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| **Primary Reviewer:** |  |  | **Date:** |
|  | ***[Name, title, and affi*** | ***liation]*** |  |
| **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:** Nontarget Insect Testing, Tier I

#### U.S. EPA OCSPP Guideline: 885.4340

PMRA Data Code: M9.5.1–Terrestrial arthropods

[***OR*** M9.6–Non-arthropod invertebrates]

OECD Data Code: IIM 8.8, IIIM 10.4

[**OR** IIM 8.9. IIIM 10.5 (earthworms)]

**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 Unpublished *[OR if published, list Journal name, vol.: pages]*.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 *[contact, acute oral or dietary]* toxicity and pathogenicity study, *[common name (scientific name)]* were exposed to a *[single OR #] [indicate exposure method]* dose of *[dose amount]* of *[formulation, note its potency, biological activity and/or concentration per unit weight or volume]* (containing % *a.i. name*). *[Include other pertinent details such as the controls used.]*

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

The *[#]*-day LC50 *[or LD50]* was *[****=, > or <****] [insert LC50 or LD50 in appropriate units]. [If the study included sublethal test endpoints and/or sublethal effects were observed and/or additional subchronic testing was triggered include the following text:* The EC50 *[or ED50]* based on sublethal effects was *[****=, > or <****] [insert EC50/ED50 in appropriate units]*. The NOEC *[or NOEL]* value, based on mortality *[and sublethal effects (if applicable)]*, was *[****=, > or <****] [insert NOEC/NOEL in appropriate units]*.

This study is classified as *[acceptable, unacceptable, supplemental].* This study was *[not]* conducted in accordance with the guideline recommendations for a *[contact, oral or dietary]* toxicity and pathogenicity study for nontarget insects (OCSPP 885.4340; PMRA: M9.5.1 and OECD: IIM 8.8, IIIM 10.4) 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 OCSPP 885.4340–Nontarget insect testing, Tier I.1* PMRA DIR 2001-02 Part 9.5.11

*Environment Canada EPS 1/RM/44 Section 13.2.31] [****OR*** *for Terrestrial non-arthropod invertebrate testing3- PMRA DIR 2001-02 Part 9.61*

*Environment Canada EPS 1/RM/44 Section 13.2.21*

*OECD 207–Earthworm acute toxicity tests2]*

*1 Guideline designed to test toxicity and pathogenicity of microbial agents.*

*2 Guideline designed to test acute toxicity of chemical agents.*

*3 Supplemental guidance for test procedures/reporting requirements are provided in seperate PRMA, Environment Canada EPS and OECD guidelines that are specific for nontarget insect testing on “Terrestrial non-arthropod invertebrate testing.” This guidance can be found under each guideline criterion (designated with underlined subheadings). If there is no additional guidance stated for “Terrestrial non-arthropod invertebrate testing,” then defer to each guideline criterion as guidance in general.*

**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 potency and/or concentration per unit weight or volume as* indicated 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):** *[Insert test species name(s).]*

***U.S. EPA OCSPP 885.4340*** *Testing should be performed on three species of insects that are important parasites or predators of the target host,* and/or representing at least two of the following groups - parasitic dipterans, predaceous hemipterans, predaceous coleopterans, predaceous mites, predaceous neuropterans, and parasitic hymenopterans. Selection of test species is the responsibility of the researcher, and a rationale for selection must be provided.

*–For viruses, testing shall be performed on three species of insects representative of those parasites and predators known or* suspected to attack the target host or to share the same ecological habitat.

*–For protozoa, at least one arthropod species should be a major (i.e. important) parasite of the target host. Many protozoans have a* wide host range. Accordingly, if possible, more than the minimum (three) species of non-target organisms should be tested.

*–For fungi, assuming testing is justified, the test species should be the major predacious and parasitic regulatory agents common to* the ecosystem where the MPCA will be applied. In addition, testing could include parasites and/or predators specific to the host of the fungal agent.

*–For bacteria, testing shall be performed on three species of insects representative of those parasites and predators known or* suspected to attack the target host or to share the same ecological habitat.

***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.

***Environment Canada EPS 1/RM/44*** *Collembolan springtail (Folsomia candida) is the test species.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***PMRA DIR 2001-02*** *Non-target non-arthropod invertebrate species selected for testing should be representative of groups that will be exposed* to the MPCA under actual conditions of use, 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.

***Environment Canada EPS 1/RM/44*** *The earthworm (Eisenia andrei) is the test species.*

***Environment Canada EPS 1/RM 43*** *The earthworms (Eisenia andrei, Eisenia fetida or Lumbricus terrestris) are the test species.*

***OECD 207*** *Eisenia fetida fetida is the preferred test species. Other species may be used if the necessary methodology is available.*

**Age at test initiation**: *[Insert age of test organisms (mean and range).]*

***U.S. EPA OCSPP 885.4340*** *No specific recommendations.*

***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.

***Environment Canada EPS 1/RM/44*** *Juvenile (10–12-day-old) springtails.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/43*** *Sub-adults or sexually mature adults with clitellum, if to be used in an acute lethality test. Clitellated* adults only for an acute avoidance or 8-week test.

***OECD 207*** *Worms should be adult (at least 2 months old, with clitellum).*

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

***U.S. EPA OCSPP 885.4340*** *Selection of the test species is the responsibility of the researcher. Rationale for selection must be provided.*

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

***Environment Canada EPS 1/RM/44*** *Laboratory-raised springtails are used.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/43*** *Cocoons, juveniles or adults from a government, private or commercial culture, all from the same source,* and identification to species confirmed. Worms cultured in 10–50 L plastic trays with perforated lid, and sides or lid transparent to allow light penetration to substrate surface. Substrate (e.g., mixture of potting soil, artificial soil and peat moss, or mixture of shredded un-inked paper, artificial soil and sphagnum peat moss) hydrated with sufficient distilled/deionized water to keep surface moist without standing water at bottom of culture chambers. Soil particles should not adhere to earthworms. Minimum substrate depth 10 cm, pH 6.0–7.5 (adjusted with CaCO3), temperature 20 ± 2 C, lighting incandescent or fluorescent 400–800 lux at substrate surface. Fixed photoperiod (e.g., 16 h light/8 h dark or 12 h light/12 h dark). Worms fed cooked oatmeal or alfalfa pellets saturated with water once per week after removing excess (unused) food from previous feeding. Substrate renewed as required, at least once every 2–3 months. The culture is healthy if worms move actively through the substrate, do not try to leave it and reproduce continually and results for reference toxicity test using worms from the culture fall with historic warning limits; discard culture if >10% of juvenile or adult worms are dead, inactive or unhealthy at any time.

***OECD 207*** *Cocoons can be purchased commercially or distributed from a central source to ensure the same strain is used.*

**Weight at test initiation:** *[mean and range]*

***Supplemental guidance for terrestrial non-arthropod invertebrates: PMRA DIR 2001-02*** *No specific recommendations.*

***Environment Canada EPS 1 RM/44*** *250–600 mg wet weight (choose worms as similar in wet weight as possible).*

***OECD 207*** *300–600 mg.*

**Date of collection:** *[Insert if applicable.]*

##### **Rationale:** *[Insert rationale for using this test organism, if applicable.]*

1. **STUDY DESIGN AND METHODS:**

*[Briefly describe the experimental design.]*

***U.S. EPA OCSPP 885.4340*** *Experimental design is highly dependent on the nature of the MPCA. Please see the guideline, and “Experimental* Methods and Conditions” below for details.

***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.

***Environment Canada EPS 1/RM/44*** *Collembolan springtails are exposed to the MPCA in the soil for 28 days, in a single-concentration test to* the maximum hazard concentration (MHC) in soil, or in a multi-concentration test to a range of at least 7 concentrations in soil, including the MHC.

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

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

***Environment Canada EPS 1/RM/43*** *Three experimental protocols are described:*

*–14-day lethality test: worms introduced into test chambers filled with a range of concentrations of the test substance mixed in soil,* mortality and number of worms on the soil surface assessed on Days 0, 7 and 14, LC50 calculated for Days 7 and 14;

*–Sublethal avoidance test: conducted in a circular container with 6 pie-shaped interconnecting compartments alternately filled with a* single concentration test soil or control soil; worms are introduced into a seventh, central, circular compartment that is not filled with soil. The compartment into which each worm migrates from the central compartment is recorded at study initiation. At 48 or 72 hours, the number of live/dead worms in each compartment is counted to calculate an EC50 for avoidance.

*–Prolonged exposure test: worms are introduced into test chambers filled with soil, in which a range of concentrations of the test* substance has been mixed. After 28 days exposure, adults are removed from soil, and mortality and growth effects recorded. Effects on reproduction are assessed after 56 days by counting the number of offspring present in the soil. The reproductive output is compared between treated and control worms to determine the NOEC and/or EC50.

***OECD 207*** *In an optional preliminary screening test, earthworms are exposed in glass vials to a test substance on moist filter paper, to identify* chemicals that are potentially toxic and will require more detailed testing in artificial soil. In the artificial soil test, the test substance is applied at a range of concentrations to aliquots of artificial soil in which earthworms are kept. Mortality is assessed 7 and 14 days after application.

## 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 885.4340*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *No specific recommendations.*

***OECD 207*** *No specific recommendations.*

## Test chamber - description and size:

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

***U.S. EPA OCSPP 885.4340*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Glass beaker or jar, 100-mL capacity, diameter ~5 cm, covered with a suitable lid.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *500 mL glass jar; perforated aluminum foil secured with a screw-top ring recommended as cover.* ***OECD 207*** *Glass containers: for filter-paper test, flat bottomed glass vials (8 cm long × 3 cm diameter); for artificial soil test, crystallizing* dishes or spoutless beakers ~1 L, covered with glass lids or perforated plastic film.

**Amount of soil per test chamber:** *[If used] [Give the mass or volume of soil.]*

***U.S. EPA OCSPP 885.4340*** *No specific recommendations.* ***PMRA DIR 2001-02*** *No specific recommendations.* ***Environment Canada EPS 1/RM/44*** *30 g (wet weight).*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *Identical wet weight, equivalent to a volume of ~350 mL (~200 g dry weight, if artificial soil).*

***OECD 207*** *None for filter paper test, 750 g of soil for artificial soil test.*

**Soil parameters:** *[If used]*

##### *[Indicate whether the test soil is natural or artificial (give soil constituents), and describe it.]*

Moisture content:

Water holding capacity (WHC): pH:

Conductivity:

Percent TOC:

Percent organic matter: Particle sizes (%sand/silt/clay):

***U.S. EPA OCSPP 885.4340*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Natural (uncontaminated) or artificial (laboratory formulated) soil. If field collected, hydrate if and as* necessary until a homogeneous crumbly texture is achieved. If artificial soil, hydrate to ~70% of water-holding capacity. The soil must enable the negative control groups included in the test to meet the validity criteria and must not cause any discernible adverse effects to test organisms. The testing facility should know the basic physicochemical characteristics of the soil (e.g., particle size distribution, percent water content, percent organic carbon, pH, metals, pesticides, petroleum hydrocarbons). The use of sterile soil is not recommended.

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***OECD 207*** *Soil prepared from 10% sphagnum peat (pH 5.5–6.0), 20% kaolin clay, 70% industrial sand (>50% of particles between 50 and 200*

*µm diameter); pH adjusted to 6.0 ± 0.5 with CaCO3. Moisture content 35% of dry weight.*

## Route(s) of exposure:

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

***U.S. EPA OCSPP 885.4340*** *Routes of exposure vary with the test substance, and with the expected environmental relationship between the* MPCA and test species.

*–For viruses, the best routes of exposure will depend on the developmental location of the non-target organism. Internal parasites* may be tested with virus-infected hosts, or if they can be cultured in vitro, the virus can be added to the diet. External stages of parasites and predators, if they are obligatory, may be fed virus-infected hosts, and others may be fed virus-contaminated media or virus suspended in sugar or honey solutions.

*–For protozoa: in addition to feeding adult predators and parasites of the target insect with the resistant stage of the protozoan,* immature stages of the predator or parasite should be exposed. Predators can be fed hosts infected with the protozoan over a period of time. The predator, at a prescribed time, should be checked for protozoan infection. The protocol for parasites is more complex. Protozoan-infected hosts can be parasitized or parasitized hosts can be fed protozoans. Parasites from protozoan-infected hosts should be examined for protozoan infection. The immature stages as well as adults should be examined. If possible, a primary hymenopterous and dipterous parasite should be examined. The best route of infection for adult Hymenoptera or Diptera is oral acquisition of protozoan spores. Predaceous insects could also be fed in this manner, but feeding them infected (live) hosts of known age is more appropriate. In either case, the actual amount of spores consumed cannot be accurately determined. If infection of the parasite or predator adult occurs, the possibility for transovarial or transovum transmission should be examined.

*–For fungi, routes of exposure should simulate field conditions as much as possible. In the case of entomopathogenic fungi,* environmental conditions (> 90 percent relative humidity) are critical at the time of exposure.

*–For bacteria, best routes of exposure will depend on developmental location of the non-target organism. Internal parasites may be* tested with bacteria-infected hosts, or if they can be cultured in vitro, the bacteria can be added to the diet. External stages may be fed bacteria- infected hosts, bacteria-contaminated media, or bacteria suspended in sugar or honey solutions.

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

***Environment Canada EPS 1/RM/44*** *Test substance is mixed in soil.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *In a single concentration study, the test material should be administered in both soil and diet. In a multi-* concentration study, the test material should be mixed in soil or diet.

***OECD 207*** *Test substance absorbed on filter paper (contact toxicity) or mixed into artificial soil.*

## Dose levels / test concentrations:

#### Nominal:

Measured: *(from confirmation of dose viability)*

##### *[List doses used, and insert calculation of the maximum hazard dose, where applicable]*

***U.S. EPA OCSPP 885.4340*** *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.

***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.

***Environment Canada EPS 1/RM/44*** *Single group tested at the maximum hazard concentration or 7 test concentrations, including the maximum* hazard concentration. The maximum hazard concentration for soil exposure is 106 active MPCA units/g soil or 1000× the EEC of the MPCA, following application to soil (whichever greater and readily attainable).

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***PMRA DIR 2001-02*** *No specific recommendations given for non-arthropod invertebrates. In general, a maximum hazard concentration is* considered to be 1000× the expected environmental concentration in a 15-cm depth of soil, or 106 MPCA units/g soil whichever is greater or achievable.

***Environment Canada EPS 1/RM/44*** *Soil exposure: Single group tested at the maximum hazard concentration or 7 test concentrations,* including the maximum hazard concentration. The maximum hazard concentration for soil exposure is 106 active MPCA units/g soil or 1000× the EEC of the MPCA, following application to soil (whichever greater and readily attainable). Dietary exposure: Single group tested at the maximum hazard concentration or 7 test concentrations, including the maximum hazard concentration. The maximum hazard concentration for dietary exposure is 100× the maximum concentration specified by the manufacturer for the tank mix is used.

***OECD 207*** *Five concentrations in a geometric series, calculated from range-finding test such that test groups include one with 0% mortality* and one with 100% mortality.

## Preparation of doses or test concentrations:

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

***U.S. EPA OCSPP 885.4340*** *The actual form of the material to be regarded as the test substance is discussed 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.*

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

***Environment Canada EPS 1/RM/44*** *For a single concentration test, a measured quantity of the MPCA representing the maximum hazard* concentration should be mixed to homogeneity in a suitable quantity of soil. In multi-concentration testing, the maximum hazard concentration and each lower test concentration should be prepared in the same manner. Mixing must be performed in a standardized manner (i.e., temperature and time).

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *Soil: for a single concentration test, a measured quantity of the MPCA representing the maximum hazard* concentration should be mixed to homogeneity in a suitable quantity of soil. In multi-concentration testing, the maximum hazard concentration and each lower test concentration should be prepared in the same manner. Mixing must be performed in a standardized manner (i.e., temperature and time). Diet: For a single concentration test, a measured quantity of the MPCA representing the maximum hazard concentration should be mixed to homogeneity in a suitable quantity of food. In multi-concentration testing, the maximum hazard concentration and each lower test concentration should be prepared in the same manner. Mixing must be performed in a standardized manner (i.e., temperature and time). The procedure for mixing the food will vary with the nature of the test material and food.

***OECD 207*** *Filter paper test: the test substance is dissolved in water (if soluble up to a concentration of 1000 mg/L) or in a suitable organic* solvent (e.g. acetone, hexane or chloroform), as appropriate, to give a range of known concentrations. One ml of solution is pipetted into each vial and evaporated to dryness under a slow stream of filtered compressed air, the vial being rotated horizontally as it dries (for substances that are relatively insoluble in either water or organic solvents this may have to be repeated several times to obtain the larger deposits required).

*After drying, 1 ml of deionized water is added to each vial to moisten the filter paper. Each vial is sealed with a cap or plastic film with a small* ventilation hole. Artificial soil test: test soils, whenever possible, should be made up immediately before the start of the test. An emulsion or dispersion of the test substance in deionized water is mixed with the artificial soil or sprayed evenly over it with a fine chromatographic or similar spray. If insoluble in water, the test substance can be dissolved in as small a volume as possible of a suitable organic solvent (e.g. hexane, acetone or chloroform). The solvent should be allowed to evaporate. If the test substance is not soluble, dispersible or emulsifiable, 10 g of a mixture of fine ground quartz sand and quantity of test substance corresponding to 750 g wet weight of artificial soil are mixed with 740 g wet

*artificial soil for each test container. Only agents which volatilize readily may be used to solubilize, disperse or emulsify the test substance. The* test soil must be ventilated before use.

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

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

***U.S. EPA OCSPP 885.4340*** *If used, must be reported, but no specific recommendations are given.*

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

***Environment Canada EPS 1/RM/44*** *No solvent other than water may be used in preparing test concentrations.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***OECD 207*** *For the filter paper test, the test substance is dissolved in water or in a suitable organic solvent (e.g., acetone, hexane or* chloroform). For the artificial soil test, only agents which volatilize readily may be used to solubilize, disperse or emulsify the test substance. The test medium must then be ventilated before use, and the amount of water evaporated replaced.

## 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 885.4340*** *The actual form of the material to be regarded as the test substance is discussed in OCSPP 885.0001.* ***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.*

***PMRA DIR 2001-02*** *Viability or potency of the MPCA in the dosing suspension should be confirmed. No specific methods are recommended.* ***Environment Canada EPS 1/RM/44*** *Analytical techniques permitting, the concentration of the MPCA in the test soil in each treatment* (including controls) should be determined at the beginning and end of the test (at minimum).

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***OECD 207*** *No specific recommendations for viability testing (guideline designed 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 885.4340*** *Any substances used to enhance virulence should be tested along with the test substance.* ***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.

***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.

***Environment Canada EPS 1/RM/44*** *The inclusion of a positive microbial control is not required and is not recommended for most* applications. In instances where a suitable pathogen is available (i.e., genetically related with known toxic/pathogenic effects), a positive microbial control might be warranted. Sensitivity of test organisms to a reference toxicant (i.e., a positive chemical control) must be determined.

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***OECD 207*** *The LC50 of a reference substance should be determined occasionally as a means of assuring that the laboratory test conditions are* adequate and have not changed significantly. A suitable reference substance is chloracetamide.

## Other controls:

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

***U.S. EPA OCSPP 885.4340*** *A concurrent control group is recommended and should be treated with microbe-free (or nonviable microbe) material from the culture system used for propagation of the microbial pest control agent.* ***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.

***PMRA DIR 2001-02*** *A negative, no-dosed control group of the non-target organism should be run concurrently with the test group.* ***Environment Canada EPS 1/RM/44*** *A negative control is required, and a non-infectious control is strongly recommended. A sterile culture* filtrate control is optional but recommended.

***Supplemental guidance for terrestrial non-arthropod invertebrates: OECD 207*** *A negative control treated with the same solvent is required.*

## Number of test organisms per chamber:

#### Control(s): Treatments:

***U.S. EPA OCSPP 885.4340*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Ten springtails per test chamber.*

***Supplemental guidance for terrestrial non-arthropod invertebrates: Environment Canada EPS 1/RM/44*** *Two worms per test chamber.*

***OECD 207*** *For the filter paper test, one worm/vial; for the artificial soil test, ten earthworms per chamber.*

## Number of replicates (chambers) per treatment:

#### Control(s): Treatments:

***U.S. EPA OCSPP 885.4340*** *No specific recommendations.*

***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.

***Environment Canada EPS 1/RM/44*** *Five chambers (fifty springtails) per treatment.*

***Supplemental guidance for terrestrial non-arthropod invertebrates: Environment Canada EPS 1/RM/44*** *Ten replicates (of two worms each) per treatment.*

***OECD 207*** *For filter paper test, 10 replicates/ treatment or control (the precision can be increased by using 20 replicates); for artificial soil* test, 4 replicates/treatment.

## Recovery of MPCA from test organisms:

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

***U.S. EPA OCSPP 885.4340*** *The recovery of the MPCA from test organisms may be required for many types of MPCA. See "Duration of the* study" below for additional details.

***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.

***Environment Canada EPS 1/RM/44*** *The recovery of the MPCA from test organisms is not required due to biomass limitations of the test organisms.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *The recovery of the MPCA from test organisms is optional based on whole-organism homogenate from* each treatment (including controls) during and/or at end of test.

***OECD 207*** *No specific recommendations (guideline designed for chemical toxicity testing).*

## Feeding:

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

***U.S. EPA OCSPP 885.4340*** *No specific recommendations- but the feeding regime is dependent on the type of MPCA (bacteria, fungi, protozoa,* and viruses. See “Route of Exposure section above for additional guidance.

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

***Environment Canada EPS 1/RM/44*** *Dry yeast, ~2 mg per test chamber on Days 0 and 14 only.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *Cooked oatmeal; 5 mL per test chamber at each feeding, placed in a shallow depression at the centre of* the soil surface and in each test chamber on Days 0. 14, 28 and 42 only.

***OECD 207*** *Worms are not fed during the study.*

## Test conditions:

#### Temperature:

Humidity:

Lighting:

***U.S. EPA OCSPP 885.4340*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Daily mean temperature 20 ± 2°C, lighting incandescent or fluorescent, 400 to 800 lux at surface of soil in* test chamber, and fixed photoperiod (e.g., 16 h light, 8 h dark or 12 h light, 12 h dark).

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***OECD 207*** *Temperature 20 ± 2°C; continuous dark for filter-paper test, continuous light for artificial soil test.*

## Duration of the study:

##### *[Specify the test duration, and comment on any observations that necessitated the extension of the test* period]

*U.S. EPA OCSPP 885.4340*

*–For viruses, control and treated insects should be observed for a period of at least 30 days after dosing, or in cases where an insect* species cannot be cultured for 30 days, until control mortality rises above 20 percent. In cases where signs, symptoms and pathologies are detected, the treated insects should be examined in detail at late stages of infection, at moribund, and at death. Such tests need not be prolonged to 30 days, if death of treated insects occurs prior to 30 days. In all cases of pathologies in the treated non-target insects, it is essential that the etiology of the infection be established.

*–For protozoa, test duration should be determined on a case-by-case basis. The most appropriate end-point for protozoan diseases for* determining pathogenic effects is the presence of the vegetative stages (shizonts or meronts) in the tissues of non-target insects. The schizonts within suspected tissues can be detected by making a smear, staining with Giemsa stain, and examining the slide with oil immersion using a compound microscope. The non-target insect should be alive when the tissues are removed for the smear because the shizonts are fragile and are usually destroyed by other microbes or are distorted upon death of the host. Protozoan spores can be used as an indicator of infection. However, if the infection is light, the few spores could have come from the inoculum. If spores are abundant (a relative term) and occur in the tissues of the non-target insect, it is likely that it is infected. Another way to confirm infection is to conduct histological studies of the tissues using standard methods and looking for spores and other pathological effects. The end-point would be just before death of the organism or a prescribed period of time. Death of the non-target insect is a good end-point if the protozoan is virulent. However, since most protozoans have chronic effects on their host, changes in behavior, size, or color could be used as an end-point. In each case, a microscopic examination to find schizonts or spores is essential to confirm the presence of the protozoan. Koch’s postulates to confirm the virulence of the isolate should be run.

*–For fungi, the test duration can be limited to 8–10 days if the MPCA is entomopathogenic. Mortality time, expressed as LT50 (time* course of population mortality), is considered the most reliable parameter for bioassaying fungi of insects in the laboratory. Pathogenicity should be confirmed by identifying the fungal agent as the original inoculum.

*–For bacteria, control and treated insects should be observed for 21 to 30 days after dosing, if this is possible. In cases where the* insect species cannot be cultured for 21 to 30 days, observation should continue until control mortality rises above 20 percent.

***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.

***Environment Canada EPS 1/RM/44*** *The test duration is at least 28 days.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***PMRA DIR 2001-02*** *The duration of the observation period depends on the mode of pesticidal action. In general, a duration of 30 days permits* time for incubation, infection and manifestation of adverse effects in the test organism. For infectivity testing, the study should continue until a pattern of microbial clearance from tissues is shown.

***Environment Canada EPS 1/RM/44*** *56 days (8 weeks).*

***OECD 207*** *48 hours for the filter paper test; 14 days for the artificial soil test.*

## 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, abnormal behavior or appearance,* fecundity, growth inhibition, individual body weights, concentration of the MPCA in diet or dosing suspension. Provide references to data summary tables, if used.]

*U.S. EPA OCSPP 885.4340*

*-For Viruses: If clinical signs are detected, the treated insects should be examined in detail at late stages of infection, moribundity and* death. Endpoints should be based on the frank development of pathology and on the early mortality of treated organisms vs. controls.

*-For Protozoa: The most appropriate endpoint for pathology is the presence of vegetative stages (shizonts, meronts) in the tissues* (Giemsa smear of tissue from live host). The presence of abundant spores is an indicator of infection. If the protozoan is virulent, mortality may be a useful endpoint, but changes in behavior, size or color can also be used. Microscopic verification of infection and confirmation of virulence using Koch’s postulates is also required.

*-For Fungi: Population mortality time, expressed as LT50 is the most reliable parameter. Pathogenicity should be confirmed by* Koch’s postulates.

***PMRA DIR 2001-02*** *Regular observations are required to record mortalities and note any behavioral, pathogenic or toxic adverse effects.* ***Environment Canada EPS 1/RM/44*** *Measurement of the temperature, moisture content (%), conductivity, pH and concentration of the MPCA* in soil of each treatment group is required. Observations for survival, total number of live second generation juveniles in each test vessel.

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *Measurement of the temperature, moisture content (%), conductivity, pH and concentration of the MPCA* in soil is required. Observations for survival of adult worms in each test chamber; number of surviving adult worms with atypical appearance and/or behavior, total dry weight, number of live juvenile worms, and number of surviving juvenile worms with atypical appearance and/or behavior.

***OECD 207*** *Measurements of artificial soil moisture content, artificial soil pH and worm live weight are required. Observations for mortality,* and behavioral or pathological signs.

## Observation/measurement intervals:

##### *[List time points at which observations or measurements were made.]*

***U.S. EPA OCSPP 885.4340*** *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.*

***PMRA DIR 2001-02*** *Regular observations are required to record mortalities and note any behavioral, pathogenic or toxic adverse effects.* ***Environment Canada EPS 1/RM/44*** *Temperature measured daily (max/min) or continuously; soil parameters (moisture content, conductivity* and pH) measured at the beginning and end of the test; concentration of the MPCA in the soil at the beginning and end of the test; and observations for mortality and reproductive success on Day 28.

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *Temperature measured daily (max/min) or continuously; soil parameters (moisture content, conductivity* and pH) measured at the beginning and end of the test for at least one replicate of each treatment; concentration of the MPCA in the soil for each treatment including controls at the beginning and end of the test (at minimum); observations on adults on Day 28; observations on juveniles on Day 56.

***OECD 207*** *Artificial soil moisture content should be determined at start and end of test. Artificial soil pH should be measured at start of test.* The average worm live weigh and number of live worms per treatment should be determined at start and end of the test. Observations for behavioral or pathological signs are made at 48 hours (optional at 72 hours for filter paper test); Days 7 and 14 for artificial soil test.

## Testing for infectivity:

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

***U.S. EPA OCSPP 885.4340*** *Infectivity testing is required for many types of MPCA. See “Duration of the study” above for additional details.* ***PMRA DIR 2001-02*** *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.

***Environment Canada EPS 1/RM/44*** *Infectivity testing is not required (impractical due to limited biomass of the test organisms).*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *Infectivity testing is optional during and/or at test end based on measured concentrations of MPCA in* whole-body homogenates.

***OECD 207*** *No specific recommendations (guideline designed 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 *[or potency]* of *[test substance]* in *[dosing suspension/diet]* administered to *[test organism]* over *[#]* days.

|  |  |  |
| --- | --- | --- |
| **Dose Group** | **Nominal Dose *[count or potency]***  **(*insert units*)** | **Measured Dose *[count or potency]***  **(*insert units*)** |
| Negative control |  |  |
| *Solvent/vehicle control* |  |  |
| *Inactivated control* |  |  |
| *Sterile filtrate control* |  |  |
| *Maximum hazard dose* |  |  |

###### *[Table suitable for microbial toxicity/infectivity/pathogenicity testing at the maximum hazard dose/concentration. Modify as* appropriate to accommodate differences in experimental design.]

1. **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 substance]* on mortality of *[test organism]* exposed by *[dosing method]* over *[#]*

days.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | | **Cumulative Mortality/Total Number of Insects** | | |
| **Negative Control** | **Killed *[test substance]* Control** | ***[Test substance]*** |
| Cumulative mortality | *Day 0* |  |  |  |
| *Day x1* |  |  |  |
| *Day x2* |  |  |  |
| *Day n* |  |  |  |
| *LD50/LC50/LT*50  *(if applicable)* | | *[insert [***>***] if greater than]* | | |
| *NOEL/NOEC* | | *[insert [***>***] if greater than]* | | |

###### *[Table suitable for maximum hazard dose testing. Modify as appropriate to accommodate differences in experimental* design.]

**TABLE *[#]*.** Effect of *[test material]* on cumulative mortality of *[common name (scientific name)]* in *[contact, acute oral or dietary]* test.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | | **No. of Test Organisms** | **Cumulative Mortality** | | | | | |
| ***Day x1*** | ***Day x2*** | ***Day x3*** | ***Day x4*** | ***Day x5*** | ***Day n*** |
| Negative control | |  |  |  |  |  |  |  |
| *Solvent control, if used* | |  |  |  |  |  |  |  |
| *Measured test concentration 1* | |  |  |  |  |  |  |  |
| *Measured test concentration 2* | |  |  |  |  |  |  |  |
| *Measured test concentration n* | |  |  |  |  |  |  |  |
| *LD50/LC50/LT*50  *[insert [***>***] if greater than]* | |  | | | | | | |
| *NOEL/NOEC*  *[insert [***>***] if greater than]* | |  | | | | | | |
| *Reference Material (if used)* | *Mortality (% or No.)* |  |  |  |  |  |  |  |
| *LD50: LC50: LT50:* | *[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.]*

1. **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 *[#[*.** Sublethal effects of *[test material]* to *[common name (scientific name)]* in a(n) *[contact, acute oral or dietary]* test.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments**  ***[indicate if nominal or measured]*** | | **Observation Period** | | | | | |
| ***Day x*** | | ***Day y*** | | ***Day z*** | |
| ***endpoint*** | **% Affected** | ***endpoint*** | **% Affected** | ***endpoint*** | **% Affected** |
| Negative control | |  |  |  |  |  |  |
| *Solvent control, if used* | |  |  |  |  |  |  |
| *measured test concentration 1* | |  |  |  |  |  |  |
| *measured test concentration 2* | |  |  |  |  |  |  |
| *measured test concentration n* | |  |  |  |  |  |  |
| *EC50/ or other sublethal endpoint*  *[insert [***>***] if greater than]* | |  |  |  |  |  |  |
| *NOEC*  *[insert [***>***] if greater than]* | |  | | | | | |
| *Reference chemical (if used)* | *LD50: LC50: LT50:* | *[insert [***>***] if greater than]* | | | | | |
| *NOEL/ NOEC* | *[insert [***>***] if greater than]* | | | | | |

###### *[Modify table as appropriate to accommodate differences in experimental design. Alternatively generate one table per* endpoint/sublethal effect, if appropriate.]

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

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

***U.S. EPA OCSPP 885.4340*** *No specific statistical recommendations. From* ***U.S. EPA OCSPP 885.0001-*** *Appropriate statistical methods are to* be used to summarize experimental data, to express trends, and to evaluate the significance of differences in data obtained from different test group and methods used shall reflect the current state-of-the art. All data averages or means must be accompanied by standard deviations 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.

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

***Environment Canada EPS 1/RM/44*** *Single concentration test: percent survival of the first-generation adults on Day 28, mean (± SD) number* of second-generation juveniles on Day 28, comparing MHC to controls. Multi-concentration test: percent survival of the first-generation adults on Day 28, mean (± SD) number of second-generation juveniles on Day 28, comparing each test chamber and treatment. Data permitting, 28-day LC50 for adults, and 28-day IC25 for number of juveniles generated, NOEC/LOEC.

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *Single concentration test: percent survival of adults on Day 28, percent of live adults showing atypical* appearance and/or behavior on Day 28; mean (± SD) number of live juveniles on Day 56, percent of live juveniles showing atypical appearance and/or behavior on Day 56, comparing MHC to controls. Multi-concentration test: percent survival of adults on Day 28, percent of live adults showing atypical appearance and/or behavior on Day 28; mean (± SD) number of live juveniles on Day 56, percent of live juveniles showing atypical appearance and/or behavior on Day 56, comparing each test chamber and treatment. Data permitting, 28-day LC50 for adults, 28-day EC50 for atypical appearance/behavior of adults, 56-day EC50 for atypical appearance/behavior of juveniles, 56-day IC25 for number of juveniles, 56-day IC25 for dry weight of juveniles.

***OECD 207*** *Mortality/concentration data should be plotted on log probability graph paper and the LC50 and its confidence limits*

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.:

LT50: 95% C.I.:

EC50: 95% C.I.:

NOEL: NOEC:

Probit Slope: 95% C.I.:

Endpoint(s) Affected:

1. **CONCLUSION:**
2. **STUDY AUTHOR CONCLUSION:** *[Summarize the study author’s conclusions- Provide the major conclusions e.g., values for LD50, LC50, LT50, NOEL, NOEC were [****=, > or <****] in appropriate units]*
3. **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 and pathogenicity study for nontarget insects (OCSPP 885.4340; PMRA: M9.5.1 and OECD: IIM 8.8, IIIM 10.4) 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 885.4340*** *No specific validity criteria. Acceptability of studies should be determined based on expert judgment.*

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

***Environment Canada EPS 1/RM/44*** *The test is invalid if mean 28-day survival of adult (first generation) springtails in negative control soil*

*<70%. Also, the test is invalid if mean reproduction rate for adults in negative control soil <10 juveniles/adult.*

*Supplemental guidance for terrestrial non-arthropod invertebrates:*

***Environment Canada EPS 1/RM/44*** *The study is invalid if 28-day survival in adults is less than 90%, if the mean reproductive rate is less than* 3 juveniles per adult and if the mean dry weight of individual live juveniles is <2.0 mg in negative control soil.

***OECD 207*** *Mortality in controls should not exceed 10% at the end of either test.*

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

##### **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 nontarget insect studies (OCSPP 885.4340). 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]* nontarget insects.

The proposed uses of *[product name]* on *[identify use sites/crops]* is not expected to result in increased exposure or adverse effects to non-target insects. *[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 nontarget insect wildlife. The *[applicant]* requests a waiver of terrestrial *[and aquatic, if applicable]* nontarget insect 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 nontarget insects.** *[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 insects/arthropods? 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 non-target insects 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 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 terrestrial nontarget insect 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 wild populations of terrestrial 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.]

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