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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:** Freshwater Aquatic Invertebrate Testing, Tier I

#### U.S. EPA OCSPP Guideline: 885.4240

PMRA Data Code: M9.5.2–Aquatic arthropods *(pelagic and benthic)*

#### OECD Data Code: IIM 8.3, IIIM 10.2

**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 Unpublished *[OR if published, list Journal name, vol.: pages]*.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 toxicity and pathogenicity study, *[common name (scientific name)]* were exposed to a *[single OR #]*

##### dose *[dose amount]* of *[formulation, note its potency, biological activity or concentration per unit weight or*

*volume; indicate if conducted at maximum hazard dose]* (containing % *a.i. name*) under *[static/flow through]*

##### conditions*. [Include other pertinent details such as the controls used.]*

*[Note if no mortality occurred. 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 ID50]* was *[****=, > or <****] [insert LC50 or ID50 in appropriate units]* **(95% C.I. if available)**. *[NOTE: 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 based on sublethal effects was *[****=, > or <****] [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 *[contact, oral or dietary]* toxicity and pathogenicity study for freshwater aquatic invertebrates (OCSPP 885.4340; PMRA: M9.5.2 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 885.4240–Freshwater aquatic invertebrate testing,* tier I1 (NOTE: This EPA template includes criteria for both benthic and pelagic aquatic arthropods; whereas, for PMRA templates- the criteria for pelagic and benthic arthropods are found in separate DER templates.)

*PMRA DIR 2001-02 Part 9.5.21*

*Environment Canada EPS 1/RM/44 Sections 10.1.21 and 10.2.21 Environment Canada EPS 1/RM/44 Section 10.1.31*

*OECD 202–Daphnia sp., acute immobilisation test2 OECD 211–Daphnia magna reproduction test2]*

*OECD 218–Sediment-water chironomid toxicity test using spiked sediment2*

*OECD 219–Sediment-water chironomid toxicity test using spiked sediment2]*

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

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

**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:** *[Complete this subsection using the information provided in the methodology section of the study report.]*
   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 indicate whether the MPCA is stable under these conditions.]*

* 1. **Test Organism**:

**Species (common and scientific names):** *[Insert test species name(s).]*

***U.S. EPA OCSPP 885.4240*** *For MPCAs where direct aquatic exposure is expected, one species of benthic invertebrate should be tested. For* MPCAs where direct aquatic exposure is anticipated, testing shall be performed on two aquatic invertebrate species, one of which is planktonic and the other benthic. The test species should bear as close a taxonomic relationship to the target host as possible. Species likely to prey upon or scavenge the diseased target host organisms should be tested, when applicable. ***From U.S. EPA OCSPP 885.4000 Background for Nontarget* Organism Testing of Microbial Pest Control Agents-** *It would be appropriate to choose an aquatic insect (e.g. caddisfly) as the nontarget* aquatic invertebrate test species when evaluating a MPCA whose target host is an insect. Daphnia, a Cladoceran, has the advantage of having considerable background data for comparative purposes and a bioconcentration effect has been observed during toxicity testing with the entomopathogen Mattesia due to the filter feeding habits of Daphnia, thus assuring the test animal ingests the microorganism. Both Daphnia and certain other aquatic insects have the advantage of a short like cycle or aquatic phase, and both undergo periods of natural stress and potential susceptibility to the microorganism as a consequence of molting.

***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.

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnia magna is the test species for freshwater tests. Paleomonetes vulgaris is the test species for marine studies.*

***OECD 202*** *Daphnia magna Straus is the preferred test species although other suitable Daphnia species can be used (e.g., D. pulex).*

***OECD 211*** *Daphnia magna Straus is the test species. Clone A is preferred. Other species meeting the reproductive output criteria (mean ≥60* offspring/surviving parent) are also acceptable.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *The chironomids, Chironimus tentans or C. riparius, or the amphipod Hyalella azteca are the test species.* ***OECD 218 and 219*** *Chironimus riparius is preferred. Chironimus tentans is also suitable but requires a longer test period. C. yoshimatsui is* also acceptable.

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

***U.S. EPA OCSPP 885.4240*** *Larval stages of invertebrates should be used whenever possible.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids should be less than 24 hours old at the start of the test. Adult shrimp should be used in marine studies.*

***OECD 202 and 211*** *Daphnids aged less than 24-h, not first-brood progeny.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Third instar C. tentans or first instar C. riparius (≤48 hour post-hatch); 2–9-day-old amphipods.*

***OECD 218 and 219*** *First instar larvae should be used.*

## Number of animals/sex:

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

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

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

***-****Pelagic Aquatic Arthropods:* ***OECD 202*** *No specific recommendations.* ***OECD 211*** *Female daphnids.*

***-****Benthic Aquatic Arthropods:*

***OECD 218 and 219*** *The sex of fully emerged midges must be recorded.*

##### **Source:** *[Insert source and/or supplier of test organisms.]*

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Test organisms should be obtained from a single healthy culture.*

***-****Pelagic Aquatic Arthropods:*

***OECD 202 and 211*** *Daphnids should be derived from a single healthy stock (i.e., showing no signs of stress such as high mortality, presence of* males and ephippia, delay in the production of the first brood, discolored animals, etc.).

***-****Benthic Aquatic Arthropods:*

***OECD 218 and 219*** *Chironomids can be cultured at the test facility. If chironominds are obtained elsewhere, the species must be confirmed* prior to testing.

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

1. **STUDY DESIGN AND METHODS:**

*[Briefly describe the experimental design.]*

***U.S. EPA OCSPP 885.4240*** *Arthropods are exposed to the MPCA as a suspension in water for at least 21 days. A single group may be tested at* the MHC. If adverse effects are observed, sequentially lower doses should be tested.

***PMRA DIR 2001-01*** *The MPCA is administered as a suspension in the water (i.e., aquatic exposure), in the diet, or as a combination of these* two routes of exposure at the maximum hazard concentration (MHC) for at least 21 days.

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids or shrimps are exposed to the MHC, or a range of concentrations, including the MHC, for 21 or* 30 days, respectively.

***OECD 202*** *In an acute toxicity study designed for chemical toxicity testing, daphnids are exposed to the test substance for 48 hours. Immobility* is the endpoint of interest.

***OECD 211*** *In a chronic toxicity study designed for chemical toxicity testing, daphnids are exposed to the test substance for 21 days. Immobility* of parental daphnids and fecundity are the endpoints of interest.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM 44*** *Single concentration test: the MPCA is mixed in both the water and sediment at the MHC. Multi-* concentration test: a minimum of 5 test concentrations, including the MHC, either mixed into the sediment or suspended in the water.

***OECD 218*** *First instar chironomid larvae are exposed to a concentration range of the test chemical in sediment-water systems. The test* substance is spiked into the sediment and larvae are introduced into test beakers in which the sediment and water concentrations have been stabilized. Chironomid emergence and development rate is measured at the end of the test.

***OECD 219*** *First instar chironomid larvae are exposed to a concentration range of the test chemical in sediment-water systems. The test starts* by placing first instar larvae into the test beakers containing the sediment-water system, and subsequently spiking the test substance into the water. Chironomid emergence and development rate is measured at the end of the test.

## Experimental Methods and Conditions:

**Acclimation:**

#### Period: Conditions: Feeding:

Health: *(any mortality observed?)*

##### *[Were they the same as those reported during the study?]*

***U.S. EPA OCSPP 885.4240*** *No specific recommendations, but acclimation parameters (i.e. lighting, acclimation, and test temperatures* (averages and range) must be reported.

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids should be acclimated for at least two generations to the test conditions (i.e., temperature,* photoperiod, food, dilution water). Shrimp should be acclimated gradually to the test conditions (salinity, temperature, and lighting) and held for at least 14 days before testing. Mortality should not exceed 10% during the final 2 days preceding testing.

***OECD 202*** *Stock animals must be maintained in culture conditions (light, temperature, medium) similar to those to be used in the test.* Otherwise, a 48-hour acclimation period is recommended.

***OECD 211*** *Stock animals must be maintained in culture conditions (light, temperature, medium) similar to those to be used in the test. If the* medium is different, a pre-test acclimation period of approximately 3 weeks (i.e., one generation) is recommended to avoid stressing the parent animals.

***-****Benthic Aquatic Arthropods:*

***OECD 218 and 219*** *4–5 days before adding the test organisms to the test vessels, egg masses should be taken from the cultures and placed in* small vessels in culture medium. Aged medium from the stock culture may be used, or freshly-prepared medium, with a small amount of food (e.g., green algae and/or droplets of filtrate from a finely ground suspension of flaked fish food) added to it. Only freshly laid egg masses should be used. Larvae begin to hatch a couple of days after the eggs are laid, and larval growth occurs in four instars, each of 4–8 days duration.

*First instar larvae are used in the test.*

## Test vessel:

##### *[Describe the test vessel.]*

Material: Size:

Fill Volume:

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For daphnids, glass beakers (50–100 mL capacity) containing 50–100 mL test solution/suspension. For* shrimp, clear glass aquaria (≥20 L)containing 15 L test solution/suspension.

***OECD 202*** *Glass or other chemically inert material. Glass test tubes or beakers, loosely covered to reduce the evaporation of water and entry* of dust. The ratio of air/water volume in the vessel should be identical for test and control groups. ≥2 mL of test solution should be provided for each daphnid.

***OECD 211*** *Glass or other chemically inert material is preferred. Glass beakers containing 50–100 mL of medium are recommended.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *300-mL high-form glass beaker, containing 100 mL wet sediment and 175 mL overlying water.*

***OECD 218 and 219*** *The study is conducted in glass 600-mL beakers measuring 8 cm in diameter. Other vessels are suitable, but they should* guarantee a suitable depth of overlying water and sediment. The sediment surface should be sufficient to provide 2–3 cm2/ larva. The ratio of the depth of the sediment layer to the depth of the overlying water should be 1:4. Test vessels and other apparatus that will come into contact with the test system should be made entirely of glass or other chemically inert material (e.g., Teflon).

## Test system:

#### Static/flow through

Type of dilution system- for flow through method Flow rate

Renewal rate for static renewal

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

***PMRA DIR 2001-02*** *High concentrations of the microbial test substance may have an adverse effect on water quality (e.g., oxygen depletion).* It is recommended that the test solution be renewed at a sufficient rate to maintain water quality and the concentration of the MPCA.

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids: static renewal; three times per week on non-consecutive days (e.g., Mon., Wed., Fri.). Shrimp:* static renewal; at least twice weekly.

***OECD 202*** *Static test. Static renewal or flow-through systems may be used when the concentration of the test substance is not stable.*

***OECD 211*** *Static-renewal or flow-through tests are acceptable.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For chironomids: static renewal, four times per week on non-consecutive days (e.g., Mon., Wed., Fri.).* For amphipods: static renewal, three times per week on non-consecutive days.

***OECD 218 and 219*** *Static systems are used. Semi-static or flow-through systems with intermittent or continuous renewal of overlying water* might be used in exceptional cases as for instance if the water quality specifications become inappropriate for the test organism or affect chemical equilibrium. However, other methods for ameliorating the quality of overlying water, such as aeration will normally suffice and be preferable.

**Sediment:** *[if used]*

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

pH:

Conductivity:

Percent TOC:

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

***U.S. EPA OPPTS 885.4240*** *No specific recommendations.*

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

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Natural (uncontaminated) or artificial (laboratory formulated) sediment. The sediment 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 sediment (e.g., for whole sediment — particle size distribution, percent water content, total organic carbon; for pore water — pH, ammonia, hardness, dissolved metals, pesticides). The use of sterile sediment is not recommended since most sterilization processes alter the physicochemical characteristics of the sediment.

***OECD 218 and 219*** *The use of formulated sediment is recommended: 4–5% peat (powder, with particle size <1 mm, pH 5.5–6.0); 20% kaolin* clay (kaolinite content >30%); 75–76% quartz sand (fine, >50% of particles 50–200 µm); deionoized water added to moisture content 30–50%; CaCO3 to adjust pH to 7.0 ± 0.5. Organic carbon content of the final mixture 2% ± 0.5%, and adjusted by use of appropriate amounts of peat and sand.

## Source of dilution water:

##### *[Insert description of the source of dilution water.]*

***U.S. EPA OCSPP 885.4240*** *No specific recommendations, but the source of the dilution water must be reported.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids: Elendt M4 or M5 media. Other media acceptable provided criteria for test validity are met.* Shrimp: natural (uncontaminated) or artificial seawater. The dilution water or medium 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.

***OECD 202*** *Surface or ground water, reconstituted water or dechlorinated tap water are acceptable if daphnids will survive for the duration of* culturing, acclimation and testing periods without showing signs of stress.

***OECD 211*** *A fully defined medium is recommended, (e.g., Elendt M4 or M7). If undefined additives are used, these must be reported in detail.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Natural (uncontaminated) or artificial freshwater. The dilution water 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.

***OECD 218 and 219*** *Any suitable water (conforming to water parameters, see below) is acceptable if chironomids will survive in it for the* duration of culturing and testing without showing signs of stress. The same type of water should be used throughout the study.

## Water parameters:

#### Dissolved oxygen pH

Temperature Hardness Particulate matter

Total organic carbon (TOC) or chemical oxygen demand (COD) Metals

Pesticides Chlorine

##### *[Intervals of water quality measurement]*

***U.S. EPA OCSPP 885.4240*** *No specific recommendations but chemical characteristics must be reported.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids: dissolved oxygen 90–100% saturation; pH 6.0–8.5; temperature 18–22°C; hardness not* specified, but must be recorded with other parameters 1×/week. Shrimp: dissolved oxygen 90–100% saturation; salinity 10–35‰, 5–25°C held at

*±2°C from mean; pH 7.0–8.5. A history of the dilution water’s basic physicochemical properties (e.g., suspended solids, ammonia, dissolved* metals, pesticides) should be known to the testing facility.

***OECD 202*** *Dissolved oxygen aerated to saturation, pH 6–9, temperature 18–22°C but it should be constant within ±1°C, hardness 140–250* mg/L for D. magna (lower for other Daphnia species), particulates < 20 mg/L, TOC < 2 mg/L, un-ionized ammonia <1 µg/L, residual chlorine

*<10 µg/L, total OP pesticides ≤ 50 ng/L, total OC pesticides/PCBs ≤ 50ng/L (or organic chlorine ≤ 25 ng/L).*

***OECD 211*** *Dissolved oxygen > 3 mg/L, pH 6–9, temperature 18–22°C but should not vary more than ± 2°C, hardness <140 mg/L, TOC <2* mg/L, TOC or COD must be reported.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Dissolved oxygen 90–100% saturation when added to test chambers; 23±1*̊*C. A history of the dilution* water's basic physicochemical properties (e.g., suspended solids, ammonia, dissolved metals, pesticides) should be known to the testing facility. ***OECD 218 and 219*** *At the start of the test, pH 6–9, total hardness ≤400 mg/L as CaCO3. However, if there is an interaction suspected between* hardness ions and the test substance, lower hardness water should be used not Elendt medium. Particulate matter <20 mg/L; TOC <2 mg/L; un- ionized ammonia 1 µg/L; residual chlorine <10 µg/L; total OPs <50 ng/L; total OCs and PCBs <50 ng/L; organic chlorine <25 ng/L.

## Aeration:

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids: test chambers must not be aerated during the test; ≥ 3 mg/L dissolved oxygen. Shrimp: aerate* gently in all test chambers to maintain a dissolved oxygen concentration ≥60% saturation.

***OECD 202 and 211*** *The dilution water may be aerated prior to use in the test. Test vessels must not be aerated during the test.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Aerate gently in all test chambers to maintain a dissolved oxygen ≥40% saturation.*

***OECD 218 and 219*** *Gentle aeration is provided through a glass Pasteur pipette fixed 2–3 cm above the sediment layer (one or a few* bubbles/sec) to maintain a dissolved oxygen concentration of ≥60% saturation. Aeration must be stopped while adding larvae to the test vessel and for the following 24 hours.

## Route(s) of exposure:

##### *[Describe the route of exposure to the MPCA.]*

***U.S. EPA OCSPP 885.4240*** *Aquatic exposure.*

***PMRA DIR 2001-02*** *The MPCA should be administered as a suspension in the dilution water (aquatic exposure), in the diet, or as a* combination of these two routes of exposure.

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For daphnids: aquatic exposure. For shrimp: the test material should be mixed in both seawater and food* for single-concentration test, and seawater or food in multi-concentration testing.

***OECD 202 and 211*** *Aquatic exposure.*

***-****Benthic Aquatic Arthropods:*

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

***OECD 218*** *Sediment exposure.*

***OECD 219*** *Aquatic exposure.*

## Test concentrations:

#### Nominal:

Measured: *(from confirmation of dose viability)*

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

***U.S. EPA OCSPP 885.4240*** *A single group tested at the MHC (106 units/mL or at least 1000× the maximum calculated concentration following* direct application to 6 inches of water, whichever is greater). If effects observed, then sequentially lower doses tested to establish LC50 /ID50 with 95% CI.

***PMRA DIR 2001-02*** *Aquatic exposure: 106 active units of the MPCA/g of soil or water, or 1000× the expected environmental concentration* (EEC) following a direct application at the maximum label rate to a 15-cm layer of soil or water, whichever is greater or achievable. Dietary exposure: arthropods can be fed a diet treated with an application of the MPCA equivalent to 100× the maximum label rate. Alternatively, organisms may be fed the target organism that has been maximally infected with the MPCA.

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids: a single group tested at the maximum hazard concentration (MHC) or ≥ 5 test concentrations,* including the MHC. Shrimp: a single group tested at the maximum hazard concentration or ≥ 5 test concentrations, including the maximum hazard concentration. The MHC for aquatic exposure is 106 active MPCA units/mL or 1000× the EEC of the MPCA, following application to a 15-cm layer of water (whichever greater and attainable) and the MHC for dietary exposure is 100× the EEC of the MPCA following application to a 15-cm layer of water.

***OECD 202*** *A limit test at 100 mg/L may be conducted using 20 daphnids (4 groups of 5). If >10% immobility is observed, a full study is* required, including a range-finding study, and definitive test using ≥5 concentrations, with ratio ≤2.2, highest concentration resulting in 100% immobility, and lowest in no observable effect.

***OECD 211*** *≥5 test concentrations in geometric series, with ratio ≤3.2. For NOEC/LOEC, in the lowest test concentration, fecundity is not* significantly different from controls, but fecundity is significantly different from controls at highest test concentration. For EC50, the range of test concentrations must permit estimation of the EC50 with confidence intervals.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *A single group tested at the MHC or ≥ 5 test concentrations, including the MHC. The MHC for water is* 106 microbial units/mL water or 1000× the EEC in the aquatic environment, whichever is greater and achievable. The MHC for sediment is 106 microbial units/g dry wt. of sediment or 1000× the EEC in the aquatic environment, whichever is greater and achievable.

***OECD 218*** *At least 5 concentrations should be used. The factor between test concentrations should be <2. A range-finding test may be helpful* to determine the range. A limit test may be performed at a concentration of 1000 mg/kg (dry weight sediment.

***OECD 219*** *At least 5 concentrations should be used. The factor between test concentrations should be <2. A range-finding test may be helpful* to determine the range. A limit test may be performed but the limit concentration is left to the discretion of the regulatory authority.

## Preparation of test concentrations:

##### *[Briefly describe the preparation of the test concentrations.]*

***U.S. EPA OCSPP 885.4240*** *The test material shall be administered directly into the dilution water. 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.*

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Aquatic exposure: 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 dilution water. 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) and may be performed by hand (glass rod or spatula), or by using mechanical stirring device (e.g., teflon-coated bar, stainless steel vortex mixer). Ultrasonic dispersion is not recommended since it may be harmful to the MPCA. Dietary exposure: 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 202*** *Test suspensions should be prepared by dilution of a stock suspension. Stock suspensions should be prepared by dissolving the test* material in dilution water. The pH should not be adjusted, but if necessary, the stock suspension concentration is not significantly changed and that no chemical reactions or precipitations occurred. HCl and NaOH are preferred. The use of solvents, emulsifiers or dispersants should be avoided.

***OECD 211*** *Test suspensions should be prepared by dilution of a stock suspension. Stock solutions should be prepared by dissolving the test* material in test (culture) medium. The use of organic solvents or dispersants may be required in some cases in order to produce a suitably concentrated stock solution, but every effort should be made to avoid the use of such materials. The maximum solvent/dispersant concentration in the final test medium is ≤0.1 mL/L.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Aquatic exposure: 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 dilution water. 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) and may be performed by hand (glass rod or spatula), or by using mechanical stirring device* (e.g., teflon-coated bar, stainless steel vortex mixer). Ultrasonic dispersion is not recommended since it may be harmful to the MPCA. Sediment exposure: 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 sediment. 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) and may be performed by hand or by using mechanical device.

***OECD 218*** *Spiked sediments of the chosen concentration are usually prepared by addition of a solution of the test substance directly to the* sediment. A stock solution of the test substance dissolved in deionized water is mixed with the formulated sediment by rolling mill, feed mixer or hand mixing. If poorly soluble in water, the test substance can be dissolved in as small a volume as possible of a suitable organic solvent. This solution is then mixed with 10 g of fine quartz sand for one test vessel. The solvent is allowed to evaporate and it has to be totally removed from sand; the sand is then mixed with the suitable amount of sediment per test beaker. Only agents which volatilize readily can be used to solubilize, disperse or emulsify the test substance. It should be born in mind that the sand provided by the test substance and sand mixture, has to be taken into account when preparing the sediment (i.e., the sediment should thus be prepared with less sand). Care should be taken to ensure that the test substance added to sediment is thoroughly and evenly distributed within the sediment.

***OECD 219*** *The test substance is added into the overlying water column 24 hours after the larvae were added to the test chambers. Small* volumes of test substance solutions are applied below the surface of the water using a pipette. The overlying water should then be mixed with care not to disturb the sediment.

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

##### *[Describe any solvent or carrier used in dose administration.]*

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

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

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

***-****Pelagic Aquatic Arthropods:*

***OECD 202*** *The concentration of test substance should not exceed the limit of solubility in the dilution water. Solvents may be required to* prepare a stock solution of suitable concentration, but as far as possible, the use of solvents, emulsifiers or dispersants should be avoided. ***OECD 211*** *The concentration of test substance should not exceed the limit of solubility in the dilution water. The use of organic solvents or* dispersants may be required in some cases to product a suitable concentrated stock solution but every effort should be made to avoid their use. Acetone, ethanol, methanol, dimethyl formamide, and triethylene glycol are examples of suitable solvents. Cremophor RH4, methylcellulose 0.01%, and HCO-40 are suitable dispersants.

***-****Benthic Aquatic Arthropods:*

*or acetone should be used. The concentration of such carriers should not exceed 0.1 mL/L.*

***OECD 218 and 219*** *The use of solvents or dispersants may be required in some cases in order to produce a suitably concentrated stock* solution. Examples are acetone, ethanol, methanol, ethylene glycol, monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide and triethylene glycol. Dispersants which may be used are Cremophor RH40, Tween 80, methylcellulose 0.01% and HCO-40. The solubilising agent concentration in the final test medium should be minimal (i.e., ≤0.1 mL/L) and should be the same in all treatments. Every effort should be made to avoid the use of such materials.

## Confirmation of MPCA viability:

##### *[Describe the methods used to confirm MPCA viability in the test material.]*

***U.S. EPA OCSPP 885.4240*** *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.*

***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 suspension in each treatment* (including controls) should be determined at the beginning and end of the test and at the beginning and end of at least one of the renewal cycles during each week of the test.

***-****Pelagic Aquatic Arthropods:*

***OECD 202 and OECD 211*** *No specific recommendations for viability testing (guideline was designed for chemical toxicity testing). However,* the concentration of test substance should be measured at minimum at the highest and lowest test concentration, at the beginning, and end.

*Results should be based on measured concentrations.*

***-****Benthic Aquatic Arthropods:*

***OECD 218 and 219*** *The use of solvents or dispersants may be required in some cases in order to produce a suitably concentrated stock* solution. Examples are acetone, ethanol, methanol, ethylene glycol, monoethyl ether, ethylene glycol dimethyl ether, dimethylformamide and triethylene glycol. Dispersants which may be used are Cremophor RH40, Tween 80, methylcellulose 0.01% and HCO-40. The solubilising agent concentration in the final test medium should be minimal (i.e., ≤0.1 mL/L) and should be the same in all treatments. Every effort should be made to avoid the use of such materials.

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

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

***U.S. EPA OCSPP 885.4240*** *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

***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. A positive chemical control is not required.

***-****Pelagic Aquatic Arthropods:*

***OECD 202*** *A reference substance may be tested for EC50 as a means of assuring that the test conditions are reliable. Toxicants used in* international ring-tests are recommended for this purpose. Test(s) with a reference substance should be done preferably every month and at least twice a year.

***OECD 211*** *No specific recommendations.*

***-****Benthic Aquatic Arthropods:*

***OECD 218 and 219*** *Reference substances may be tested periodically as a means of assuring that the test protocol and test conditions are* reliable. Examples of reference toxicants used successfully are lindane, trifluralin, pentachlorophenol, cadmium chloride and potassium chloride.

## Other controls:

##### *[Describe the other controls used.]*

***U.S. EPA OCSPP 885.4240*** *A concurrent, negative, non-dosed control group should be included in addition to another control group in which* invertebrates are exposed to sterile culture filtrate from production cultures. ***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, non-dosed control group of the non-target organism should also be run concurrently with the test group.*

***-****Pelagic Aquatic Arthropods:*

***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.

***OECD 202*** *One dilution-water control series and also, if relevant, one control series containing the solubilizing agent (solvent control) at the* level used in treatments.

***OECD 211*** *One test-medium control series and also, if relevant, one control series containing the solvent or dispersant should be run in* addition to the test series. When used, the solvent or dispersant concentration should be the same as that used in the vessels containing the test substance.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Each test must include a negative control comprised of clean sediment and clean overlying water. Use of* a non-infectious control is strongly recommended. The use of a sterile filtrate control is optional.

***OECD 218 and 219*** *Control vessels without any test chemical but including sediment should be included in the test with the appropriate number* of replicates. If a solvent has been used for application of the test substance, as sediment solvent control should be added.

## Number of replicates/groups:

#### Control(s): Treatments:

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For daphnids, at least ten replicates per treatment concentration and control. For shrimp, 3 replicates per* treatment concentration and control.

***OECD 202*** *Preferably four replicates per treatment or control.*

***OECD 211*** *For static-renewal testing, ten replicates per treatment or control. For flow-through testing, ≥2 replicates (4 recommended).*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Five replicates per treatment concentration and control.*

***OECD 218 and 219*** *The number of replicates depends on the desired output. For analysis by regression (calculation of an ECX), ≥3 replicates* are required. Use additional replicates to estimate 10-day larval survival and growth. For estimation of a NOEC or LOEC, ≥4 replicates are required for statistical power. For a limit test use ≥6 replicates.

## Number of organisms per replicate /groups:

#### Control(s): Treatments:

***U.S. EPA OCSPP 885.4240*** *20 invertebrates per test concentration for multiple dose testing. 50 invertebrates per test concentration for single-* dose testing.

***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 (e.g., for D. magna 50 arthropods recommended).

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For daphnids: one per replicate (≥10 per test concentration). For shrimp: 10 per replicate (30 shrimp/treatment)*

***OECD 202*** *At least 20 daphnids per treatment, or control.*

***OECD 211*** *For static-renewal testing, one daphnid per replicate (10 per test concentration). For flow-through testing, ≥20 daphnids (40* recommended) per test concentration, in ≥2 replicates (4 replicates recommended).

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Ten organisms per replicate (50/test concentration)*

***OECD 218 and 219*** *20 larvae per replicate.*

## Biomass loading rate:

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *One daphnid per 50 –100 mL; 0.8 mg/L shrimp*

***OECD 202*** *≥2 mL solution/daphnid.*

***OECD 211*** *One daphnid per 50–100 mL.*

***-****Benthic Aquatic Arthropods:*

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

***OECD 218 and 219*** *No specific recommendations.*

## Recovery of MPCA from tissues:

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

***U.S. EPA OCSPP 885.4240*** *A detailed description of steps taken to determine microorganism dissemination, replication and survival in test* animal tissues, organism and fluids is required.

***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*** *Analytical techniques permitting, the recovery of the MPCA in whole-body homogenate of parental* daphnids or shrimp from each treatment is optional during and/or at test end.

***-****Pelagic Aquatic Arthropods:*

***OECD 202 and OECD 211*** *No specific recommendations (guideline designed for chemical toxicity testing).*

***-****Benthic Aquatic Arthropods:*

***OECD 218 and 219*** *No specific reference to viability testing (guideline designed for chemical toxicity testing).*

## Feeding:

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

***U.S. EPA OCSPP 885.4240*** *No specific recommendations- usually ad libitum*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids should be fed a concentrated suspension of living algal cells (as per OECD 211) once daily or* at least 3×/week (at renewal) throughout the test. Shrimp should be fed ad libitum once daily using a commercially prepared fish food diet.

***OECD 202*** *Daphnids should not be fed during the test.*

***OECD 211*** *Preferably daily for static-renewal, but at least 3×/week (corresponding to media changes) Living algal cells (Chlorella sp.,* Selenasrum capricornatum, or Stenedesmus subspicatus) are fed as a concentrated suspension at 0.1–0.2 mg/daphnid/day. If a larger volume is used, the ration given to daphnids may need to be increased to ensure food availability.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Three times/week, on non-consecutive days (e.g., Mon, Wed, Fri), with ground tropical fish food flakes.* For chironomids, 15.0 mg dry solids in a 3.75 mL suspension added to each test chamber. For H. azteca, 6.3 mg dry solids in a 3.5 mL suspension added to each test chamber during each feeding.

***OECD 218 and 219*** *Larvae are fed daily, or at least 3 ×/week. Fish food (a suspension in water or finely ground food, for example Tetra-Min* or Tetra-Phyll) in the amount of 0.25–0.5 mg/larva/day is adequate for the first 10 days 0.5–1.0 mg/larva is recommended for the duration of the test.

## Lighting:

##### *[Describe lighting conditions used during the test.]*

***U.S. EPA OCSPP 885.4240*** *No specific recommendations, but the lighting must be reported.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For daphnids, cool fluorescent or full spectrum light at an intensity of ≤15–20 µEm-2s-1with a gradual* transition period between dark and light is preferred. For shrimp, cool-white or full spectrum at an intensity of 300–1000 lux at the water surface; 16±1 hour light/8±1 hour dark with a gradual transition period between dark and light.

***OECD 202*** *A 16-hour light, 8-hour dark cycle is recommended. Complete darkness is also acceptable, especially for test substances that are* susceptible to light.

***OECD 211*** *16-hour light at an intensity ≤15–20 µEm-2s-1.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Overhead full spectrum (fluorescent or equivalent). 500–1000 lux at water surface. Photoperiod of 16 ±* 1 hour light, 8 ± 1 hour dark.

***OECD 218 and 219*** *A 16-hour photoperiod is used and the light intensity should be 500 to 1000 lux.*

## Duration of study:

***U.S. EPA OCSPP 885.4240*** *The test duration should be at least 21 days. If pathogenicity and/or toxicity are apparent at day 21, observation* should continue until recovery, mortality or unequivocal moribundity is established.

***PMRA DIR 2001-02*** *The test organism should be exposed for at least 21 days, or until mortality in the control group increases to a significant* level.

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For daphnids: the test duration is 21 days. For shrimp: the test duration is 30 days for shrimp.*

***OECD 202*** *The test duration is 48 hours.*

***OECD 211*** *The test duration is 21 days.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *The test duration is at least 10 days for Chironomids and at least 14 days for H. azteca.*

***OECD 218 and 219*** *The maximum exposure duration is 28 days for C. riparius and C. yoshimatsui, and 65 days for C. tentans. If midges* emerge earlier, the test can be terminated after a minimum of 5 days after emergence of the last adult in the control.

## Other methods or conditions, if any:

* 1. **Observations:**

**Parameters measured including mortality, 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 885.4240*** *Measurements of temperature, dissolved oxygen content, pH, dissolved salts, lighting, and concentrations of MPCA* are required. Include any observations for mortality and/or sublethal effects (e.g., changes in life cycle duration, fecundity, morphology).

***PMRA DIR 2001-02*** *Regular observations are required to record mortalities and note any behavioral, pathogenic or toxic adverse effects.*

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids: temperature, pH, hardness and dissolved oxygen concentration and, analyses permitting,* concentration of the MPCA in each treatment (incl controls). Survival of parental daphnids and cumulative number of live young produced per parental daphnid; for any chamber wherein the parental daphnid dies, exclude this replicate from analysis. Shrimp: temperature, pH, salinity, dissolved oxygen concentration and, analyses permitting, concentration of the MPCA in each treatment (incl controls). The survival, appearance and behavior of shrimp in each test chamber.

***OECD 202*** *Measurements of temperature, dissolved oxygen, pH and concentration of the test substance are required. Observations for* immobilization and abnormal behavior.

***OECD 211*** *Measurements of temperature, dissolved oxygen, hardness, pH, light intensity and concentration of the test substance are required.* Offspring are counted and removed. Only living offspring should be counted, but the presence of aborted eggs/dead offspring is recorded.

*Mortality among parent animals is recorded when offspring are counted. Other parameters (body length, time to production of first brood, no.* and size of broods per animal, no. of aborted broods, presence of males or ephippia or intrinsic rate of population increase) can be used to define sublethal effects.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Chironomids: Measurements of the dissolved oxygen, temperature, pH, conductivity, ammonia, mean dry* weight of surviving midges and concentration of the MPCA in the test suspension and in sediment of each treatment group are required.

*Observations for the number of midge larvae on sediment surface, appearance, and survival. Amphipods: Measurements of the dissolved oxygen,* temperature , pH, conductivity, ammonia, mean dry weight of surviving amphipods and concentration of the MPCA in the test suspension and in sediment of each treatment group are required. Observations for the number of amphipods on sediment surface, appearance, and survival.

***OECD 218 and 219*** *Concentration of the test substance, physical and chemical parameters of the test/overlying water. Emergence, number of* male and female adults. Abnormal behavior (e.g., sediment avoidance, swimming). Egg masses recorded and removed, number of visible pupae that have failed to emerge. Growth and survival (if 10-day growth and survival are to be tested, additional test vessels should be included at the start).

## Observation/measurement intervals:

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

***U.S. EPA OCSPP 885.4240*** *Detailed description of the effects of exposure to the test substance, including:(i) The criteria used to determine the* effects; (ii) percentages of organisms that died or showed effects of treatment; (iii) A summary of these observations, including changes in life cycle(duration, fecundity, and morphology). ***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.*

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *For daphnids: temperature, pH, hardness, and dissolved oxygen concentration should be measured at* least once a week in fresh and old test suspensions of each control and the highest test concentration. Analyses permitting, the concentration of the MPCA in each treatment and control group should be determined at the beginning and end of the study, and at the beginning and end of at least one renewal cycle during each week of the study. Survival and number of live young should be observed daily (young should be removed

*after counting). For shrimp: temperature, pH. dissolved oxygen concentration should be measured at the beginning and end of each renewal for* at least one replicate of each treatment. Analyses permitting, the concentration of the MPCA in each treatment and control group should be determined at the beginning and end of the study, and at the beginning and end of at least one renewal cycle during each week of the study.

*Survival, appearance and behavior must be observed daily and at test end.*

***OECD 202*** *Observation for immobility and abnormal behavior at 24 and 48 hours. The dissolved oxygen and pH are measured at the beginning* and end of the test in the control(s) and in the highest test substance concentration. The temperature is usually measured in control vessels or in ambient air and it should be recorded preferably continuously during the test or, as a minimum, at the beginning and end of the test. The concentration of the test substance should be measured, as a minimum, at the highest and lowest test concentration, at the beginning and end of the test.

***OECD 211*** *Offspring counted daily, mortality in parent animals assessed daily. Dissolved oxygen concentration, temperature, hardness and pH* values should be measured at least once a week, in fresh and old media, in the control(s) and in the highest test substance concentration. In static-renewal studies where the concentration of the test material is expected to remain within 20% of the nominal concentration, the concentration in highest and lowest test concentrations should be analyzed when freshly prepared and at least once weekly at time of renewal. In static-renewal studies where the concentration is not expected to remain within 20% of the nominal concentration, the concentration in all chambers should be analyzed when freshly prepared and at all renewals. In flow-through studies, concentrations should be determined at least weekly, however, the flow-rate of diluent and test substance must be checked daily.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Temperature, dissolved oxygen measured 3×/week; pH, conductivity and ammonia at start and end of test* and each renewal; concentration of the MPCA at beginning and end of test, and in water, at one renewal cycle per week. Observations of test organisms at each renewal. The mean dry weight of surviving test organisms measured at test end.

***OECD 218 and 219*** *Test vessels should be observed at least 3 times per week for abnormal behavior. During the period of expected* emergence, daily counts are required (sex and number). Prior to the beginning of the test, the concentration of the test substance in sediment should be determined in at least one vessel per treatment. It is recommended that, as a minimum, samples of the overlying water, the pore water and the sediment be analyzed at the start and at the end of the test, at the highest concentration and a lower one. Physical and chemical parameters measured at the beginning and end of the test and at suitable intervals.

## Testing for infectivity/pathogenicity:

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

***U.S. EPA OCSPP 885.4240*** *A detailed description of steps taken to determine microorganism dissemination, replication and survival in test* animal tissues, organism and fluids is required.

***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 optional during and/or at test end based on measured concentrations of new microbial* substance in whole-body homogenates.

***OECD 202, 211, 218, & 219*** *No specific recommendations (guideline designed for chemical toxicity testing).*

## Necropsy:

##### *[Indicate on which groups of test animals necropsies were performed, and list observations made at* necropsy (gross lesions, histological examination).]

***U.S. EPA OCSPP 885.4240*** *No specific recommendations.*

***PMRA DIR 2001-02*** *Gross necropsy and histopathological examination should be performed on exposure site tissues and other organs or* tissues showing anatomical or physiological abnormalities in adversely affected test organisms. In cases where tissue preferences are known or suspected, the tissues should be examined whether or not gross anatomical or physiological changes are seen.

***Environment Canada EPS 1/RM/44*** *All dying as well as those surviving at test termination must be necropsied; organs and tissues must be* examined for evidence of lesions and abnormalities. Selected tissues must be collected for future microscopic examination where necessary. ***OECD 202, 211, 218, & 219*** *No specific recommendations (guideline designed for chemical toxicity testing).*

## Water quality was acceptable? (Results and Discussion Section# 1) Were raw data included?

**Other observations, if any:**

1. **RESULTS**
2. **WATER QUALITY PARAMETERS:**

##### *[Summarize water quality measurements and discuss the acceptability.]*

**TABLE *[#]*.** Dissolved oxygen, temperature and pH in test suspensions during the *[X]*-day exposure of *[test organism]* to *[concentration]* of *[test substance]* under *[static-renewal/flow-through]* conditions.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Replicate** | **Day 0** | **Day 2** | | **Day 4** | | ***Day –2*** | | ***Day n*** |
| **New** | **Old** | **New** | **Old** | **New** | **Old** | **New** | **Old** |
| Dissolved oxygen (% saturation or mg/L) | 1 |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |
| Temperature (°C) | 1 |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |
| pH | 1 |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |

###### *[Table suitable for microbial infectivity/pathogenicity and toxicity testing of the maximum hazard concentration. Modify as* appropriate to accommodate differences in experimental design or delete if acute toxicity test or multiple concentrations are used.]

**TABLE *[#]*.** *[Dissolved oxygen concentration/Temperature/pH]* in *[units]* in test suspensions during the *[X]*- day exposure of *[test organism]* to *[test substance]* under *[static- renewal/flow-through]* conditions.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Day** | **Suspension** | ***[Parameter (units)]*** | | | | | |
| **Nominal Concentration of Test Suspension (CFU/L)** | | | | | |
| ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | **Negative Control** |
| 0 | new |  |  |  |  |  |  |
| 2 | old |  |  |  |  |  |  |
| new |  |  |  |  |  |  |
| *–2* | old |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Day** | **Suspension** | ***[Parameter (units)]*** | | | | | |
| **Nominal Concentration of Test Suspension (CFU/L)** | | | | | |
| ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | **Negative Control** |
| new |  |  |  |  |  |  |
| *n* | old |  |  |  |  |  |  |

###### *[Table suitable for microbial infectivity/pathogenicity and toxicity testing of multiple concentrations. Modify as appropriate* to accommodate differences in experimental design or delete if acute toxicity test or maximum hazard concentration is used.]

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

**TABLE *[#]***. *[Method (e.g., Viable count)]* verification of test and control suspension concentrations during an

*[X]*-day exposure of *[test organism]* to *[test substance]*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Day** | **Suspension** | **Measured Concentration of Test Suspension (CFU/L)** | | | | | |
| **Nominal Concentration of Test Suspension (CFU/L)** | | | | | |
| ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | ***X × 10X*** | **Negative Control** |
| 0 | new |  |  |  |  |  |  |
| 2 | old |  |  |  |  |  |  |
| new |  |  |  |  |  |  |
| *–2* | old |  |  |  |  |  |  |
| new |  |  |  |  |  |  |
| *n* | old |  |  |  |  |  |  |

##### **MORTALITY:** *[Briefly summarize mortality; indicate if there was a dose-response effect; slope* values, if provided. Compare the mortality with control treatment and/or the reference chemical (if used). Data may be summarized in a table such as those presented below. Modify tables 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 *[#]***. Cumulative mortality *[or number of immobilized] [test organism]* exposed to *[test substance]* for

*[test duration]* under *[static-renewal/flow-through]* conditions.

|  |  |
| --- | --- |
| **Day** | **Cumulative Mortality *or [Number of Immobilized] [test organism]*** |
| **Measured Concentration of Test Suspension (CFU/L)** |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***X × 10X*** | | ***X × 10X*** | | ***X × 10X*** | | ***X × 10X*** | | ***X × 10X*** | | **Negative Control** | |
| **A** | **B** | **A** | **B** | **A** | **B** | **A** | **B** | **A** | **B** | **A** | **B** |
| 1 | *X/Y* |  |  |  |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |  |  |  |  |  |  |
| *N* |  |  |  |  |  |  |  |  |  |  |  |  |
| **Total Mortality [Immobility]** |  | |  | |  | |  | |  | |  | |

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

**TABLE *[#]*.** Mean percent survival in *[test organism]* exposed to *[test substance]* for *[test duration]* under

*[static-renewal/flow-through]* conditions.

|  |  |
| --- | --- |
| **Treatment (CFU/L)** | **Mean Percent Survival (%)** |
| *Test concentration 1* |  |
| *Test concentration 2* |  |
| *Test concentration 3* |  |
| *Test concentration 4* |  |
| *Test concentration n* |  |
| Negative control |  |
| LC50  *[insert [>] if greater than]* |  |
| NOEC  *[insert [>] if greater than]* |  |

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

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

1. **SUBLETHAL TOXICITY ENDPOINTS:** *[Include if study design used subchronic assessment test end-points and/or if any sublethal effects are observed (e.g. abnormal observations of offspring produced by female and/or effects on fecundity). Briefly summarize behavioral abnormalities or other signs of toxicity. Indicate effects that were related to the test-material. Compare the sublethal observations 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 *[#]*.** Mean cumulative number of offspring produced per female *[test organism] during [test duration] [acute/chronic]* exposure to *[test substance]* under *[static-renewal/flow-through]* conditions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Day** | **Mean Cumulative Number of Offspring Produced per Female** | | | | | |
| **Mean Measured Concentration (CFU/L)** | | | | | |
| ***X.XX × 10X*** | ***X.XX × 10X*** | ***X.XX × 10X*** | ***X.XX × 10X*** | ***X.XX × 10X*** | **Negative Control** |
| 1 |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |
| 4 |  |  |  |  |  |  |
| 5 |  |  |  |  |  |  |
| 6 |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |

###### *[Table suitable for testing at multiple concentrations. Modify as appropriate to accommodate differences in experimental* design, otherwise delete if maximum hazard concentration was used.]

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

**TABLE *[#]*.** Sublethal effects *(e.g., growth, ephippia, etc.)* in *[test organism]* during *[test duration] [acute/chronic]* exposure to *[test substance]* under *[static-renewal/flow-through]* conditions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mean Measured Concentration** | **Observation Period** | | |
| ***endpoint 1* at *Day x1*** | ***endpoint 2* at *Day x2*** | ***endpoint n* at *Day x n*** |
| **% Affected** | **% Affected** | **% Affected** |
| *Test concentration 1* |  |  |  |
| *Test concentration 2* |  |  |  |
| *Test concentration 3* |  |  |  |
| *Test concentration 4* |  |  |  |
| *Test concentration n* |  |  |  |
| EC50  *[insert [***>***] if greater than]* |  |  |  |
| NOEC  *[insert [***>***] if greater than]* |  |  |  |
| LOEC  *[insert [***>***] if greater than]* |  |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Mean Measured Concentration** | **Observation Period** | | |
| ***endpoint 1* at *Day x1*** | ***endpoint 2* at *Day x2*** | ***endpoint n* at *Day x n*** |
| **% Affected** | **% Affected** | **% Affected** |
| Positive control, if used  % sublethal effect: EC50:  *[insert [***>***] if greater than]* |  |  |  |

###### *[Table suitable for microbial infectivity/pathogenicity and toxicity (maximum hazard dose) testing. Modify as appropriate to* accommodate differences in experimental design or delete if acute toxicity test is used.]

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

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

***U.S. EPA OCSPP 885.4240*** *Satisfactory data must establish whether or not the test substance is pathogenic to the test organisms during a* sufficiently long period of exposure and observation, or the test must establish a definitive LC50 value with 95% confidence intervals or that the LC50 is greater than the highest dose. ***U.S. OCSPP 885.0001 Overview for microbial pest control agents (MPCA)****- Appropriate statistical* analyses 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.

***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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Daphnids: Single concentration test: percent survival of parental daphnids in each treatment at test end;* mean (±SD) no. young/parental daphnid, for each treatment. Multiple concentration test: percent survival of parental daphnids in each treatment at test end; mean (±SD) no. live young produced per parental daphnid, for each treatment; data permitting–21-day LC50 for parental daphnids, 21-day IC25 for number of live young produced per parental daphnid, NOEC/LOEC. Shrimp: Single concentration test: percent survival of shrimp in each treatment at test end; percent of shrimp showing atypical behavior and/or atypical appearance of organs and/or tissues, comparing MHC to controls. Multiple concentration test: Single concentration test: percent survival of shrimp in each treatment at test end; percent of shrimp showing atypical behavior and/or atypical appearance of organs and/or tissues, comparing MHC to controls.30-day LC50, 30- day EC25 based percent of shrimp showing atypical behavior and/or atypical appearance of organs and/or tissue, NOEC/LOEC.

***OECD 202*** *Calculate the slopes of curves and the EC50 with 95% confidence intervals. Where standard methods of calculating the EC50 are not* applicable, the EC50 should be considered to be the geometric mean of the LOEC and the NOEC.

***OECD 211*** *Consult the guideline for recommended statistical methods.*

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Single concentration test: percent survival, percent surviving test organisms showing atypical appearance* (necropsy) or behavior at test end, mean dry weight of survivors, comparing MHC to controls; Multiple concentration test: percent survival, percent surviving test organisms showing atypical appearance (necropsy) or behavior at test end, mean dry weight of survivors, comparing each test chamber and treatment. Data permitting, calculation of 10- or 14-day LC50, 10- or 14-day EC50 for atypical appearance, 10- or 14-day IC25 for mean dry weight of survivors, NOEC/LOEC.

***OECD 218 and 219*** *Analysis by regression (EC50), estimation of an NOEC/LOEC, or a limit test are all acceptable. For estimation of* NOEC/LOEC, based on development rate, an ANOVA is usually appropriate, such as Dunnett-test and Williams-test. In the emergence ration the Cochrane-Armitage, Fisher’s exact (with Bonferroni correction) or Mantel Haentzal tests may be used. For a limit test, with metric response (development rate and weight), the t-test is suitable if data meet the requirements of normality and homogeneous variance. The unequal-variance t-test or a non parametric tests such as the Wilcoxon-Mann-Withey test may be used. For the emergence ration, the Fisher exact test is appropriate.

## VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

##### *[Report the statistical methods used by the reviewer to verify the applicant’s results, if applicable. If* values for LC50 and/or NOEL are greater than the MHD level, use ***<*** *symbol.]*

LC50: 95% C.I.:

NOEL:

Probit Slope: 95% C.I.:

1. **CONCLUSION**
2. **STUDY AUTHOR CONCLUSION:** *[Summarize the study author’s conclusions- Provide the major conclusions e.g., LC50, LT50, NOEC [insert value] was [****=, > or <****] [insert final dose concentration/level (in appropriate units). Include Probit slope (95% confidence interval) and if sublethal effects were observed.]*
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 toxicity and pathogenicity study for freshwater aquatic invertebrates (OCSPP 885.4340; PMRA: M9.5.2 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 885.4240*** *No specific validity criteria.*

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

***-****Pelagic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *Invalid if <80% of parental daphnids survive to the end of the test or if < 60 live young are produced per* adult in negative controls. Study is invalid in shrimp if <80% survival in negative control <80% at test end.

***OECD 202*** *The study is invalid if more than 10% of control daphnids are immobilized or if dissolved oxygen concentrations are less than 3* mg/L in control or test vessels.

***OECD 211*** *The study is invalid if there is greater than 20% mortality in parental daphnids, or if the mean number of live offspring is less than* 60/parental daphnid.

***-****Benthic Aquatic Arthropods:*

***Environment Canada EPS 1/RM/44*** *The chironomid test is invalid if mean 10-day survival in negative control is <70%, and if mean dry weight* at test end for negative controls is > 0.6 mg for C. tentans) or >0.5 mg for C. riparius). The H. azteca test is invalid if 14-day survival in negative control is <80%, or if individual mean dry weight for negative controls at test end is <0.1 mg.

***OECD 218 and 219*** *Emergence in controls must be ≥70% at the end of test (emergence expected between 12 and 23 days for C. riparius and C.* yoshimatsui; 20–65 days for C. tentans). DO should be 60% saturation and pH should be 6–9 at the end of the test in all test vessels. The water temperature should not differ by more than ±1̊*C.*

## 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 freshwater aquatic invertebrate studies (OCSPP 885.4240). 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 aquatic 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 aquatic invertebrates.

The proposed uses of *[product name]* on *[identify use sites/crops]* is not expected to result in increased exposure or adverse effects to freshwater aquatic invertebrates. *[If environmental concentration will show a substantial increase in aquatic environment, 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 aquatic invertebrates. The *[applicant]* requests a waiver of freshwater aquatic invertebrate 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 aquatic exposure to *[name of active ingredient]*, due to use of the end-use product *[product name]*, will be minimal to aquatic invertebrates.** *[Applicant should provide further elaboration: Describe the natural habitat of the MPCA. 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 aquatic arthropods. *[Does timing of application preclude direct exposure to aquatic insects/arthropods? Discuss crop use sites and application methods and its effects on limiting runoff, if applicable. Give 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 aquatic arthropods.* Would any of the MPCA that reaches the soil/water behave as it would in the wild? State whether the

*MPCA does/does not survive or persist in aquatic ecosystems.]*

* 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/aquatic fate and interactions with aquatic arthropods. *[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 aquatic arthropod species? Have any cases or evidence of adverse effects, due to the MPCA, been reported in the literature?]*

##### *[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 aquatic insect/arthropod pathogens, particularly crustaceans? Provide the lists examined. Have any adverse effects to wild populations of aquatic 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- Provide the major conclusions e.g., values for LC50, ID50, EC50, NOEC, etc.* were *[****=, > or <****] insert final dose concentration/level (in appropriate units).]*
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]*
2. **METHOD** *[Describe the experimental procedure]*
3. **RESULTS** *[Summarize the results using appropriate headers*

e.g*.,* ***A. GENERAL OBSERVATIONS:***

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

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)***

**I. 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 Müeller (1785), three approaches have been used in Canada to examine the effects of this MPCA on non-target stream invertebrates, including pelagic organisms. 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)*
  2. ***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)]* | |
| **II.**  **A.** | **CONCLUSION**  **LITERATURE REVIEW CONCLUSION:** | | | *[Summarize the study author’s conclusions]* |

1. **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]**

**III. 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.*]**