bench top sprayer could provide an application that would be similar to a typical liquid spray in the field. Would the bench top sprayer also provide an application that would be similar to a typical aerosol spray? Please discuss the potential of bridging efficacy data between aerosol and liquid sprays.

Response 2: The Panel concluded that a metered benchtop sprayer would not generally be similar to typical aerosol or liquid insecticide applications in the field.

a. A metered bench top sprayer is a good tool to provide even and consistent distribution of spray deposits on substrates for residual efficacy studies because volume, rate, droplet size, etc. can be specified and reproduced. Use of a compressed air sprayer (pump sprayer) with appropriate tank pressure and nozzle; direct aerosol spray (known time, application volume, and actuator characteristics); and deposition from a total release aerosol would all be appropriate application methods depending on the type and claims of the product. Formulations for liquid sprays and aerosols are different. Aerosol sprays usually contain propellant and other inert materials, which may provide a much faster knockdown and kill, especially in a direct spray study. Efficacy data between aerosol and liquid spray should not be bridged and need to be tested separately using methods appropriate to the formulations and claims.

Question 3: Sections (i), (j), (k), (l), (o), (p), and (q) indicate that pests should be moved to untreated containers as soon as practical but no longer 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. Please discuss: a. Whether these time constraints are reasonable for most public health premises pests to predict efficacy under actual use, and why or why not. If not, what standards are recommended? Should there be differences for specific pests or residual surface types? If so, please recommend time constraints for specific pests or residual surface types.

Response 3: The time constraints proposed, particularly the 1 and 4-hour period to remove the insects from a treated surface, depends on the species of insect being tested and the actual claims of the product. Several Panel members agreed that a 1 to 4-hour period between direct treatment and removal to an untreated container is not reasonable and does not mimic actual direct spray use. In that case, insects should be removed as soon as practically possible from the treated area or surface. In the field, insects not immediately knocked down or killed would crawl or fly away from the treated area. If insects are to be treated directly with a formulation, they should be removed and placed into an untreated container as soon as possible. Barber *et al* (2007) recommends one to five minutes, or up to 15 minutes, between direct treatment and removal of insects. If the product claims include treatment into a harborage or enclosed exposure such as total release aerosol, or use of insecticidal strips in a closed room, then the exposure time should be adjusted to reflect the claims of the product.

Continuous exposure is a good comparative test method, but not realistic unless trying to make claims about killing resistant insects. Limited exposure tests, i.e., timed exposures to treated surfaces (both porous and non-porous), would be more realistic. Depending on the species of target insect, the species behavior (crawling or flying), and product claims, exposure times should be adjusted. For example, the recommended 1 to 5-minute exposure to a treated surface may then be followed by the removal of exposed insects to a clean container. Insect mortality should be recorded at several intervals (every 10-15 minutes for the first hour and hourly thereafter). Depending on the species tested, water, food, and an appropriate harborage should be supplied within the clean container.

Efficacy:

Question 4: Sections (j) and (k) describe studies to test the residual efficacy of premises pesticide products and include specific substrates for testing outdoor versus indoor products; sections (k), (r) and (t) propose that indoor aging of treated surfaces or baits simulating outdoor conditions may be used in lieu of actual outdoor aging. Please discuss:

a. Whether there is a single surface type that could be used as a standard, representative surface for testing product residual activity in lieu of testing multiple surfaces as recommended in the draft guidelines. If so, please recommend a single surface type and discuss why it is representative of other surfaces.

b. Whether the methodology in section (k) for evaluating pesticide residues on leaves in a Petri dish is appropriate. Is a specific species of plant necessary or recommended, and if so, why?

c. Appropriate methods to simulate outdoor aging in an indoor testing environment.

Response 4:

a. The Panel unanimously concluded that there is no single surface type that would represent

both porous and non-porous substrates. However, the Agency should consider three standards, unpainted wood for porous, glazed ceramic tile or stainless steel for non-porous, and foliage for organic substrates. Substrates must be relevant to the proposed application and target pest. If a company wants to make claims for specific types of substrates, such as concrete, then it would be reasonable to require evaluations with concrete.

b. Placing treated leaves in a Petri dish is one of many reasonable approaches to examining the efficacy of residuals on leaves. Cut leaves may not have the same effect as using intact or dry leaves and would introduce physiological variables of the plant to the insecticide bioassay. Probably no single plant could be used as a typical species. The Panel suggested identifying two to three species for use that account for natural variation in waxiness, hairiness, and metabolism. These should be species that are easily propagated and common in a wide range of locations; justification could be made based on target pest. For residual tests on plants, removing leaves from live plants after treatment is be preferable to removing leaves at the time of treatment and storing them. The Panel recommended treating surfaces of leaves, allowing the residual to dry, and then confining insects to the treated surface. There are several designs of "clip cages" that could be used for this purpose (Haas, 2018).

c. Many factors could affect the longevity of a product in an outdoor environment. Simulated outdoor aging in an indoor testing environment could provide insight on the product performance. However, simulations will not truly represent an outdoor environment. Simulation of outdoor aging of an insecticide residual would need to mimic or account for diel cycles of photoperiod, temperature, and RH, as well as, periodic washing to simulate rain. The light source would need to include both UV A and B. There are accelerated aging protocols for paint and other outdoor surfaces that might be appropriate. Testing laboratories may not have or be unable to acquire appropriate equipment for simulated outdoor aging, therefore several aging trials should be conducted in different geographic regions depending on product claims.

Question 5: Section (q) describes field methods for assessing efficacy of outdoor misting systems. The methods currently proposed focus on determining efficacy of direct contact with the spray only. Please discuss:

a. Whether the experimental design can be used to adequately evaluate outdoor misting systems: Given the nature of how these products are used in the field, should population reduction over time or residual efficacy in the treated area also be considered? If so, please recommend appropriate test methods.

Response 5: The Panel members agreed that the basic experimental design can evaluate misting systems with the following changes to the protocols:

a. One Panel member noted that the performance guidelines should use either metric or imperial measurements in the protocols, not both. Another Panel member expressed concerns that this performance protocol did not encompass the 25(b) pesticide products (i.e., those products exempt from FIFRA Section 3 registration requirements where claims of toxicity and/or population reduction are not required). While recognizing the dichotomy of requirements between the groups of 25(b) and Section 3 products it should be noted here that the charge to the Panel focused on reviewing the performance standards for premise treatment testing, not to provide a review of the regulations and requirements governing product registration under FIFRA. Therefore, to clarify this point, the Panel recommended to the Agency to add a section emphasizing that these are guidelines, rather than absolute requirements for data packages, and any need or intent to vary from the outlined experimental designs must be justified in those data submissions. Another suggestion made by the Panel to prospective registrants, is to provide

examples of variances (e.g., differences in sample/replicate sizes and the number(s) of both product and control replicates) to assist the registrants in preparing their submissions. General consensus was reached that misting systems target mosquitoes, however, other pests may be considered, such as house flies and other small biting flies.

The Panel discussion focused on (framed in terms of mosquitoes) the following details of the experimental design:

Design standards for the adult holding containers: In general, the cages holding test insects and the potential for the mesh screen to reduce the passage of spray particles were discussed. The Panel suggested standardizing the adult holding containers, such as those described in Barber et al. (2007) which used bronze containers 15 cm in diameter and 2.5 cm deep with a mesh screen of 14 X 18 cm on both vertical circular surfaces.

The height of the misting system nozzles in the test area: There is a need to specify a height of nozzles and test cages, since these devices may be installed at different heights depending upon the surface to which they are attached (e.g., a roof overhang, fence or in-ground in a yard). The Panel considered that five feet has been a commonly-used standard height (Cilek, 2008) although some systems have been observed attached at heights greater than 5 feet. The increased height could impact the amount of particle drift. The Panel recommended that tests be conducted in a manner that would make them more consistent with other mosquito adulticiding evaluations (to facilitate relative comparisons), and should include wording such as "the targeted specimens should be exposed to the misting system in a manner that is consistent with its expected operational installation." The "operational installation" description should also include some reference to the expected results.

Positioning of adult containers: The Panel agreed that the adult containers should be positioned 90^{o} downwind of the nozzle array to account for the drift of the product. This arrangement would mimic the operational installation expected on a residential property. Panel members recommended the following designs to consider: a) a nozzle array twice as long as the line of cages, and b) a grid design (Barber *et al*, 2007) which could account for vortices of air flow (potentially created by the test area or actual landscape) that might move insecticide particles in unpredictable waves potentially causing some cages to escape treatment.

Measuring droplet size/dispersal: The Panel agreed that spray impingers should be included in the design to determine the dispersal and size of insecticide particles from the emitter nozzles. In the opening presentation by the Agency, there was a diagram of the misting system experimental design showing the placement of the impinger at the end of the row of adult cages. One Panel member recommended that the impinger should be placed in the middle of the row of adult cages because it would better represent the size and number of droplets to which the pest was exposed. Untreated control replicates should be placed upwind far enough to prevent any possibility of exposure.

Environmental data: The Panel agreed that experimental data should include environmental factors such as temperature, RH, wind speed and direction, and time of day (TOD), since each of these factors can affect particle drift. TOD is especially important as it relates to the behavior of particular mosquito species of public health concern. The Panel did not provide specific ranges for temperature, RH, and TOD, but instead recommended that trials be conducted to a standard "under conditions representative of times when the mosquito species of concern would normally be active and also consistent with the registrant's desired application". Regarding wind-related factors, one Panel member pointed out with typical adulticide efficacy tests for mosquitoes, applications are made when wind speeds are 2-10 mph.

The Panel recommended this same standard apply to misting systems unless the registrant has clearly defined the need for an exception.

Insecticide resistance: One Panel member expressed concern about insecticide resistance in the populations of test insects (across all species tested) and differentiating whether these are laboratory-reared or field collected. It is important to have this information prior to conducting tests, since it could directly impact efficacy results, particularly if there are claims by the registrant of pest population reduction.

Data collection: The Panel recommended for the purpose of determining direct exposure toxicity (kill effect) with misting systems, the test insects should be transferred to clean containers within 15 minutes rather than the proposed standard of 1 hour for crawling insects and <4 hours for flying insects. If test insects are immobilized using CO_2, untreated control replicates should be handled similarly. The Panel members had differing opinions (no consensus reached) on the number of insects per replicates and the number of replicates. The Panel majority recommended following the proposed standard of 10 insects per cage, while another Panel member recommended 15 insects/replicate as suitable. The current design calls for five replicates. One Panel member suggested that this was acceptable pending the discussion of Charge Question 11. Another Panel member asked the Agency to consider allowing the registrant to test three different species simultaneously, but acknowledged that test area size may be a complicating factor in this approach. Ultimately, based on subsequent information provided by an Agency statistician during the meeting, the Panel concurred with the use of the unbalanced design. They also recommended that registrants be permitted to test multiple species simultaneously against the same product, if the size of the test area can accommodate the number of test replicates without compromising the validity of the test then this strategy should be employed while it potentially helps to reduce the number of insects needed per test.
Experimental design applicability for registrant claims of "pest population reduction": It was presented by the Agency that most product claims have been directed to product toxicity (i.e., "kills mosquitoes"); however, both data submissions and claims by product vendors at professional meetings are that the product/system "reduces the population". Based on these currently labeled product claims, the consensus of the Panel was that the primary intent of misting systems has been knockdown of pest populations in the immediate vicinity of the system (e.g., a residential property). Furthermore, the Panel agreed that the proposed experimental design is not adequate if a registrant's intent is to claim population reduction. The Panel recommended that, in order for the registrant to claim population reduction, the experimental design must include: a) assessment of what is considered "residual activity" of the product (this relates to charge question #4 concerning surface residual activity), and b) 'pre' and 'post' assessments of mosquito populations from the surrounding area of the test site that use currently accepted standards, preferably those that do not rely on traditional landing counts in light of the potential risk for mosquito-borne disease transmission. Allow for the registrant to describe how they are going to perform the test.

Regarding the potential for the use of misting systems for arthropods other than mosquitoes, the current experimental design should suffice. Albeit, larger cages or cages of different design may be required and requirements such as the height of cages would largely depend on the type of arthropod and its mobility (e.g., crawling versus flying). The Agency is advised to take into account that larger cages may limit droplet contact with the target. Mosquito control districts with extensive experience in testing, such as New Jersey Mosquito Control, should be consulted about designs for misting experiments.

Question 6: Section (s) describes methods for evaluating efficacy of flushing products. Please discuss:

a. Whether the experimental design can be used to adequately evaluate flushing products. Please

consider the concept of using placebo versus water-only controls and determine which allows for better determination of flushing action in the treated groups.

Response 6: There was consensus among Panel members on several issues with the experimental design, particularly on two points:

a. Panel members agreed that the tower design was suitable. However, the cardboard tube does not adequately mimic a "typical" harborage for insects such as cockroaches. One Panel member noted that the measure of effectiveness of this design was the number of insects escaping the harborage, and time of escape for those insects using the hole, over a 15-minute testing period. As cardboard is not transparent, it would be difficult to observe insects prior to and during treatment. This makes it difficult to determine if injury and/or death of test specimens might have occurred before insects had time to be flushed out. Some of the flushing agents currently used have some toxic effect on test insects, and so it is important to distinguish between mortality and poor flushing quality. Further, cardboard is an absorbent material, the Panel provided clarification on how differences in cardboard used for preparation of the tubes should be assessed and reported, while demonstrating the impact on product efficacy, with the following recommendations:

Test harborages should have a maximum diameter of 5 mm to simulate typical situations that we see with roaches (Appel, 1996). Size can be adjusted appropriately and as needed for other test species of concern.

The reference cited by the Agency (Hostetler, 2014) may not be the most appropriate choice for this document because it is an unpublished study report from a private testing company. While the technique may be good for the study reported, it is not a common or easily accessible reference. If this technique is going to be the method of choice, then there should be more accessible examples of the test in use. One example offered was the design used by Elbert and Behrenz (1986) which consisted of plastic boxes and two aluminum plates covered with a two-component polyurethane paint. This arena allows adjustment of both depth and height of a harborage and might make a more suitable standard arena. Reid and Bennett (1988) used a similar method based on Masonite Panels, instead of aluminum plates, which are easily cleaned.

The Panel recommends allowing the test insects to acclimate in the experimental tower for two or more days prior to the test. Although this extends the time to complete a test, it is particularly important for cockroaches in order to allow them to acclimate, deposit fecal smears and odors.

b. Panel members agreed that water was not the best choice for a control. Since most flushing agents are aerosols, the Panel suggested two possible placebo treatments: a) using only the inert ingredients (including propellant) for the test product, and b) a non-toxic aerosol product such as "keyboard cleaner" which is often recommended in place of a pesticidal flushing agent for use in sensitive environments such as schools, nursing homes, etc. Another Panel member questioned "aerosol flushing agents". The Agency clarified that there can be non-aerosol flushing agents. The Panel agreed that with non-aerosol products, a water-based placebo could be appropriate. One Panel member mentioned that some flushing agents are dry formulations. The Panel recommended the following possible placebo treatments:

1. Silica gel (positive control)

2. Boric acid (negative control strictly for its flushing effect, not ingested toxicity)

3. The test product's inert ingredients

Regarding the earlier point of flushing agents that also produce mortality, one Panel member raised concerns about how such data would be analyzed under the current design regarding whether mortality should be considered given that the intent is to evaluate flushing characteristics and efficacy. The Panel recommended that post-treatment mortality data be collected and presented along with flushing efficacy data in response to said Panel member's concern.

Question 7: Currently only laboratory studies are proposed for assessing cockroach, fly, and ant baits (sections (r), (t), and (u)) and outdoor residual foggers (subset of section (k)). Field studies are proposed for assessing direct contact outdoor foggers (section (p)). Please discuss:

a. Whether it is necessary to also assess cockroach, fly, and ant baits and outdoor residual foggers in field studies in addition to the proposed lab studies, and why or why not. If so, please recommend appropriate test methods.

b. Whether a field study as proposed is necessary to assess efficacy of direct contact outdoor fogger products, and why or why not. If not, please recommend appropriate laboratory test methods for outdoor foggers.

Response 7: The Panel offered differing opinions on the need for field tests to prove efficacy of insect baits applied outdoors:

a. Panel members noted that outdoor tests are more complicated and difficult to execute, making it difficult to comply with GLP standards. Therefore, indoor baiting tests are suitable for product registration purposes to prove that the baits are effective in killing the test species. Panel members in favor of outdoor tests noted that outdoor environments (as compared to indoor settings) can offer different challenges in terms of complexity and competing/attractive food resources particularly for cockroaches, ants, and flies. On that basis, the Panel recommended requiring outdoor efficacy testing before approving a product for site use. One Panel member offered the example of designing laboratory experiments for cockroach bait testing. Typically, in these tests, small arenas can significantly affect their outcome where the absence of a harborage can significantly reduce cockroach feeding activity and results in a much larger LT_{50} value and reduced kill. Another Panel member expressed the opinion that with filth fly management, the goal of using baits is not to control but rather reduce the population as a component of an overall integrated management approach. Another Panel member noted that with flies (primarily house flies), the toxic action of the chemical can affect how data are collected. Acutely toxic chemicals are easier to evaluate because of the immediacy of the killing action, which makes determination of fly mortality simple. In comparison, the newer chemistries that exhibit delayed toxicity allow flies to feed briefly and then move away before dying. This makes bait efficacy more difficult to assess. The Panel recommended the following guidance (Hogsette, 2018, in press) for testing fly baits:

Studies should first be done in the laboratory to see if flies will consume the bait and die in a reasonable period of time. Studies should then be repeated in large windowless (preferably) rooms to determine whether flies can and will locate the bait, then feed on it and die. This more closely simulates what may be seen in the field. The following are specific Panel recommendations:

Laboratory studies in cages
Purpose: To determine if flies will eat the bait and die in sufficient numbers (see **Semi-field studies in**

large windowless rooms for fly interaction with the bait in the field)

Cage size: 18 inches (46 cm) on a side. These are large enough and are available commercially (e.g., Bioquip).

Flies: Flies should be from healthy colonized stock and should be 3 to 5 days old. Older flies react differently to attractants and should not be used. For house flies, only female insects should be used. Tests with other fly species can employ both females and males. The standard number of flies should be 100 per cage, counted and sexed (house flies) while anaesthetized either with CO_2 or by chilling. Regardless of the method used, flies should be allowed to recover for at least 30 minutes prior to initiating a test. Flies anaesthetized for too long a period of time will result in increased control mortality.

Baits: Baits provided to flies should not be restricted to label rates. This method depends on ample amounts of bait to kill the flies. Even if every fly fed to repletion, there should be ample bait left over in the treatment cages. For application purposes, volumetric measurement of the bait is preferred although the registrant can choose to weigh out the bait material. Containers for presenting bait to test insects can be the choice of the registrant with a suggestion of using aluminum weigh boats (3-inch diameter); other shallow containers should test 100 flies per 15 to 20 ml of bait.

Fly food: Control and treatment cages of flies should receive liquid food in the form of 10% sucrose solution, which can be applied to several saturated cotton balls placed in the same containers as used for presenting the bait to the test insects.

Replication: Each cage of flies is a replication and 6 replications should provide suitable data.

Methods: Arrange cages in a testing area maintained at 28°C and ambient RH, with continuous lighting, and cages 15 cm apart to allow for air flow. Place flies directly in cages to allow recovery from anesthesia. When the fly recovery period has passed, place weigh boats with sucrose solution in all cages followed by placement of bait application containers in treatment cages. Make mortality counts at 15, 30, 45 and 60 minutes after bait was introduced, then at 24, 48, 72, and 96 hours post treatment. This is an acceptable schedule particularly for some of the new toxicants, which have rather slow rates of kill. If the bait being evaluated is known to have a fast rate of kill, additional mortality count intervals could be added along with a justification statement by the registrant. When mortality counts are made, remove dead flies from the cage to avoid confusion with later counts. This test can be conducted during a single 96-hour period if enough cages and flies are available. Otherwise the test can be replicated over time. Criterion for assessment of death is complete cessation of movement.

Semi-field studies in large windowless rooms

Purpose: To determine if the flies will locate and feed on the bait and subsequently die in sufficient numbers. This test more closely simulates how flies will interact with the bait in the field.

Room size: Rooms should be approximately 165 ft^2 (e.g., 16.5 x 10 ft) maintained at 28°C, ambient RH and continuous lighting. If rooms have windows, windows should be covered so they are completely dark. Rooms should be completely empty so baits can be placed on the floor as they might be when in the field.

Flies: Follow exact procedures found above in **Laboratory studies in cages**, with the exception that a replicate should consist of 200 flies per room.

Baits: Baits should be provided to flies at label rates based on the area of the floor in the room. Aluminum weigh boats (3-inch diameter) or other similar shallow containers as noted in the laboratory evaluation can be used to introduce the bait. Based on the amount of bait to be used for the room size, divide the bait into four equal amounts and place each part in a weigh boat. Place weigh boats with bait on the floor of the room and center them along each of the four walls.

Fly food: Follow exact procedures found above in **Laboratory studies in cages**, with the exception that a minimum of two containers with fly food should be placed on the floor, more-or-less evenly spaced, in the center of the room along the long axis. Additional sucrose solution can be added during the test as needed.

Replication: Each room of flies is a replication and 6 replications should be required per bait.

Methods: Follow exact procedures found above in **Laboratory studies in cages**, with the exception that there should be no cage arrangement nor continuous lighting and 15cm separation for air flow. When the fly recovery period has passed, place two weigh boats with 10% sucrose solution down the center of the floor in all rooms followed by placement on the floor of four weigh boats with bait centered along the walls in the treatment room. If only a single room is available, complete all untreated replications first, followed by the treated replications.

One Panel member noted that although the proposed design requires evaluation of the effects of bait exposure to outdoor conditions (sunlight and rainfall), most modern baits are packaged in (or added to) bait stations which exposes the baits only indirectly to these environmental factors. The Agency responded that the working assumption is unless the proposed product label specifies keeping the product out of direct sunlight, then the product could be placed where it would be easily exposed to these conditions. Therefore, the product needs to be tested in these conditions following the most conservative approach in terms of how it might be exposed to outdoor conditions. Similarly, a Panel member questioned the need for measuring bait consumption since the objective is to measure toxic effects. Further discussion among Panel members, revealed that a change in bait weight may be difficult to accurately measure especially in the event test insects are killed when provided with sufficient bait as per the product instructions. This issue is in part, addressed for flies in the protocol previously described above. The Agency provided clarification to the Panel that some registrants claim that the bait is a "preferred food source" or "more attractive" in which case it is important to measure consumption of bait versus the alternative food source in order to prove this claim.

b. The Panel provided guidance on designs for outdoor tests for cockroach and ant baits including parameters for assessing efficacy. In situations where the registrant claims that the product controls the pest population, the Panel asked whether the test should include various types of ant queens and how should such tests be conducted? The Agency clarified for the Panel that for the purposes of data submission, the product labels currently specify four species: red imported fire ant, pharaoh ant, harvester ant and carpenter ant.

One Panel member cited that because of the complexity and difficulty of outdoor testing, it can also be difficult to adhere to GLP requirements. The Agency acknowledged the difficulty of adhering to GLP requirements outdoors and requested that the registrants report any deviations from GLP.

Regarding the testing of outdoor foggers, the Panel considered the design appropriate in terms of the use of caged test insects, and provided guidelines for recommended heights and positioning (rows) of cages similar to the protocol discussed for misting systems (Question 5). There were differing opinions among

Panel members about the need for outdoor testing given the difficulty of outdoor trials. If the intent of the product is to cause immediate toxicity and quick knockdown, then indoor tests could demonstrate this adequately. One Panel member suggested the use of large outdoor cages for such studies. Others held the opinion that outdoor testing is important given the variable and dynamic nature of natural environments and this variability could negatively impact product efficacy. Panel consensus was reached that claims of residual activity should require outdoor testing. As with the misting systems (Question 5), concerns whether registrants were claiming population reduction arose. The Agency clarified for the Panel that claims of residual activity do not necessarily imply claims of population reduction but are intended to address registrant claims such as "May work for xx days".

Questions arose in regards to section (p) as to whether it applied to non-residual or residual products or both. Currently, there are a wide range of available OTC fogger products. Some products, such as citronella candles and thermal foggers for mosquitoes and biting flies emit very small particles (smoke). This contrasts with the relatively larger particles produced by foggers such as the *Cutter* or *OFF!* brands of backyard mosquito control aerosol cans. The latter are designed to target the actual insect within the harborage (per label claims) versus quick kill, non-residual products like foggers. Panel members noted that it is necessary to have field or simulated-field studies to evaluate all of these types of these products.

The protocol presented in section (p) seems appropriate as written when describing the fogger type products that are carried and moved by the end-user. The Panel pointed out that in contrast, insecticidal "candles" and torch products, which are typically placed in fixed locations during their use, makes them more equivalent to the misting systems in terms of experimental design. The Panel reminded the Agency to be mindful that these products are typically designed to repel insects not control them. The Panel recommended separate sections for these types of products or perhaps group them with the protocols for misting systems. Comments for mister systems would mostly apply to these fixed emitter products. This protocol, as currently written, does not specify appropriate mesh-sizes for the test cages (14X18 cm screening, Barber *et al*, 2007) or the other environmental data recommended by the Panel for misting systems (including time to run the application, wind speed and direction, temperature and time of day). The Panel raised another consideration was raised regarding the biological background of the test insects (e.g., lab-reared or field-collected) in terms of insecticide resistance.

Panel consensus with the misting systems was that all claims of residual activity and/or pest population reduction must be adequately proven. The current protocol does not address how population reduction would be measured but it should involve pre-treatment and post-treatment sampling which could possibly be done by sweeps or traps (such as the CDC trap). The Panel agreed that recommending an interval between the pre and post samples (e.g., 1-2 days' post-treatment) is desirable. Population reduction for a misting system should be measured within 24 hours since adulticide applications cannot prevent re-infestation.

Question 8: Sections (v) and (w) describe methods for assessing the efficacy of direct treatment of the nest/hive/colony and bait treatment of stinging, flying Hymenoptera (except ants). It is proposed that nest excavation/dissection should be conducted within 24 hours of the final at-nest assessment with zero activity because: (1) paper nests break down quickly once the majority of the worker force has been incapacitated (i.e., killed, moribund, or knocked down), and (2) product performance claims for nest kill are typically associated with the final at-nest assessment time point (e.g., kills the nest by 7 days), since there will have been zero foraging activity for two consecutive days and it is generally assumed that the colony is dead/dying or vacated. Please discuss:

a. Whether a 24-hour window is an acceptable length of time to allow for nest excavations and in-field

dissections, and why or why not.

b. Whether colony mortality should be defined as 100% mortality of the colony members, and why or why not. If not, what is an acceptable definition or threshold to define mortality of a colony?

Response 8:

a. The Panel agreed that after treatment with baits, it is reasonable and appropriate to utilize a 24-hour window following lack of activity in nests/hives/colonies of stinging, flying Hymenoptera. The lack of colony activity, i.e., no adults entering or leaving the colony, should indicate whether or not it is safe to dissect a nest. For closed nest species (e.g., hornets) mortality should be assessed 24 hours after the label claims for a bait product. Prior to dissection of the nest, the nest should be agitated to assure mortality (no insect activity) from a safe distance with an extended tool (e.g., long pole) to assure mortality (no insect activity). Even though there are no foraging activities after the application, newly emerged insects could be active inside nests.

In contrast, the Panel found that the 24-hour window was not acceptable when using direct spray methods on nests/hives/colonies of stinging, flying Hymenoptera and a single time window should not be applied uniformly to different species. Using direct spray methods, which are required to have a 10 second knockdown, mortality of nests/hives/colonies should be assessed immediately or at longest within one hour after application of the product for open nest species (e.g., paper wasps). If the product claims long term action, mortality should also be assessed at 24 hours after treatment to account for returning foragers. For closed nest species (e.g., hornets) mortality should be assessed 24 hours after application of a spray product. Foraging adults trying to re-enter the colony should be dissuaded by the pesticide applied to the nest, although during that period, returning foragers may need to be knocked down with an aerosol. Prior to dissection, the nest should be agitated to assure mortality (no insect activity) from a safe distance with an extended tool (e.g., long pole) to assure mortality (no insect activity). Even though there are no foraging activities after the application, insects could be active inside nests. Regardless, the sooner the nest can be removed from where returning foragers expect it to be, the sooner the nest can be safely dissected.

b. The Panel recommended that the acceptable threshold be 100% mortality of adults (foragers). It was decided that the definition of colony members was unclear. If colony members include adults, pupae, larvae and eggs, then 100% mortality of the colony members may not be realistic. Pupae, larvae and eggs may not be exposed to an insecticide, either bait or spray, during the application. Label claims for the given application need to be considered if there is to be a long-term action by the pesticide. For closed nest species (e.g., hornets), if the pesticide does not kill pupae, newly emerged adults could be active inside nests over time. Some insect species, i.e., fly pupae, are impervious to pesticide sprays.

Question 9: Section (x) describes methods to determine the resistance ratio of a population. EPA's current bed bug guideline (OPPTS 810.3900) specifies a resistance ratio equal to or greater than 100 when testing against resistant strains is performed. Please discuss:

a. Whether the resistance ratio of 100 should also apply to the pests covered in the premises guideline, and why or why not. If not, what might be an appropriate resistance ratio and why? Please comment specifically on an appropriate resistance ratio for flies, cockroaches, and mosquitoes.

b. Whether the recommended methods are appropriate for flying and crawling species, and why or why not. If not, please recommend other methods that may be more appropriate.

Response 9: The Panel agreed that a resistance ratio of 100 should not apply to the pests covered in premises guideline. Specific points are as follows:

a. The levels of resistance against the major pesticides were reported for common household pests and should be used as reference for the insects of concern (Naqqash *et al*, 2016). In addition, the Panel recommended that the Agency should allow flexibility for methods to determine the resistance ratio as the reported apparent level of resistance may vary among the various methods employed.

b. The Panel majority noted that resistance ratios should be determined with topical application of technical insecticide dissolved in acetone. Resistance ratios can also be determined using limited exposure of insects to different concentrations of an insecticide applied directly to a glass Petri dish. The use of filter paper should be avoided, since it could affect surface availability of the insecticide and confound the test. Concentrations that result in 10-90% or 20-80% mortality should be used in the bioassays, and those results would be subject to an appropriate statistical analysis. Historically, Probit analysis has been used (Robertson *et al*, 2007). Resistance ratios using LD_{50} or LC_{50} data are preferred over resistance ratios using LT_{50} data to avoid the statistical issues of multiple observations on the same individuals. LT_{50} values tend to be much greater than LD_{50} values, at least for cockroaches. However, LT_{50} data can be properly analyzed using methods and programs developed by Throne *et al* (1995).

Questions 10: Please provide comments on the overall clarity, accuracy, and completeness of the draft premises guidelines. Please provide any additional comments that highlight areas of the draft guidelines that may need to be clarified and note any critical topics that are missing. Please include references to published literature that could help improve the completeness and clarity of the draft guidelines.

Response 10: The Panel recommended considering spatial repellent products in the testing guidelines unless they are accounted for in a separate set of guidelines, as well as a number of page by page comments and editorial points discussed below:

Sections a-h; Concerning field and laboratory studies: The Panel concluded that data required from the field and laboratory are not the same and this should be made clear in the guidelines.

A section missing in the guideline is guidance on handling and immobilizing insects for handling during testing. Carbon dioxide should be acceptable for most insects, however there may be exceptions. The Panel noted again that the Agency should check for consistency when referencing metric or imperial measurements in the protocols, use one or the other not both.

Page 4 (b, i); Considerations should include the requirement of full GLP protocols for any test being submitted to EPA, which will increase cost of testing and reduce number of labs willing to conduct tests. The Panel recommended that EPA identify some essential GLP elements that must be adhered to in all testing, and make only these mandatory, since full GLP certification is burdensome and expensive. (c.) Resistance management considerations should include the Agency provided list of requirements for IRAC labeling of all public health pests for which there is a concern about resistance. The Panel was unsure what "strongly encouraged to adhere to" really means – this should be clarified for the submitter.

Page 5 (g, k); The value in distinguishing between knockdown and moribund insects for efficacy tests was not clear to the Panel. This should be clarified for the submitter. Knockdown (extending the test length by 24 hours) is more useful for flying insects, but in an insecticide efficacy test, it seems like knockdown and morbidity represent similar responses. A Panel member also noted that some moribund cockroaches and other pests may not turn over.

Page 6 (r); add: "…, usually expressed numerically as a ratio (e.g., 14L: 10D)."

Page 9 (a, vii, 5); The Panel suggested a sentence change from "A positive control is recommended only when determining a resistance ratio." to "Positive controls are not normally required for product approval, unless needed to determine resistance ratios, per under Section (x)."

Page 11 (e, iii); The section on use of turf or leaves as test surfaces seems out of place, the Panel suggested to move it to the Experimental Units section.

Page 15 (b, iv); Treated surfaces should be aged for a minimum of 24 hours outdoors in indirect sunlight, and fully dried. Direct sunlight is too unpredictable and variable.

Question 11: Historically, the Agency has often received basic laboratory studies which utilize 5 replicates of 10 individuals. Based on the statistical document provided to the Panel, that replication provides power of 0.8 with 15% or 20% precision. However, with a precision of 10%, the same replication only provides a power of 0.6. This level of replication is the default recommendation in the draft premises guideline, though other levels of replication may be acceptable if submission of information from a power analysis or other justifications are provided. The Agency is specifically considering available methods to increase the statistical power of each test. Please discuss and provide comment on:

a. The statistical methods and simulations EPA has developed to estimate the power of the proposed design, and specifically to achieve an adequate estimate of precision around the estimated mortality rate in the treated group.

b. Using the assumptions described in the Sample Size document, options with 10% precision and power of 0.8 are 5 replicates of 15 individuals, 7 replicates of 10 individuals per replicate, or 35 replicates with 1 individual per replicate. Please comment on this conclusion and provide alternative approaches, if appropriate. Please also comment on the use of power of 0.8 and precision of 10% as generally acceptable standards. Do the Panel's recommendations vary based on species and/or test? Please specify.

Response 11: Overall the Panel concurred with the statistical model assessing the power of the proposed design and precision of statistical analyses with the following suggestions:

a. A singular protocol is desirable and should be applied to insects that are easily reared and tested under laboratory conditions. However, this set of conditions is not always achievable because not all arthropods can be established in standardized laboratory conditions or assessed with the same protocol. Therefore, other methods must be used to achieve the end result of statistical reliability. The statistical model and simulations presented assumed parametric data, and where possible this approach should be applied. However, there are equally valid methods of statistical analysis when the data are non-parametric. As alternatives, the Agency should consider using meta-analysis or Bayesian Bootstrap power analysis (Huson, 2009) to provide a validation of data from rare or difficult species or variable field studies where it is not possible to achieve the power level of a parametric study as suggested. Additionally, even with a standardized test subject and years of operational experience, some variability is inevitable even in standardize laboratory testing. This concept is implicitly understood in the field of "bioassay" work. As a result, while 90% mortality is a great objective, it should not always be the defining factor as to whether an experiment or product trial was effective. In the end, the acceptability of any statistical analysis should be consistent with the claims of the product. Based on the statistical

simulation for one treatment and one control the SAS program clearly results in the desired level of power. However, the current analysis does not include more than one treatment, blocking, split-plot designs, or repeated measures designs and is thus limited in its usefulness. In addition, the analysis provided seems to be designed for one experiment with no provision for replication or additional experiments. There is also no mention of normality and equal variance testing (assumptions of parametric tests) and no mention of the possibility of non-parametric tests. There should be an easily used program/test provided by the Agency for testing the statistical power of results. Alternatively, specific software and built-in routines should be suggested.

b. The Panel noted that in the past, the Agency often accepted data obtained by using 5 replicates, each of 10 insects. The draft guidelines suggest a standard of "5 replicates of 15 individuals, 7 replicates of 10 individuals, or 35 replicates of 1 individual per replicate", and this would increase the statistical power of the test. This increase is reasonable for the most common pests that are easily reared/collected but could be challenging for other targets that are costly or are difficult to collect and house. For rare pests, or those that are more difficult to handle, a smaller number should be allowed within the guidelines. It is apparent that test species matters, and the experimental design should be accepted on a case by case basis. Latitude within the guidelines must be developed and expected.

The Panel recommended the adoption of the following 2-list approach for test species described below, in which the new, higher standard is applied to those common pests that are either easily collected or available from established colonies.

Common Pests (but not limited to)

- Fleas
- Cockroaches (American and German)
- Flies (house, flesh, blow, face, stable, horn)
- Biting midges
- Mosquitoes (Aedes, Anopheles, Culex)
- Ants (fire, pharaoh, harvester, carpenter)
- Ticks (brown dog, American dog, deer, lone star)

The second list would include more challenging targets for which the historical standard would be applied, with the caveat that the statistical power is expected, if found to be lower, is still acceptable in the range of historic standards.

Rare Pests (but not limited to)

- Scorpions
- Spiders (brown recluse, black widow)
- Centipedes
- Wasps, Hornets, Bees

TOPIC: FIRE ANTS

This guideline provides recommendations for the design and execution of laboratory and field studies to evaluate the performance of pesticide products for the treatment of red imported fire ants (*Solenopsis invicta*) 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, granular or bait, if intended to be applied for control of red

imported fire ant colonies (i.e., workers, queen(s) and brood) or as a barrier treatment. 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. For example, tests for additional formulations of products targeting red imported fire ant individuals (rather than colonies), such as direct spray testing and indoor/outdoor residual applications targeting workers/foragers, should refer to the Premises Guideline for appropriate testing.

Question 1: The draft guidelines describe test methods for evaluating the efficacy of a variety of pesticides to control fire ants. Please discuss:

a. Whether, given the objectives and the types of products being evaluated, the test methods are appropriate to evaluate the efficacy of premises products and to support pesticide-labeling claims related to kill, knockdown, residual control, and/or flushing.

b. Whether there are additional or alternative test methods beyond those discussed in the draft guidelines for testing the efficacy of premises pesticide products.

Response 1: Consensus was reached on the following conclusions:

a. The draft guidelines were a significant improvement over the 1998 guidelines. The 2018 documents were well written with clear objectives. However, there were several areas where the test methods, other than those proposed, might provide adequate efficacy data using alternative test methods.

b. The Panel members proposed the following options for consideration:

Rename Guidelines to 'Treatments for Imported Fire Ants…." to include red, black and hybrid imported fire ants.

Sections a-h; On general test conditions: Clarify whether conditions refer to laboratory or field.

Page 4; Add red, black and hybrid imported fire ant to the list of definitions

"Mound" vs. "Colony" – The definition of "mound" is presented as being the visible (above ground) part of the "nest". However, "mound treatments" may encompass more than just the above ground portion. A mound drench or similar treatment is designed to extend deeper into the "nest". The mound usually refers to the structure above and below ground that the ants live in while the colony refers to the individuals that inhabit the mound (Vinson, 1997).

The definition of moribund should be reconsidered. There should be a reasonable time defined in the scenario where if an adult test subject has not recovered, the determination will be made that such subject will not recover. This may be different with larvae where the determination may not be failed to recover after a given period, but rather a failed molt to the next instar.

Page 5; Definition of Positive Control needs to be added: Positive controls are necessary, especially in field trials, where environmental conditions can result in trial failures or reduced efficacy (e.g. drought, high temperatures for long periods, rain, etc.).

In section (d) a. i. Regarding the statement: "For products that control red imported fire ants, the objective is to determine if the pesticide application has residual efficacy." The Panel agreed that none of the highly effective baits work because of residual efficacy. They work because they successfully kill entire colonies and it takes fire ants time to recolonize a depopulated area. Even residual insecticides used as individual mound treatments (IMTs) do not work because of residual efficacy, rather because of their ability to kill entire colonies. A residual product can remain in the soil a long time after mound treatment. However, if it does not kill the entire colony of red, black, or hybrid fire ants, survivors will move a few meters and reestablish.

Residual efficacy is primarily important for products claiming season-long or longer control. Currently this includes only certain broadcast residual granular or liquid products. The Panel suggested alternative language to read: "For products designed to control fire ants, the objective is to effectively reduce active fire ant colonies and foraging for the length of time claimed on the label. In all cases, the scientific objectives and label claims should be clearly stated, and all treated areas should be compared to areas that have received no treatment or a diluent-only treatment."

The Panel highlighted that looking only at colonies/mounds/nests is less instructive than numbers of mounds and ants in an area. It is possible to treat a mound and have it remain vacant, when ants have merely been displaced and pest control in the treatment area has not been achieved. The Panel recommended for a broadcast treatment, mounds per unit area would provide more informative data.

In section (d) a. ii. The Panel notes there is no need for products to be stored at ambient temperature and humidity at least one day before use. Most products are in sealed, moisture-proof containers, so acclimatizing really has no function. Fire ant baits are temperature and moisture sensitive and remain in better condition if stored in cool, dry conditions.

Page 6; (d) a. iv. The Panel explained that maintaining the RH of a test at 70 to 90% is unrealistic. For the RH to be maintained at the Agency recommended level, would require a growth chamber and then the material normally used to keep ants inside the test arena would break down (products like fluon are water soluble). The Panel suggested that an alternative to this, while keeping the ants in a healthy environment, would be to provide an artificial nest lined with moistened dental stone or plaster of Paris. This would maintain a nest chamber at a humidity found in a normal nest. Thus, the test chamber mimics nature where there is a nest at relatively high humidity, but foraging is in variable levels of humidity. Williams (1989) describes this type of nest chamber.

In Section (d) a. v. Add parentheses to clarify which target endpoint target (e.g., Mortality, No. of Active Mounds, etc.) refers to lab studies or field studies. For example, Mortality (lab studies), Number of Active Mounds (field studies), Forager numbers (field studies).

Page 7; (d) a. vi. Under test organisms, include *S. richteri*.

The Panel recommended changing red imported fire ants to imported fire ants. Multiple species are inferred in the last sentence "All sources of red imported fire ants should be listed in the study methods along with species". Red imported fire ant is the approved common name of *S. invicta*. Otherwise, include *S. richteri* and the hybrid, *S. invicta* x *S. richteri*. If referring to red imported fire ants, no species name is necessary.

In section (d) a. vii. The Panel suggested adding a positive control using an industry standard for field tests. This is standard for most field trials to account for conditions such as drought, excess heat, excess

rain, etc. Environmental conditions can sometimes result in failures in the field that are not related to the product being tested. This provides a baseline of efficacy and can provide information indicating whether the product should be retested. A positive control is more important for bait trials than those with contact insecticides, but should be considered with both.

In section (d) a. viii. The Panel noted QA plans and GLP are more relevant for laboratory studies than for field studies. The required use of GLP standards should not be strictly stated in the protocol. The Panel has in several of their recommendations mentioned including outlining an alternative to GLP methods.

In section (d) c. Randomization of field trials should be based on numbers of mounds. Plots are arranged according to numbers of mounds and then blocked from highest to lowest numbers. This blocking system is similar to animal feeding studies where animals are assigned to replicates or blocks according to weight (Snedecor and Cochran, 1989).

Page 8; (g) c. The Panel finds the section on testing conditions provides a good example of information that 'should' as opposed to 'may' be reported. The Panel recommended language which indicates that "should" be reported where applicable. One Panel member noted, this was well written on p. 6, section (d) a. iv. (p. 8)

Page 9; (i) Area applied products includes two categories of fire ant control product: broadcast baits and broadcast residual soil treatments. The Panel suggested addressing these separately.

(i) a. Change red imported fire ants to imported fire ants
(i) b. i. If fire ant mounds are present and active, it is unnecessary to have a one year waiting period after the last pesticide application on the site. The Panel noted, if ants are present and active, it is a good indication that there is no residual pesticide on the site.

Page 10; (i) b. ii. Plots should all be the same size. A minimum number of 10 mounds per plot should be adequate for an efficacy trial. For granular and liquid contact application products, a minimum plot size of 0.25 acre should be adequate if a minimum of 10 mounds can be included in a central area of each plot. There should be a buffer between the edge of the treated plot and the data collection area in the center to prevent edge effects. A 0.5-acre plot with a 0.25 data collection area in the center is preferable, as recommended by several Panel members. For bait products, a minimum plot size of 0.5 acres should be used (0.75 to 1 acre is preferable if there is enough area at the test site) with data collected from a 0.25-acre circle in the middle to eliminate edge effects. In most southern states, a 0.25-acre circle (ca. 58' radius) is usually required to obtain enough plots for a test that has 10 or more mounds in the circle. If plots are 0.5 acres, the edges of the center circle should be a minimum of 50 feet apart to prevent ants within the circle from foraging into adjacent plots. This arrangement is similar to data collection in row crops. In cotton, plots are treated and data are collected from the middle rows beginning at least 10 feet into the plot to reduce edge effects (Graham *et al*, 1984 and 1987; Darnell *et al*, 2016).

In section (i) b. v. The Panel recommended for granular and liquid contact application products a minimum of 60 days may be long enough, but 90 days is a preferred length of time. Products such as fipronil have taken 30-45 days to show reductions is mound numbers. For bait products, especially insect growth regulators, the tests should continue for 4-6 months with 6 months being the minimum for IGRs.

In section (i) b. vi. The Panel suggested sampling intervals of 1, 2, 3, 4 and 6 weeks and monthly thereafter. If fast control is claimed on the label, earlier sample(s) should be included to match the claims. If quick kill is the basis for the product, a 30-day minimum is acceptable for sampling. Other

Panel suggestions included, endpoints for residual claims should be a minimum of 90 days for contact insecticides and 4-6 months for baits. Products claiming longer than 90-day control must have sample data is consistent with claimed length of control.

The Panel raised concerns about the sole use of hot dogs (or other food attractants) to evaluate the effectiveness of fire ant control products. Using forager counts is one way to evaluate control, but it is not the only valid way. Fire ants recruit workers to discovered food resources and it is not uncommon to find hundreds of ants recruited to one vial, while another nearby vial equidistant from a mound has no or few ants. Recruitment is also highly dependent on time of day and being shielded from the sun. There are other effective methods for evaluating fire ant bait products including mound counts and ratings (Drees *et al*, 2013). The Panel acknowledged that using mound counts and ratings is not perfect, but it has been used successfully to test fire ant baits and individual mound treatments for many years. It provides a good way to discriminate between products that work well and those that do not (Collins *et al*, 1999). Mound data counts have been the standard for USDA-APHIS protocols when testing products for use in the fire ant quarantine program. (Loftin *et al*, 2016 and 2017; and Callcott, 2014).

In section (i) b. vi. 1. If foragers are used as a sampling tool, the Panel recommended that care should be taken to ensure data are accurate. The Panel noted that vials should be shaded while in the field to prevent reaching internal temperatures that limit foraging. As a precaution, soil temperatures should be monitored to ensure ants are foraging. During the hot summer months, the window for sampling by foraging is usually from sunrise until ca. 10:00 a.m. and from ca. 4:00 p.m. in the evening until sunset. In addition, to assure adequate access to the vials they should be placed in contact with the soil and not placed on top of grass, etc. While Drees *et al* (2013) recommend a 45 to 60-minute timeframe for baiting foragers, baits have been removed and/or eaten by ants in trials in Alabama when left longer than 30 minutes. A sampling period of 30 minutes should be long enough for ants to recruit to the bait and not consume or remove the bait. If a longer period is used, care must be given to ensure that enough bait placed at each station.

In section (i) b. vi. 2. The number of active mounds per plot may be used as a measure of treatment efficacy to supplement forager data, and to establish homogeneity among plots at a site. Randomization of field trials should be based on number of mounds, arranging plots according to numbers of mounds and then blocking from highest to lowest numbers (Snedecor and Cochran, 1989). There is no need to scratch the surface of the mound with forceps or a similar tool. This is a difficult sampling method for a large field trial. A probe, up to 3/8 inches in diameter, may be used to probe the mound to elicit a response without significantly disturbing the mound and will produce the same results while lessening the stress on the researcher. During hotter weather months, if sampling continues into the hotter portion of the day the probe may need to be inserted 6-8 inches in depth because ants will locate deeper into the soil.

The majority of Panel members indicated that using active mounds alone is an efficient and effective way to determine efficacy of a product. This method does not have to include adding foragers (see (i) b. v. and (i) b. vi. above for discussion).

Page 11; (i) b. vi. 3. If brood are used for an assessment, there is no need to remove a small portion of the mound. Simply opening a mound with a probe or narrow shovel to observe ant activity is less disturbing and will suffice to determine whether brood is present.

Based on the mode of action of the IGR (to block reproduction in the adult and inhibit larval growth/metamorphosis) the Panel indicated that treatment of an active mound would not stop the

development of pupae which do not feed. In order to ensure efficacy of the IGR treatment, sufficient time would need to be incorporated to assure inhibition of reproduction of adults that eclose from pupae that were formed following treatment. A minimum of 120-day post treatment to assess mound activity should be sufficient to assure the effective action of the IGR treatment.

The Panel had different opinions on IGR studies and the presence of brood:

Some Panelists noted that the presence of brood must be confirmed in 100% of treated fire ant mounds prior to application. Inclusion of 10% unconfirmed mounds would bias the test towards efficacious treatment if only 90% of mounds were permitted. If possible, mounds that cannot be confirmed to contain a brood should be excluded from the test. In order to have an appropriate statistical assessment for the test, the untreated controls cannot be biased and inclusion of 10% unconfirmed mounds would increase the variability and decrease the confidence of the test.

Other Panelists noted that there is no need to determine the presence of brood for each assessment if the test is run for a long enough duration for the IGR to work. Presence of brood is affected by season and environment and will vary during the year. Looking for brood increases cost considerably for a test due to the increased time for the assessment and the fact that assessments can only be done during the cooler times of the day in the morning and evening. The product should be tested under field conditions to determine if it works, but the determination of efficacy is not the presence or absence of brood, but whether the mound dies. This can be determined by extending the time of the study. The determination of whether the product kills a brood should be determined in the laboratory by the Applicant submitting those data. The deciding factor is whether or not the mound is killed. This determination can be made with a 180-day post treatment count.

In section (i) c. iii. One Panel member indicated that if a Poisson distribution has not been established as the proper underlying distribution for forager recruitment to hot dog vials, then the presence/absence of ants in vials might be a better measure of ant foraging. Several studies have shown that using foraging numbers for a trial is an acceptable method of assessing imported fire ant populations (Jones *et al*, 1998).

In section (i) c. iv. The number of active mounds should be reported for each assessment, but there is no need to record the number of inactive mounds. The Panel noted that this, in fact, contradicts the statement made on p. 6. (d) a. v. 2 of the original document that the numbers of active mounds in plots should be reported. No mention is made of inactive mounds.

In section (i) c. v. Unless the assessment is for speed of activity of the product, the Panel agreed there is no need to assess brood at each data collection for an IGR if the study is conducted for a long enough sampling period. The purpose of an efficacy trial is not to determine the fate of the brood, but to determine if the product kills the mound.

Page 12; (j) b. i. References to section 18 of draft guideline:

In section (j) b. ii. The definition of a plot is not clear. With individual mound treatments, plot size may vary, but each replicate or plot should have the same number of mounds. Individual mound treatments should have a balanced design with equal numbers of mounds in each replicate. Ten should be the minimum.

In section (j) b. vi. Soil temperatures are listed for foraging data, not for determining mound activity. Drees *et al* (2013) recommends collecting foraging data when air temperatures are between 18.3° C and

35° C or 65° F and 95° F. The Panel agreed that these temperatures should be adequate for collecting mound data, and further noted if soil temperatures are taken, the protocol should specify soil temperatures at 1" (2 cm) (Porter, 1987).

Panel consensus was reached that if a product claims quick kill, sampling data should be taken earlier than seven days and data collection times should depend on the claim.

Page 13; (j) b. vi. 1. The Panel cautioned that an individual mound treatment should not make claims of preventing new mounds from forming. The number of all active mounds per plot per treatment within a site should be reported for each assessment to account for possible colony displacement as opposed to colony elimination. More than one satellite mound may form from a colony that was not completely killed if the queen escaped. These will usually reform to one mound with time.

In section (j) b. vi. 2. See comments for page 11 (i) c. iv.

Page 14; (k) b. i. One Panel member suggested the following language: replace "via plastic tubing (e.g., Tygon® tubing)" with "via a suitable bridge (e.g., paper strips or Tygon® tubing)".

In section (k) b. iv., the Panel noted that with baits, the acclimation time for the ants placed into a test arena should be 72 hours not 24 hours to minimize aberrations that may be due to handling and disruptions in their foraging behavior due to earlier handling.

In section (k) b. v., the Panel discussed the following concerns about "If re-baiting occurs, how re-baiting is conducted and thresholds for re-baiting should be recorded". This is vague but does it reflect the possible variation in the modes of action of the baits? For example, if the bait is an IGR, adults would (presumably) continue to feed to the end of the test (when brood disappear) but an adulticidal bait would be expected to be effective in a more definitive time. For example, termiticide baits are often replaced when 50% has been consumed. Would "percent bait remaining" work? It would seem 'form-dependent' particularly with liquids and scattered granules.

The Panel noted the amount of bait specified for a lab trial is insufficient. The current protocol suggests that the minimum label rate should be used and the amount of bait should be extrapolated from the label rate based on the treatment area. When this is calculated for the area used in test boxes, it would result in the use of a miniscule amount of bait being used for a test. This is unrealistic because in nature fire ants forage over a large area to collect the food that is needed for the colony, until the colony is satiated. The Panel recommended that bait be provided *ad libitum*, as should the alternate food source, for the specified exposure time for the trial.

Page 15; (l) b. i. The Panel suggested specifying the minimum width of barrier for the assays for consistency.

If multiple queens are included in a laboratory trial, then the death of one of those queens in a control should not invalidate the trial. Multiple queens are sometimes used in each replication and a single queen death when more queens are still viable should not in itself constitute a failure. In multiple queen colonies, queens are occasionally eliminated by the colony and natural mortality is also possible. With other queens surviving in the test, the death of one queen is not critical as long as the mortality rate of the entire test colony remains acceptable.

There is the question of tying the term "control" to a product having a residual effect. "Control" is not defined in this document and to some extent it is a "moving target". On one hand, "control" may be perceived as when RIFA activity ceases and there are no live workers/brood/queen found in a mound. If "control" means no new mounds, then is "new" referring strictly to no secondary/satellite mounds originating from the treated colony, e.g., where ants left because of the disruption of the mound during or subsequent to the treatment and were unable to return because of the pesticide's residual effect? Satellite mounds resulting from a treatment are somewhat easier to identify since they typically appear near the original mound. Identifying satellite mounds is important in an individual mound treatment test. The Panel would like clarification in the guideline on these points.

The discussion of control raises the complexity of whether to distinguish monogyne and polygyne mounds because genetic analysis is more reliable than visual determinations such as size and distribution of mounds in a given area. There is still the matter of possible intrusion of new reproductives from a nearby, untreated colony because mound drenches target an existing mound which would not likely be reused by a new colony. If "control" implies a residual then the term should be more clearly defined, such as no new mounds forming within 12 months or "next year" or "next season" or the length of time the product claims on the label.

Question 2: Fire ant product field tests are described in sections (i) and (j). Please discuss:

a. Whether data should be collected from locations identified with uniquely monogyne and uniquely polygyne populations, and why or why not. If social form is not a factor in the field study design, please discuss:

i. Whether field studies should be conducted at geographically disparate sites, and why or why not.

ii. If there is a biological reason (i.e. not a statistical reason) that more than two sites should be added to the design.

b. Section (i) describes area-applied product field tests for fire ants. Please discuss:

i. Whether a 60-day duration is an acceptable minimum time frame to run an area-applied product study for fire ant control, and why or why not.

ii. What the minimum number of active fire ant mounds that should be included in each plot is if the plot size is determined by the investigator. For example, if an investigator decided to set up a study in an urban area where plot sizes may be 0.1 acre, what is the minimum number of mounds that should be included in a plot? Please provide a justification for the recommended number.

iii. Whether, when sampling foragers using vials containing a food lure, the placement of the farthest vial from the center at 90% of the radius of the plot is an acceptable distance. Would a smaller sampling radius be acceptable, and why or why not?

c. Section (j) describes mound-applied product field tests for fire ants. Please discuss: i. whether a 30-day duration is an acceptable minimum time frame to run a mound-applied product study for fire ant control, and why or why not.

d. Sections (i) and (j) describe IGR product field tests for fire ants. Please discuss:

i. Whether it is unreasonable to think that all mounds should have brood at the beginning of an IGR study; that is, do they all have to have brood or would it be acceptable if at least 90% of the active mounds had brood? Please provide a justification for the recommendations.

ii. Whether, when considering duration of an IGR product field study, if a minimum 60-day duration is an acceptable time frame to see an IGR-effect, and why or why not.

Response 2: The Panel agreed that there is no need to include social form as a factor in the field study design. The biology of monogyne and polygyne forms of *Solenopsis invicta* are identical, and biological factors are extremely close among all of the *Solenopsis saevissima* complex (includes two fire ants in the United States, *S. invicta* and *S. richteri*, and several South American species). The only difference between the social forms of *S. invicta* is the aggressive interactions between monogyne colonies. However, this would have no impact on field tests because either form is still able to forage effectively whether in a field of monogyne or polygyne colonies. It was noted that *S. invicta* would not be an equivalent proxy for trials of the tropical fire ant, *Solenopsis geminata*, or the southern fire ant, *Solenopsis xyloni*.

a (i). The Panel did not see any need to require that tests be conducted at geographically disparate sites. The biology and behavior of these ants do not change in different geographical settings, thus there is no need to require difficult and costly requirements to include disparate sites. If the intent is to prevent pseudo-replications, there is still no need to require different geographical sites that include different ecotypes.

(ii). This question was answered by the Panel as part of the previous discussion and the conclusion was that there is no biological reason for more than two sites to be added to the design of field tests.

b (i). The Panel generally thought that a 60-day duration for tests may not be sufficient in some cases, however the length of these tests really depends upon the required endpoint. If colony elimination or complete control is the desired endpoint, then 60 days may not be sufficient for slow acting baits and would almost certainly not be enough for insect growth regulators (IGRs). If colony reduction for management is the desired end-point, then 60 days may be sufficient except for IGRs but the decision on duration should be determined from the mode of action of the proposed control.

(ii). The Panel agreed that the standard given in Drees *et al* (2013) of a minimum of 20 mounds per acre for a test appears to be a reasonable threshold for a successful test. Because foraging can occur at a distance of up to 200 feet (~65 meters), small plots are not ideal. Distance between plots should also take into consideration the foraging ability of imported fire ants and there should be ideally, a 200-foot barrier between test plots. If areas smaller than one acre are used, the standard of 20 mounds per acre could be extrapolated downward but the number should never be less than would give statistical power to the analysis of the test. This situation is especially pertinent to doing tests in urban areas where it is not possible to get plots anywhere near an acre in size. The Panel suggested in an urban test, where lawns are used as test plots, the number of ant mounds per test should probably not go below 5 or 6 mounds per plot. The Panel deferred to EPA statisticians to determine how many mounds would make each plot a significant test.

(iii). Because of the above stated foraging ability of imported fire ants, the Panel thought that the placement of baits at 90% of the radius was problematic. Reducing the sampling area would help to eliminate false negative results of the test due to untreated foragers from outside the test plot being

collected at food lures within the test plot. The most commonly suggested distance for the maximum placement of baits was no more than 50% of the radius of the test plot. Although not shared by other Panelists, one Panel member suggested that because of the foraging dynamics of this species, baits might not be a useful tool in documenting effects on ants within the test plot.

c. The Panel noted that there is a discrepancy in timing of tests between field and mound treatments, and there does not appear to be a good reason for this. In the protocol, field tests are given a 60-day test duration and mound treatments are given 30 days. The timing of these tests and specifically for this response for mound tests depends upon the desired endpoint as well as the nature of the active ingredient. If the endpoint is colony elimination or complete control, then a 30-day duration is probably not long enough. The duration for this endpoint would likely need to be more than even 60 days. If the endpoint is to reach at least 90% control, which would suggest that the colonies are declining towards elimination, then a 30-day test duration would be sufficient unless a slow-active ingredient or IGR are being used.

d (i). The Panel agreed that it is a reasonable assumption that all mounds should naturally have brood at the beginning of a test. All mounds that are large enough to be identified within a test plot will be mature colonies. It is the nature of red imported fire ants to maintain brood throughout the year when a colony is mature. If this were not the case, the inclusion of up to 10% mounds that did not have brood would be problematic for the assessment of the test. Assuming that all mature natural colonies will have brood should be better than trying to assess whether colonies have brood to start a test. Significant mound disturbance is needed to ensure that brood is observed in a colony and even with this disturbance, brood might not be apparent. Brood are moved within the nest structure as conditions change and at the time of a pre-test disturbance, brood could be deep within the nest requiring near complete nest destruction to find if they are present. The assumption that all mature (observable) mounds have brood is a better alternative to substantial mound disturbance.

(ii). The Panel concluded that a 60-day test duration will not be sufficient for accurate assessment of an IGR test. At 60 days it may be possible to document an IGR effect on the colony, but it does not guarantee success of the test. An IGR could subdue production or development of brood, but if the reproductive ability of the colony is not permanently eliminated, the colony could bounce back. The Panel suggested that a minimum of 120 days may be needed to fully insure that the IGR was effective in controlling the colony.

Question 3: Fire ant product lab tests are described in sections (k), (l), and (m). Please discuss:

a. Whether the arena size used to establish test colonies should be standardized, and if so, what the size should be and why.

b. Whether there should be a standardized length of Tygon tubing between the nest arenas and foraging arenas, and if so, what the length should be and why.

c. Whether an acclimation period of 24 hours is enough time for smaller sized test colonies (e.g. 100 workers) and 72 hours is enough time for larger test colonies (e.g. 10,000 workers), and why or why not.

d. Whether, when considering duration of an IGR product lab study, a minimum 30-day duration is an acceptable period to see an IGR effect, and why or why not. Should one expect to see absence of brood, deformities of brood, dead brood, and/or changes in caste structure within 30 days?

e. Whether 100 workers per replicate is enough individuals to initially support brood and queen(s) in a 2-week lab test, and why or why not.

f. Section (l) describes barrier product lab tests for fire ants. Please discuss:

i. Whether 100 workers per replicate is enough individuals for a 2-week lab test, and why or why not.

Response 3:

a. The Panel concluded that specifying a test arena size may not be needed as long as the arena provides adequate space for the colony size being used in the test, which will obviously be variable among tests. Since the protocol states that a minimum of 100 worker ants be used for tests, the standard 12" x 15" plastic box used in many tests is sufficient.

b. The Panel questioned whether even using an arena that connects two test arenas with Tygon tubing is appropriate. Tygon tubes connecting a nesting area with a foraging area does not represent a natural setting. In nature, ants leave a nest and can begin foraging immediately without crossing an additional barrier. A single arena, a nest structure that maintains proper nest humidity located within the arena provides a more natural setting and should be preferred. Baits, both test and alternative, are placed an equal distance from the nest. The exception to this simple arena model is if the bait is volatile, and the active ingredient could vaporize. Then some bridging or tubing might be necessary for lab arena design. The consensus of the Panel was not in support of the two arena, Tygon tubing design, therefore, no suggestion on the length of Tygon tubing was recommended. One Panel member suggested that simpler arenas were also desirable because there is no reason to increase the cost of the test with more complicated and unwarranted test arenas.

c. The Panel concurred that the times provided in the Agency document would be suitable since colonies appear able to organize in this amount of time. However, it was the consensus of the Panel that 10,000 workers are many more than are needed for any test, including an IGR test. Far fewer workers can maintain an ample brood for a test. The Panel noted that less than 200 ants have been capable of maintaining brood for an IGR test. Suggestions of colony size mentioned by Panel members ranged from 200 to 1,000 for IGR studies.

d. The Panel agreed that thirty days should be sufficient to document some effects on the brood structure of the colony, but this time is probably not enough to show colony collapse or ultimate effects of the IGR. There is always the possibility that the IGR could stop reproduction only temporarily. The Panel noted that duration is dependent on the desired endpoint that is desired for registration of a product. If that endpoint is colony elimination, then 30 days would not be enough, and if this is the case the time needed may be as much as 120 days. If the endpoint is showing that the IGR is having an effect on the colony structure, then 30 days would be sufficient.

e. The general consensus of the Panel was that 100 workers can support brood and a queen for a two-week laboratory test. This also indicates the idea that 10,000 workers are not needed for a successful IGR test.

f. The Panel concluded that 100 workers per replicate is enough for a 2-week laboratory test whether only workers or a colony structure with brood is used for the test. With regard to barrier tests, there are situations where there might need to be two test arenas, with some bridging between the arenas. The bridges or tubes should be long enough to minimize the effects of any volatiles from the treatment.

If there is not a volatile component, then this bridging structure may not be needed. One component that is missing in the protocol is mention of the width of the barrier. This distance could be different between products but its testing should be consistent with the way it would be applied in a non-laboratory setting.

Question 4: Please provide comments on the overall clarity, accuracy, and completeness of the draft fire ant guidelines. Please provide any additional comments that highlight areas of the draft guidelines that may need to be clarified and note any critical topics that are missing. Please include references to published literature that could help improve the completeness and clarity of the draft guidelines.

Response 4: The Panel reached consensus on the following points unless otherwise noted:

Sections A-H cover field and laboratory testing. Data required from the field and data required from the laboratory are not the same and this should be indicated in the guidelines.

If multiple rates, ages of baits, or multiple products are tested at the same time, the Panel recommended making a clear statement that only one replicated control group is needed, not one for each rate, etc. Although not clear in the protocols, this is common in field trials.

There is no need to scratch the surface of the mound with forceps or a similar tool. This is a difficult sampling method for a large field trial. A probe, up to 3/8 inches in diameter, can probe the mound to elicit a response without significantly disturbing the mound and will produce the same results. Mounds are routinely disturbed in trials by mowers, cattle, vehicles, etc. The disturbance from sampling mounds is much less intrusive and works well to determine activity.

There are other effective methods for evaluating fire ant bait products than foraging including mound counts and ratings (Drees *et al*, 2013). While this method is not perfect, it has been used successfully to test fire ant baits and individual mound treatments for many years. It provides a good way to discriminate between products that work well and those that do not (Collins *et al*, 1999).

The majority of the Panel members indicated that there were no real differences between the biology of the red and black imported fire ant and their hybrid, so there was no real reason for a species determination before a field trial was initiated. They also indicated that there was no reason to test a product in both monogyne and polygyne sites. The biology of the two forms is similar and the only way to get a definitive confirmation of polygyny in many cases is by genetic analysis. If information on polygyny is required for the study, a genetic analysis is recommended in most states in the southeast. With the exception of Texas and Florida, polygyne sites are small and ephemeral, and are usually not large enough to initiate a field trial, especially with baits.

The black imported fire ant and hybrid (and possibly European fire ant (*Myrmica rubra*) and little fire ant (*Wasmannia auropunctata*)) should be added to the list of public health pests. The rationale for this is that the latter two are emerging fire ant pests in the NW/NE and Florida areas, respectively. Another advisable comment was that voucher specimens could be preserved in 70% ethanol so that if species identity is later questioned the voucher specimens could be referenced. This methodology would be particularly useful for new and invasive species.

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