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| **Primary Reviewer:** |  |  | **Date:** |
|  | ***[ Name, title, and aff*** | ***iliation]*** |  |
| **Secondary Reviewer:** |  |  | **Date:** |
|  | ***[Name, title, and affi*** | ***liation]*** |  |
| **[FOR JOINT REVIEWS ONLY- *otherwise delete*]** | | | |
| **Approved by:** |  |  | **Date:** |
|  | ***[Name, title, and affi*** | ***liation]*** |  |

**DATA EVALUATION RECORD**

***[*NOTE TO REGISTRANT/APPLICANT: PLEASE DISREGARD *the header, footer, and reviewer information; reviewers’ comments in the conclusion section; and study classification statement. These sections are for EPA, PMRA, and OECD data entry only and will be populated upon Agency review.]***

|  |  |  |
| --- | --- | --- |
| **REQUIREMENT:** | Honey Bee Testing, Tier I  U.S. EPA OCSPP Guideline: | 885.4380 |
|  | PMRA Data Code: OECD Data Code: | M9.5.1–Terrestrial arthropod IIM 8.7, IIIM 10.3 |

**TEST MATERIAL (PURITY):** *[use name of material tested as referred to in the study and include its*

##### *potency, lot no., biological activity or concentration per unit weight or* volume (% active ingredient name in parenthesis)] or [insert TGAI and EP names if a waiver request is made]

**SYNONYMS:** *[other names, code names and acronyms]*

**CITATION:** Author(s). *[Year]*. Study Title. Laboratory name and address. Laboratory report number, full study date. Unpublished *[OR if published, list Journal name, vol.: pages]*. MRID No. *[no hyphen],* PMRA *[number if applicable]*.

##### **SPONSOR:** *[Name and address of Study Sponsor - indicate if different from Applicant]*

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were *[not]* provided. The study was *[not]* conducted in compliance with GLP [40 CFR § 160]. *[Discuss deviations from regulatory requirements]* This DER does *[not]* contain FIFRA CBI.

**EXECUTIVE SUMMARY:** *[Describe the study and its findings.]*

In a *[#]*-day *[oral or dietary]* toxicity and pathogenicity study, *[common name (scientific name)]* were exposed to a *[single OR #]* dose of *[dose amount]* of *[formulation, note its potency, biological activity or concentration per unit weight or volume]* (containing % *a.i. name*) by *[indicate exposure method]*. *[Include other pertinent details such as the controls used.]*

##### *[Describe findings briefly, including mortality, behavioral abnormalities, and other signs of toxicity or* pathogenicity. If no effects were observed, state that there was no test material-related toxic or pathogenic effect.]

The *[#]*-day LC50 was *[****=, > or <****] [insert LC50 in appropriate units]* (95% C.I. *-if applicable*). *[If the study included sublethal test endpoints and/or sublethal effects were observed and/or additional subchronic testing was triggered include the following text (otherwise-delete):* The EC50 based on sublethal effects, were *[insert EC50 in appropriate units.]* The NOEC value, based on mortality *[and sublethal effects]*, was *[****=, > or <****] [insert NOEC in appropriate units]*.

This study is classified as *[acceptable, unacceptable, supplemental].* This study was *[not]* conducted in accordance with the guideline recommendations for a *[oral or dietary]* toxicity and pathogenicity study for honey bees (OCSPP 885.4380; PMRA: M9.5.1 and OECD: IIM 8.7, IIIM 10.3) in the *[species]*. *[If it does not satisfy the requirement, concisely list only major deficiencies or refer to deficiency section.]*

## CLASSIFICATION: [ACCEPTABLE / UNACCEPTABLE / SUPPLEMENTAL, but UPGRADEABLE]

***Use the following template if a study report (i.e. toxicity test) was submitted. If a request for the use of alternative data is submitted in lieu of a new study, delete study template section and proceed to last section of DER template for alternative data requests)***

# *(NOTE: Guidance on populating the DER are reflected as [red italics]- please* replace this text with requested data. Excerpts of study recommendations/criteria are reflected as blue italicized text from the respective OSCPP Guideline and should be deleted upon completion of the DER template. For best preparation of data submission- refer to respective OSCPP Guideline and use both the DER template and guideline criteria. However, the overall structure of the templates should not be altered and data evaluation elements reflected in black *text should* not be deleted (i.e. headings, test parameters, tables, results section). Also- note for data elements of the template that are not applicable- insert “not applicable.” For unavailable information- insert “not available” with a brief explanation for the omission of data.)

## MATERIALS AND METHODS:

1. **GUIDELINE FOLLOWED:** *Indicate which guideline was followed most closely in testing. Such as:*

##### *U.S. EPA 885.4380–Honey bee testing, Tier I1*

*U.S. EPA 850.3020–Honey bee acute contact toxicity2*

*PMRA DIR 2001-02 Part 9.5.11*

*OECD 213–Honeybees, acute oral toxicity test2 OECD 214–Honeybees, acute contact toxicity test2]*

*1 Guideline designed to test acute oral infectivity and pathogenicity of microbial agents.*

*2 Guideline designed to test acute toxicity of chemical agents. Note: The U.S. EPA OCSPP Guideline 850.3020 guideline is only appropriate for MPCA's under certain circumstances and should not be used in place of 885.4380 guideline without consultation with EPA. The U.S. EPA has two other honey bee guidelines designed for toxicity testing of chemical agents (OCSPP 850.3030 Honey bee toxicity of residues on foliage and OCSPP 850.3090 Field testing for pollinators). These are not included in this review template.*

**Deviations from guideline:** *[Indicate if there were any deviations from the test procedures and reporting requirements stated in guideline(s).This information is usually stated in the Good Laboratory Practices (GLP) and Quality Assurance (QA) statements in the introductory section of the study report. State the reasons for such deviations and its overall effect on the validity of the study.]*

1. **MATERIALS:**
   1. **Test Material:** *[Name of test material as cited in the study report.]*

##### **Description:** *[e.g., Physical-chemical state of the test material.]*

**Lot/Batch #:** *[Insert the test material’s lot or batch number.]*

##### *[NOTE: Verify that test material is derived from same source (i.e. lot/batch # or certificate of analysis) of* MPCA (TGAI, MP or EP) that was previously characterized and data were acceptable]

**Purity:** *[Insert the test material’s nominal potency and/or concentration per unit weight or volume (i.e., as specified by the study sponsor).]*

**Storage conditions:** *[Indicate how the test material was maintained, i.e., frozen, refrigerated, maintained in the dark, etc., and comment on the stability of sample under these conditions.]*

* 1. **Test Organism:**

**Species (common and scientific names):** *[Indicate the species used.]*

***U.S. EPA OCSPP 885.4380*** *Testing shall be performed on the honey bee (Apis mellifera).*

***U.S. EPA OCSPP 850.3020*** *Honey bee (Apis mellifera) is the test species.*

***PMRA DIR 2001-02*** *Non-target arthropod species selected for testing should be representative of groups that will be exposed to the MPCA* under actual conditions of use, and that have some important relationship with the target pest species, and should be representative of species found in the ecozone(s) of intended use. See DIR 2001-02, Appendix XI for suggestions.

***OECD 213 and 214*** *Honey bee (Apis mellifera) is the test species. Bees from the same race should be used.*

**Age at test initiation**: *[Give the age of the test organisms.]*

***U.S. EPA OCSPP 885.4380*** *When the MPCA may be expected to affect insect larvae, test insects should include honey bee larvae.*

***U.S. EPA OCSPP 850.3020*** *Test should be conducted on worker bees 1–7 days-old at test initiation.*

***PMRA DIR 2001-02*** *The life stage that is most likely to be exposed to the MPCA, or the life stage that is most susceptible to the MPCA should* be chosen.

***OECD 213 and 214*** *Young adult worker bees of similar age should be used.*

##### **Strain/Source:** *[Report the strain, supplier and/or source of the test organism.]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *Bees may be obtained from on-site colonies or from a commercial apiary, either directly from hives or from frames* kept in an incubator. All control and treatment bees should be from the same disease-free colony.

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213 and 214*** *Bees should be obtained from adequately fed, healthy, as far as possible disease-free and queen-right colonies with known* history and physiological status. Bees should not be taken from the colony in early spring or late fall, but at these times, bees can be emerged in an incubator and reared for one week with pollen and sucrose solution. Bees treated with chemical substances, such as antibiotics, anti-varroa, etc., should not be used for 4 weeks from the time of the last treatment.

**Date of collection:** *[Insert the date of collection, if applicable.]*

## STUDY DESIGN AND METHODS:

##### *[Briefly describe the experimental design.]*

***U.S. EPA OCSPP 885.4380*** *Honey bees are dosed orally with the MPCA and observed for toxic and/or pathogenic effects for at least 30 days* after dosing. Testing in the hive may be necessary.

***U.S. EPA OCSPP 850.3020*** *Test bees are immobilized, and the test substance is administered topically at a range of concentrations (or a single* concentration in a limit test) either suspended in liquid (topical drop) or whole-body exposure to impregnated dust. Bees are observed closely for 4 hours after exposure, and then for mortality and signs of intoxication at 24 and 48 hours after exposure.

***PMRA DIR 2001-02*** *Test arthropods should be exposed to the MPCA at the maximum challenge concentration (MCC) in a manner consistent* with the route of exposure, mode of action and greatest degree of susceptibility under natural environmental conditions, either topically, in the environment (e.g., mixed in the soil), in the diet, or as a combination of these routes of exposure. For topical exposure, the test organisms should be treated for 5 successive days and then observed for an additional 16 days. For environmental or dietary exposure, the non-target arthropod should be exposed to or fed the MPCA for at least 21 days, or until mortality in the control group increases to a significant level.

***OECD 213*** *Adult worker honeybees are exposed to a range of doses of the test substance dispersed in sucrose solution. The bees are then fed* the same diet, free of the test substance. Mortality is recorded daily during at least 48 hours (maximum 96 hours) and compared with control values. The results are analyzed in order to calculate the LD50 at 24 and 48 hours and, in case the study is prolonged, at 72 and 96 hours.

***OECD 214*** *Adult worker honeybees are exposed to a range of doses of the test substance dissolved in appropriate carrier, by direct application* to the thorax (droplets). The test duration is 48 hours. If the mortality rate is increasing between 24 and 48 hours whilst control mortality remains at an accepted level, i.e., ≤10%, it is appropriate to extend the duration of the test to a maximum of 96 hours. Mortality is recorded daily during at least 48 hours. The results are analyzed in order to calculate the LD50 at 24 and 48 hours, and in case the study is prolonged, at 72 and 96 hours.

## Experimental Methods and Conditions:

**Acclimation:**

#### Duration:

Feeding:

Water:

Temperature:

Relative humidity:

##### *[Insert acclimation conditions. Were they the same as those reported during testing?]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *No acclimation period is necessary.*

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213 and 214*** *No acclimation period recommended. Moribund bees should be rejected and replaced before starting the test. If tests have* to be conducted in early spring or late autumn, bees can be emerged in an incubator and reared for one week with “bee bread” (pollen collected from the comb) and sucrose solution.

## Test chamber - description and size:

##### *[Insert details of cage size and construction]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *Tests should be conducted indoors with bees maintained in small test chambers made of metal, plastic, wire mesh* or cardboard. Chambers must be constructed so that a vial containing sugar water may be attached.

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213 and 214*** *Easy-to-clean and well-ventilated cages may be made from any appropriate material, e.g., stainless steel, wire mesh,* plastic, disposable wooden cages, etc. The size of test cages should be appropriate to the number of bees.

## Route(s) of exposure:

##### *[Describe route of exposure and topical application apparatus, if applicable.]*

***U.S. EPA OCSPP 885.4380*** *When the MPCA may be expected to act by a dietary route of exposure or are particles of such a size that they* might be carried back to the hive like pollen, the honey bees must be dosed orally. Testing in the hive may be necessary.

***U.S. EPA OCSPP 850.3020*** *Topical application.*

***PMRA DIR 2001-02*** *The MPCA can be administered topically, in soil, in the diet, or as a combination of these routes of exposure.*

***OECD 213*** *Dietary (oral) exposure.*

***OECD 214*** *Topical application.*

## Dose levels / test concentrations:

#### Nominal:

Measured: *(from confirmation of dose viability)*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations in this guideline. From* ***U.S. EPA OCSPP 885.4340 Nontarget Insect Testing*** *The dosage should be in suitable increments up to 100× the LD50 or LC50 of the pathogen in its natural host, or 10–100× the recommended field dosage.* ***From U.S. EPA OCSPP 885.4000 Background for Nontarget Organism Testing of Microbial Pesticide Control Agents*** *For Tier I tests, the Agency suggests that a maximum hazard dosage be administered. For all testing, the maximum dose should be no less than the maximum hazard dose as defined in the testing guideline. If the MPCA produces significant toxic or pathogenic effects at the maximum hazard dose level, testing at lower doses would be indicated. Sufficient doses and test organisms would be required to determine an LD50/LC50 value, if possible.*

***U.S. EPA OCSPP 850.3020*** *A minimum of five dosage levels of the test substance should be used in the definitive test. These levels should be* spaced geometrically. The recommended spacing is for each dosage level to be at least 60 percent of the next higher level. Ideally, dosage levels should be spaced so that at least three levels result in mortality between 0 and 100 percent. For test substances expected to have relatively low toxicity, a limit test may be conducted at 25 mg per bee. The LD50 may be reported as greater than 25 mg per bee if 20 bees are dosed at 25 mg per bee, if no mortality occurs, and if test procedures, number of controls, and duration are the same as in a definitive test. Signs of intoxication should be reported. If no mortality occurs, further testing is not required.

***PMRA DIR 2001-02*** *For topical exposure, arthropods should be exposed to a concentration of the MPCA that is equivalent to 100× the* maximum rate of application. For application to soil, a concentration ≥106 active units of the MPCA per gram of soil is used, or 1000 × the expected environmental concentration immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water, whichever is greater or achievable. For dietary administration, feed should be spiked with the maximum concentration of the MPCA expected in the target organism (or treated at 100× the maximum label rate). Alternatively, target organisms that have been maximally infected with the MPCA may be fed to the test organism.

***OECD 213*** *The number of doses and replicates should meet statistical requirements for determination of an LD50 with 95% confidence limits.* Normally 5 doses in a geometric series, with a factor not exceeding 2.2, and covering the range for LD50 are required. Alternatively, a limit test using 100 µg a.i./bee may be conducted to demonstrate an LD50 < 100 µg/bee. If mortalities occur, a full study should be conducted. Each test group of bees may be starved for up to 2 h prior to the introduction to the test chamber of 100–200 µL of 50% sucrose solution in water, containing the test substance at the appropriate concentration. A larger volume may be required for products of low solubility, low toxicity or low concentration in the formulation. The amount of treated diet per group should be monitored. Once consumed (usually within 3–4 h), the feeder is removed from the cage and replaced with one containing 50% sucrose solution alone.

***OECD 214*** *The number of doses and replicates should meet statistical requirements for determination of an LD50 with 95% confidence limits.* Normally 5 doses in a geometric series, with a factor not exceeding 2.2, and covering the range for LD50 are required. Alternatively, a limit test using 100 µg a.i./bee may be conducted to demonstrate an LD50 < 100 µg/bee. If mortalities occur, a full study should be conducted.

*Anaesthetized bees are individually treated by topical application. A 1-µL volume of solution containing the test substance at the suitable* concentration should be applied with a microapplicator to the dorsal side of the thorax of each bee. Other volumes may be used, if justified.

## Preparation of dose or test concentration:

##### *[Briefly describe methods for dose preparation.]*

***U.S. EPA OCSPP 885.4380*** *The actual form of the material to be regarded as the test substance is discussed in in OCSPP Guideline: 885.0001- under section (g)(1)(i-vi).* ***From U.S. EPA OCSPP 885.4000 Background for Nontarget Organism Testing of Microbial Pesticide Control Agents*** *Testing the technical grade of the active ingredient (TGAI) applies in all tests except the simulated and actual field testing (OPPTS 885.4900), where the use of the formulated product applies in order to simulate or reproduce actual field use. In some cases the technical grade of the active ingredient and the formulated product may be identical.*

***U.S. EPA OCSPP 850.3020*** *The test substance is generally administered in a solvent but a dust diluent is also acceptable. Maximum dosage* volume should not exceed 5µL per bee to allow for adequate volatilization of the solvent.

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213*** *If the test substance is water miscible, this compound may be dispersed directly in a 50% sucrose solution. For technical products* and substances of low water solubility, vehicles may be used. A volume of 100-200 µL of test solution at a suitable concentration is to be provided to the replicate chambers. A larger volume, however, may be required for products of low solubility.

***OECD 214*** *The test substance is to be applied as solution in a carrier, i.e. an organic solvent or a water solution with a wetting agent. A volume* of 1 µL of test solution at a suitable concentration is to be applied to the dorsal side of the thorax of each bee.

**Solvent/vehicle:** *[if used]*

##### *[Describe the type and percentage, if used]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *Acetone is preferred, but other volatile organic solvents have been used. If a dusting apparatus is used, a nontoxic* dust diluent will be required as a carrier.

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213*** *Where the test substance is a water miscible compound this may be dispersed directly in 50% sucrose solution. For technical* products and substances of low water solubility, vehicles such as organic solvent, emulsifiers or dispersants of low toxicity to bees may be used (e.g., acetone, dimethylformamide, dimethylsulfoxide). The concentration of the vehicle depends on the solubility of the test substance, and should be the same for all concentrations tested. A concentration of 1% is generally appropriate and should not be exceeded.

***OECD 214*** *The test substance is to be applied as a solution in a carrier, i.e., an organic solvent or a water solution with a wetting agent. As* organic solvent, acetone is preferred but other organic solvents of low toxicity to bees may be used (e.g., dimethylformamide, dimethylsulfoxide). For water dispersed formulated products and highly polar organic substances not soluble in organic carrier solvents, solutions may be easier to apply if prepared in a weak solution of a commercial wetting agent.

## Confirmation of MPCA viability:

##### *[Describe methods used to confirm the concentration and/or viability of the MPCA in the dosing* suspensions.]

***U.S. EPA OCSPP 885.4380*** *No specific recommendations, however, the viability of the MPCA in the test substance should be confirmed.* ***From***

***U.S. EPA OCSPP 885.4000 Background for Nontarget Organism Testing of Microbial Pesticide Control Agents*** *The concentration of MPCA in the water or food must be monitored to ensure that the test organisms are exposed to a sufficient MPCA level throughout the test period.*

***U.S. EPA OCSPP 850.3020*** *No specific recommendations (guideline developed for chemical toxicity testing).*

***PMRA DIR 2001-02*** *Viability or potency of the MPCA in the dosing suspension should be confirmed. No specific methods are recommended.*

***OECD 213*** *No specific recommendations (guidelines developed for chemical toxicity testing).*

***OECD 214*** *No specific recommendations (guidelines developed for chemical toxicity testing).*

## Positive control / reference material: *[if used]*

##### *[Insert a description of the reference material, with the number of arthropods treated and frequency of* testing (if not concurrent).]

***U.S. EPA OCSPP 885.4380*** *No specific recommendations in this guideline.* ***From U.S. EPA OCSPP 885.0001 Overview for Microbial Pest* Control Agents** *Positive controls generally are not required unless to serve as internal quality controls, demonstrate known test organism* sensitivity and respond to known toxic or infective agents, and/or to ascertain if a strain or species reacts similarly to another strain or species when exposed to the same known or standard toxicant or infective agent.

***U.S. EPA OCSPP 850.3020*** *A concurrent positive control with a substance of known toxicity is not required. However, a quarterly or* semiannual test with a laboratory standard (reference toxicant) is recommended as a means of detecting possible interlaboratory or temporal variation. A laboratory standard is also recommended when there is any significant change in source of bees.

***PMRA DIR 2001-02*** *No reference toxicant substance is required, but for all tests, the activity level of the MPCA should be related to its* pesticidal capability by running parallel studies in which target pests or hosts are exposed to the MPCA. Alternatively, the activity of the MPCA, in terms of viability can be assessed by another technique, e.g., culturing on a synthetic medium. In either case, the activity of the MPCA used in the test must be equal to or greater than the activity of the MPCA in the EP to be registered.

***OECD 213 and 214*** *A toxic standard (e.g. dimethoate; 24 h LD50 ~0.10–0.30 µg/bee) should be included in the test series, with at least 3 doses* to cover the expected LD50 value.

## Other controls:

##### *[Insert a description of any other control group included in the test.]*

***U.S. EPA OCSPP 885.4380*** *A concurrent control group should be treated with microbe-free (or nonviable microbe) material from the culture* system used for the propagation of the MPCA. ***From U.S. EPA OCSPP 885.0001 Overview for Microbial Pest Control Agents*** *All controls shall,* to the extent possible, be from the same source, be of the same age, receive the same care, and receive the same nutrients as the animals or plants receiving the test substance. To prevent bias, a method to assign organisms to treatment and control groups randomly is required and must be referenced in the report.

***U.S. EPA OCSPP 850.3020*** *Two concurrent controls are required during the test: a negative control and a solvent (or carrier) control, if a* volatile organic solvent or dust diluent is used. Negative control bees should receive no treatment. Solvent (carrier) control bees receive a volume of solvent or vehicle equal to the largest volume administered to test bees (maximum 5 µL/bee.). The use of shared controls is acceptable for concurrent tests as long as the same solvent or carrier is used for all the tests.

***PMRA DIR 2001-02*** *A negative, no-dosed control group of the non-target organism should be run concurrently with the test group.*

***OECD 213*** *Appropriate control solutions should be prepared, i.e. where a solvent or a dispersant is used to solubilize the test substance, two* separate control groups should be used: a solution in water, and a sucrose solution with the solvent/carrier at the concentration used in dosing solutions.

***OECD 214*** *Appropriate control solutions should be prepared, i.e. where a solvent or a dispersant is used to solubilize the test substance, two* separate control groups should be used, one treated with water, and one treated with the solvent/dispersant.

## Number of bees per chamber:

#### Control(s): Treatment(s):

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *Bees at a treatment level may be divided into replicates if desired.*

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213 and 214*** *Ten bees per replicate (chamber) are preferred.*

## Number of replicates (chambers) per treatment:

#### Control(s): Treatment(s):

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *At minimum of 25 bees is needed for each treatment group and control.*

***OECD 213 and 214*** *A minimum of 3 replicate test groups should be dosed with each test concentration, and three replicate controls are* required.

***PMRA DIR 2001-02*** *A sufficient number of test organisms must be treated to allow for adequate controls, statistical analysis, interpretation of* data and for interim sacrifice, if applicable. The number in each test group will depend on the species to be tested, the expected duration of the study, whether single or multiple groups are to be treated.

**Recovery of MPCA from bees:** *[if applicable]*

##### *[Describe methods used to recover the MPCA from collected samples.]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *No specific recommendations (guidelines developed for chemical toxicity testing).*

***PMRA DIR 2001-02*** *Required for MPCAs that are pathogens. Various methods for detection may be employed to detect the MPCA but it* should be appropriate for both the organism (e.g., bacterium) and the mode of action of the MPCA.

***OECD 213 and 214*** *No specific recommendations (guidelines developed for chemical toxicity testing).*

## Feeding:

##### *[Describe the feeding regime used during the experiment.]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations. usually ad libitum*

***U.S. EPA OCSPP 850.3020*** *A 50% sugar/water solution should be provided ad libitum throughout the holding and test periods. Purified or* distilled water should be used for the sugar solution.

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213*** *Sucrose solution in water with a final concentration of 500 g/L (50% w/v) is used as food. After given test doses, food should be* provided ad libitum. The feeding system would allow recording food intake for each cage. A glass tube (ca. 50 mm long and 10 mm wide with the open end narrowed to about 2 mm diameter) can be used.

***OECD 214*** *Sucrose solution in water with a final concentration of 500 g/L (50% w/v) should be used as food and provided ad libitum during the* test time, using a bee feeder. This can be a glass tube (ca 50 mm long and 10 mm wide with the open end narrowed to about 2 mm diameter).

**Test conditions:** Temperature: Humidity: Lighting:

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *Temperature between 25°C and 35°C, relative humidity between 50% and 80%. Bees should be maintained in the* dark except during dosing and observation.

***PMRA DIR 2001-02*** *No specific recommendations.*

***OECD 213 and 214*** *Bees should be held in the dark in an experimental room at a temperature of 25 ± 2°C with a relative humidity of 50% to* 70%.

## Duration of the study:

***U.S. EPA OCSPP 885.4380*** *Control and treated bees should be observed for at least 30 days after dosing.*

***U.S. EPA OCSPP 850.3020*** *The definitive test consists of the administration of the test substance followed by an observation period of 48 h.* ***PMRA DIR 2001-02*** *For topical exposure, the test organism should be treated daily for 5 days, then observed for an additional 16 days. For* dietary or environmental exposure, the test organism should be exposed for at least 21 days, or until mortality in the control group increases to a significant level.

***OECD 213*** *The duration of the test is 48h after the test solution has been replaced with sucrose solution alone. If mortality continues to rise by*

*>10% after the first 24h, the test duration should be extended to a maximum of 96h, provided that the control mortality does not exceed 10%.* ***OECD 214*** *The duration of the test is 48h. If mortality increases by more than 10% between 24h and 48h, the test duration should be extended* up to a maximum of 96h provided that control mortality does not exceed 10%.

## Other methods or conditions, if any:

* 1. **Observations:**

**Parameters measured including sublethal effects/toxicity symptoms:**

##### *[List the parameters measured during the experiment, e.g., mortality, survival, abnormal behavior or* appearance, fecundity, growth inhibition, concentration of the MPCA in the test suspensions. Provide references to data summary tables, if used.]

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.*

***U.S. EPA OCSPP 850.3020*** *Measurements of approximate room temperature, humidity and lighting are required. All signs of intoxication,* abnormal behavior and mortality should be recorded throughout the test period and reported by dosage level and by time of occurrence. Signs of intoxication may include ataxia, lethargy and hypersensitivity. Dead bees should not be removed from the test chambers until the test is terminated.

***PMRA DIR 2001-02*** *Regular observations are required to record mortalities and note any behavioral, pathogenic or toxic adverse effects.* ***OECD 213*** *Measurements of temperature, test room humidity, and group diet consumption are required. Observations for mortality and* abnormal behavior of bees in each chamber.

***OECD 214*** *Measurements of temperature and test room humidity are required. Observations for mortality and abnormal behavior of bees in* each chamber.

## Observation/measurement intervals:

##### *[List time points for each parameter measurement and observation.]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations.* ***From U.S. EPA OCSPP 885.0001 Overview for Microbial Pest Control Agents***

*Method, frequency, and duration of observations made during the study are to be reported.*

***U.S. EPA OCSPP 850.3020*** *No recommendations are given for environmental parameters such as temperature or humidity. Bees should be* observed for mortality and toxicological responses at approximately 4, 24 and 48h after dosing.

***PMRA DIR 2001-02*** *Regular observations are required to record mortalities and note any behavioral, pathogenic or toxic adverse effects.* ***OECD 213 and 214*** *No recommendations are given for environmental parameters such as temperature or humidity. The amount of diet* consumed should be determined following treatment (OECD 213 only). Mortality is recorded 4 h after dosing and thereafter at 24 h and 48 h. If a prolonged observation period is required, further assessments should be made at 24 h intervals, up to a maximum of 96h, provided that control mortality does not exceed 10%.

## Testing for infectivity/pathogenicity:

##### *[Briefly describe how infectivity was tested, and list the organs, tissues or fluids tested, if applicable]*

***U.S. EPA OCSPP 885.4380*** *No specific recommendations. From* ***U.S. EPA OCSPP 885.0001 Overview for microbial pest control agents (MPCA)****- infectivity tests often require sophisticated assessment methods for detecting sublethal pathogenic effects. These methods may include serological or nucleic acid technology.*

***U.S. EPA OCSPP 850.3020*** *No specific recommendations (guidelines developed for chemical toxicity testing).*

***PMRA DIR 2001*** *For MPCAs that are pathogens, pathogenicity testing should be performed. The specific test method used should match the* infectivity requirements of the pathogen and host and should be capable of detecting both infection and disease symptoms. When the MPCA is not a pathogen, applicants can rely on standard toxicity test methods.

***OECD 213 and 214*** *No specific recommendations (guidelines developed for chemical toxicity testing).*

## Were raw data included? Other observations, if any:

1. **RESULTS**
2. **VIABILITY OF DOSING SUSPENSIONS:** *[Summarize the dose verification data and indicate if the tested sample was still viable.]*

**TABLE *[#]*.** Viability of *[test substance]* in the *[dosing suspension/diet]* administered to honey bees (*Apis mellifera*) in a *[contact, acute oral or dietary]* test.

|  |  |  |
| --- | --- | --- |
| **Dose Group** | **Nominal Concentration *[units]*** | **Measured Concentration *[units]*** |
| *Solvent/vehicle control* |  |  |
| *Inactivated control* |  |  |
| *Sterile filtrate control* |  |  |
| *Maximum hazard dose* |  |  |
| Negative control |  |  |

##### **MORTALITY:** *[[Briefly summarize mortality results (if any). If values for LD50, LC50, LT50,* NOEL, NOEC are greater than the MHD level, use ***<*** *symbol. Comment on dose response relationship;* Slope of response, if provided. Compare the mortality with control treatment and/or the reference chemical. Data may be summarized in a table such as those presented below. Modify table to accommodate differences in experimental design.]

***From U.S. EPA OCSPP 885.0001 Overview for Microbial Pest Control Agents*** *The Agency realizes that it would be very difficult to establish* specific LC50, ED50, or LD50 values (e.g. LD50 = 1,000 mg/kg) and 95 percent confidence limits for most MPCAs whose mechanism of action is pathogenicity, because test data are not likely to exhibit a log-probit dose-response relationship that is typical of chemical pesticides. Therefore, data that establishes an LC50, ED50, or LD50 that is greater than the maximum hazard dosage level (e.g. LD50 >1,000 mg/kg) would often be adequate for the purposes of hazard assessment and reporting in this section.

**TABLE *[#]*.** Effect of *[test material]* on cumulative mortality of honey bees (*Apis mellifera*) in a *[contact, acute oral or dietary]* test.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments *[indicate if nominal or measured (measured***  ***should be used, if provided)]*** | | **No. of Bees** | **Observation Period** | | | | | |
| ***Day x1*** | | ***Day x2*** | | ***Day n*** | |
| **No. Dead** | **%**  **Mortality** | **No. Dead** | **%**  **Mortality** | **No. Dead** | **%**  **Mortality** |
| Negative control | |  |  |  |  |  |  |  |
| Solvent control, if used | |  |  |  |  |  |  |  |
| *test concentration 1* | |  |  |  |  |  |  |  |
| *test concentration 2* | |  |  |  |  |  |  |  |
| *test concentration3* | |  |  |  |  |  |  |  |
| *test concentration 4* | |  |  |  |  |  |  |  |
| *test concentration n* | |  |  |  |  |  |  |  |
| LD50/LC50  *[insert [***>***] if greater than]* | |  | | | | | | |
| NOEL/NOEC  *[insert [***>***] if greater than]* | |  | | | | | | |
| *Reference chemical* | *Mortality (% or No.)* |  |  |  |  |  |  |  |
| *LD50: / LC50:* | *[insert [***>***] if greater than]* | | | | | | |
| *NOEL / NOEC* | *[insert [***>***] if greater than]* | | | | | | |

*[a Use superscript and footnote to indicate values that are statistically significantly different from control.]*

##### **SUB-LETHAL TOXICITY EFFECTS:** *[Include if any sublethal effects are observed- Briefly* summarize behavioral abnormalities or other signs of toxicity. . Indicate effects that were related to the

*test-material. Compare sub-lethal effects with control treatment and/or the reference chemical. Data may be summarized in a table such as those presented below. Modify tables to accommodate differences in experimental design. For acute oral and dietary, provide information about palatability of the treated diet, rate of consumption of diet in treated and untreated groups.]*

**TABLE *[#]*.** Effect of *[test material]* on *[endpoint]* of honey bees (*Apis mellifera*) in a *[contact, acute oral or dietary]* test.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments *[indicate if nominal or measured (measured***  ***should be used, if provided)]*** | | **Observation Period** | | | | | |
| ***Day x1*** | | ***Day x2*** | | ***Day n*** | |
| ***endpoint 1*** | **%**  **Affected** | ***endpoint 2*** | **%**  **Affected** | ***endpoint n*** | **%**  **Affected** |
| Negative control | |  |  |  |  |  |  |
| Solvent control, if used | |  |  |  |  |  |  |
| *test concentration 1* | |  |  |  |  |  |  |
| *test concentration 2* | |  |  |  |  |  |  |
| *test concentration3* | |  |  |  |  |  |  |
| *test concentration 4* | |  |  |  |  |  |  |
| *test concentration n* | |  |  |  |  |  |  |
| ED50/EC50 or other sublethal endpoint *[insert [***>***] if greater than]* | |  | | | | | |
| NOEL/NOEC  *[insert [***>***] if greater than]* | |  |  |  |  |  |  |
| *Reference chemical* | *LC50/ LC50* | *[insert [***>***] if greater than]* | | | | | |
| *NOEL/ NOEC* | *[insert [***>***] if greater than]* | | | | | |

##### **REPORTED STATISTICS:** *[If applicable- List the parameters that were analyzed and the statistical* tests that were performed.]

***U.S. EPA OCSPP 885.4380*** *No specific recommendations in this guideline. From* ***OCSPP 885.0001 Overview for microbial pest control agents (MPCA)****- Appropriate statistical shall reflect the current state-of-the art. All data averages or means must be accompanied by standard deviation and the standard errors of the means should also be calculated; however, notations of statistically significant differences accompanied*

*by the confidence level or probability should also be used in place of standard error determinations. Other methods of expressing data dispersion* may also be used when appropriate.

***U.S. EPA OCSPP 850.3020*** *Probit analysis, moving averages or binomial probability is used to calculate LD50, with 95% confidence limits and* the slope of the dose-response curve for mortality at test end.

***PMRA DIR 2001-02*** *All relevant analyses of results must be provided. May attach a copy of the statistical methods from the study with a* statement that the reviewer has no objections to the analyses used

***OECD 213 and 214*** *Data should be summarized in tabular form, showing for each treatment group, as well as control and toxic standard* groups, the number of bees used, mortality at each observation time and number of bees with adverse behavior. Mortality should be analyzed using appropriate statistical methods (e.g., Probit analysis, moving-average, binomial probability. Plot dose-response curves at each recommended observation time (i.e., 24, 48, and if relevant 72 and 96 hours) and calculate the slopes of the curves and the median lethal doses with 95% confidence limits. Corrections for control mortality could be made using Abbott’s correction. Where treated diet is not completely consumed, the dose of test substance consumed per group should be determined.

1. **VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:** *[If applicable- Report the statistical methods used by the reviewer to verify the applicant’s results.][ If values for LD50, LC50, LT50, NOEL, NOEC are greater than the MHD level, use* ***<*** *symbol.]*

|  |  |  |
| --- | --- | --- |
|  | LD50: | 95% C.I.: |
| LC50: | 95% C.I.: |
| NOEL: |  |
| NOEC: |  |
| Probit Slope: | 95% C.I.: |
| **III.** | Endpoint(s) Affected:  **CONCLUSION** |  |
| **A.** | **STUDY AUTHOR CONCLUSION:** | *[Summarize the study author’s conclusions- Provide the major* |

##### *conclusions e.g., values for LD50, LC50, LT50, EC50, NOEL, NOEC, etc.* were *[****=, > or <****] insert final dose* concentration/level (in appropriate units).]

1. **REVIEWER’S COMMENTS:** The reviewer agrees *[does not agree]* with the study author’s conclusion. *[Provide additional comments that do not appear under other sections of the template. Discuss the specific methods/ results/findings that may affect the validity of the study and overall acceptability of the study.]* The study was *[not]* conducted in accordance with the guideline recommendations for a *[contact, oral or dietary]* toxicity study for honey bee testing (OCSPP 885.4380; PMRA: M9.5.1 and OECD: IIM 8.7, IIIM 10.3) in the *[species]*.

##### **DEFICIENCIES:** *[List each deficiency with the required data to resolve the deficiency or if no* data can be provided to satisfy the deficiency.]

***U.S. EPA OCSPP 885.4380*** *No specific validity criteria.*

***U.S. EPA OCSPP 850.3020*** *The test is unacceptable if there is more than 20% mortality in controls.*

***PMRA DIR 2001-02*** *No specific validity criteria.*

***OECD 213 and 214*** *For a test to be valid, the following conditions apply: the average mortality for the total number of controls must not exceed* 10% at the end of the test, and the LD50 of the toxic standard meets the specified range.

## CLASSIFICATION: [ACCEPTABLE / UNACCEPTABLE / SUPPLEMENTAL, but UPGRADEABLE]

##### **IV. REFERENCES:** *[Provide full citations of references that were cited in the study report: methods,* SOPs protocols, references to other relevant study reports in the submission or other studies conducted by the applicant.

[***NOTE: If methods/protocols contain specific methodology that is not reported in detail in study report as requested in DER- include specific literature of method/SOP/protocol attached as an appendix and attached to the study report for the reviewer’s reference and verification of rationale. If no extra references were used, state “No references were cited.”].***

***(This section of the DER represent the format for submitting alternative data for satisfying data requirement and supporting scientific rationale to justify the use of alternative data Alternative data include: waiver request(s), published study, and/or mini-literature review.***

***(Formatting instructions: Use cover page (first page of template) and include a brief executive summary of the waiver request/published study/OR mini- literature review (see example below) and its classification. Delete study template and proceed to the following sections)***

**EXECUTIVE SUMMARY *[FOR EXAMPLE]:*** *[Applicant]* is submitting a justification for a data waiver from honey bee testing studies (OCSPP 885.4380). The waiver request is based on the rationale that *[name of active ingredient]* is a naturally-occurring *[soil/water/plant-surface. etc.]* colonizer, whose level in the environment will not significantly increase with the use of *[product name]* and that an extensive literature search yielded no *[or no significant]* reports of adverse effects in terrestrial *[and aquatic, if applicable]* honey bees.

The proposed uses of *[product name]* on *[identify use sites/crops]* is not expected to result in increased exposure or adverse effects to honey bees. *[If environmental concentration will show a substantial increase, give the rate of environmental reduction to background levels in days/weeks/months].* Therefore, additional testing is not considered necessary to assess the risks of the *[product name]* to honey bees. The *[applicant]* requests a waiver of honey bee testing.

# *(For a waiver request, otherwise delete)*

##### **WAIVER RATIONALE:** *[Summarize the information and/or data presented by the author* justifying why the required data element should be waived for the MPCA, TGAI, MP, or EP.]

The waiver request is based on the following rationales:

* 1. **Increased environmental exposure to *[name of active ingredient]*, due to use of the end-use product *[product name]*, will be minimal to** *honey bees****.*** *[Applicant should provide further elaboration: Describe the natural habitat of the MPCA. Is it an obligate parasite/epiphyte? Is it ubiquitous in nature (give geographical distribution)? Has the MPCA, and/or phylogenetically close species/strains, been isolated from soil/streams/ponds/lakes and a variety of plant surfaces including (identify names) crops/vegetables/fruits? Provide the known natural concentration of the MPCA in CFU/(weight-volume- surface area) in these environmental niches.]*

##### Use of *[product name]* will be limited to *[soil, seed, foliar, greenhouse, etc.]* applications *[by spray, dip,* soil incorporation, aerial, etc.] on *[name crops/use sites]*, thus minimizing direct exposure to non-target insects. *[Does timing of application preclude direct exposure to honey bees? Discuss crop use sites and* application methods and its effects on limiting runoff, if applicable. Provide the rate in environmental reduction of the MPCA to background levels in days/weeks/months, if available. Include any other factors that would limit exposure to honey bees and other terrestrial arthropods. Would any of the MPCA that reaches the soil/water behave as it would in the wild?]

* 1. **No evidence of adverse effects.** A literature search of the *[e.g., AGRICOLA, TOXLINE, BIOLOGICAL ABSTRACTS, CHEMTOX, PUBMED, HAZARDOUS AND REGULATED CHEMICALS DATABASE or OTHER]* databases for the period *[year range]* was conducted. In this literature search, *[name of MPCA]* and other phylogenetically close species/strains in the *[family/genus/species-group, etc., as appropriate]*, as well as synonyms *[name of synonyms of MPCA, if any]* were used as the search words. The searches were also used to ascertain the known production of *[genotoxic, carcinogenic, allergenic, mutagenic, toxic]* metabolites, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with honey bees and other terrestrial nontarget insects. *[Include the metabolites found to be produced - does the MPCA strain also produce these or other metabolites? Have natural populations of the MPCA or its metabolites been associated with adverse effects in honey bee species?]*

##### *[Discuss whether runoff or overspray would result in effects not seen from the naturally occurring MPCA* levels. Does the MPCA appear on any authoritative list of insect/arthropod pathogens, particularly honey bees and other pollinators as well as beneficials such as predators and parasites? Provide the lists examined. Have any adverse effects to honey beers or other wild populations of terrestrial nontarget arthropods been reported due to naturally occurring populations of the MPCA?]

**[*NOTE: All statements used as justification to support the scientific rationale for the waiver rationale should be individually supported by a reference (i.e. studies in the open literature, references to other study reports in the submission and/ or other studies conducted by the registrant/applicant). Include specific details and/or excerpts of relevant data/information from individual references. Supporting data include: background information of MPCA (e.g. previously reported characterization data related to its identity, mode of action, its nature, prevalence and/or interactions in the environment), supporting evidence/rationale for lack of adverse effects and lack (or minimal) environmental exposure to nontarget species, history of safe use, and/or significant similarities to other microbial strains.*]**

1. **CONCLUSION**
2. **STUDY AUTHOR CONCLUSION:** *[Summarize the study author’s conclusions]*
3. **REVIEWER’S COMMENTS:** *[Note if in agreement with study authors.]*

##### **DEFICIENCIES:** *[List each deficiency with the required data to resolve the deficiency or if no data* can be provided to satisfy the deficiency.]

1. **CLASSIFICATION: [ACCEPTABLE / UNACCEPTABLE / SUPPLEMENTAL, but UPGRADEABLE]**
2. **REFERENCES:** *[List references that were cited in the study report]*

***[NOTE: Depending on the level of relevance- copies of published literature and any other supporting literature that support the use of alternative data/waiver rationale (including other studies reporting similar findings) should be provided as an appendix and attached to the study report for the reviewer’s reference and verification of rationale.]***

***(For a published study, otherwise delete)***

1. **PURPOSE:** *[Indicate the purpose of the study]*

##### **METHOD:** *[Describe the experimental procedure]*

1. **RESULTS:** *[Summarize the results using appropriate headers e.g.,* ***A. GENERAL OBSERVATIONS:***

***B. DETECTABLE LEVELS OF MPCA ON INSECT:]***

1. **CONCLUSION**
2. **STUDY AUTHOR CONCLUSION:** *[Summarize the study author’s conclusions]*
3. **REVIEWER’S COMMENTS:** *[Note if in agreement with study authors.]*

##### **DEFICIENCIES:** *[List each deficiency with the required data to resolve the deficiency or if no data* can be provided to satisfy the deficiency.]

1. **CLASSIFICATION: [ACCEPTABLE / UNACCEPTABLE / SUPPLEMENTAL, but UPGRADEABLE]**
2. **REFERENCES:** *[Provide references that were cited in the study report: methods, studies in the open literature, references to other study reports in the submission or other studies conducted by the applicant.].*

**[*NOTE: Include a copy of the published study and/or previously conducted unpublished study in the study report as an appendix attached to the study report for the reviewer’s reference and verification of study details. Any additional statements used as justification to support the use of alternative data should be individually cited- including the specific background information, details and/or excerpts of relevant data/information from individual references. Depending on the level of relevance- copies of published literature and any other supporting literature that support the use of a published study or previously conducted study as alternative data (including other studies reporting similar findings) should also be provided in the appendix.*]**

***(For a mini literature review, otherwise delete)***

1. **REVIEW OF PUBLISHED LITERATURE:** *[Summarize the background information and published studies covered in this mini literature review. Grouping related papers for discussion under specific subheadings may be useful.*

*e.g., MPCA-based products are widely used in forest management to control forest pests in Canada and the United States ... As noted by Lubbock (1870), three approaches have been used in Canada to examine the effects of this MPCA on non-target invertebrates. These include acute toxicity testing, experiments in outside mesocosms, and in the field.*

* 1. *.,* ***A. ACUTE TOXICITY TESTING:***
     1. ***Article 1:*** *(summarize and report findings)*
     2. ***Article 2:*** *(summarize and report findings)*

### *MESOCOSM TESTING:*

* + 1. ***Article 1:*** *(summarize and report findings)*
    2. ***Article 2:*** *(summarize and report findings)*

### *FIELD TESTING:*

* + 1. ***Article 1:*** *(summarize and report findings)*

***2 Article 2:*** *(summarize and report findings)]*

1. **CONCLUSION**
2. **LITERATURE REVIEW CONCLUSION:** *[Summarize the study author’s conclusions]*
3. **REVIEWER’S COMMENTS:** *[Note if in agreement with study authors.]*

##### **DEFICIENCIES:** *[List each deficiency with the required data to resolve the deficiency or if no data* can be provided to satisfy the deficiency.]

1. **CLASSIFICATION: [ACCEPTABLE / UNACCEPTABLE / SUPPLEMENTAL, but UPGRADEABLE]**
2. **REFERENCES:** *[Provide references that were cited in the study report: methods, studies in the open literature, references to other study reports in the submission or other studies conducted by the applicant.].*

**[*NOTE: Depending on the level of relevance- copies of published literature, previously conducted unpublished study and any other background literature that support the use of a literature review as alternative data (including other studies reporting similar findings) should be provided as an appendix attached to the study report for the reviewer’s reference and verification of study details.*]**