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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:** Estuarine and Marine Animal Testing, Tier I

#### U.S. EPA OCSPP Guideline: 885.4280

PMRA Data Code: M9.4.2–Estuarine or marine fish PMRA Data Code: M9.6–Non-arthropod invertebrates OECD Data Code: IIM 8.2, IIIM 10.2

**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 Unpublished *[OR if published, list Journal name, vol.: pages]*.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 were no test material-related adverse effects.]

The *[#]*-day LC50 was *[****=, > or <****] [insert LC50 in appropriate units]* for *[formulation, note its potency, biological activity and/or concentration per unit weight or volume]* (containing % *a.i. name*). *[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 (if applicable)]*, 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 estuarine and marine animal testing (OCSPP 885.4280; PMRA: M9.6 and OECD: IIM 8.2, IIIM 10.2) 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 885.4280–Estuarine and Marine Animal Testing1* PMRA DIR 2001-02 Part 9.4.2 1Estuarine or Maine Fish

*PMRA DIR 2001-02 Part 9.61 Non-arthropod invertebrates NOTE : For benthic and pelagic arthropod species- see individual*

*PMRA DER templates or EPA DER template – Freshwater Aquatic Invertebrate Testing*

*Environment Canada EPS 1/RM/44 Section 11.21 OECD 203–Fish acute toxicity test2*

*OECD 204–Fish prolonged toxicity test: 14-day study2]*

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

*2 Guideline designed to test acute 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:**
   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 if the MPCA is stable under these conditions.]*

* 1. **Test Organism:**

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

***U.S. EPA OCSPP 885.4280*** *Testing is preferred for one species of shrimp, preferably Paleomonetes vulgaris and one estuarine or marine fish* species. Testing of additional estuarine and marine animal species may be required, preferably those that are likely to prey upon or scavenge the ***PMRA DIR 2001-02*** *Testing should be performed on one estuarine or marine fish species, preferably sheepshead minnow (Cyprinodon* variegatus).

***Environment Canada EPS 1/RM/44*** *Seawater-acclimated rainbow trout (Oncorhynchus mykiss), chum salmon (Oncorhynchus keta), and pink* salmon (Oncorhyncus gorbuscha) are the recommended test species for psychrophilic microorganisms. Seawater-acclimated inland silverside (Menidia berylina), sheepshead minnor (Cyprinodon variegatus), topsmelt (Atherinops affinis) and threespine stickleback (Gasterosteus aculeatus) are preferred for warm-water tests with mesophilic organisms.

***OECD 203 & 204*** *No estuarine/marine species are recommended.*

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

***U.S. EPA OCSPP 885.4280*** *Testing of young (all of the same year class), actively feeding fish is preferable. Very young, spawning, or recently* spawned fish should not be tested.

***PMRA DIR 2001-02*** *Actively feeding juvenile fish, 3–6 months old should be treated.*

***Environment Canada EPS 1/RM/44*** *Juveniles in exponential growth phase.*

***OECD 203 & 204*** *No specific recommendations.*

**Weight at test initiation (mean and range):** *[Insert the weight of the test organisms.]*

***U.S. EPA OCSPP 885.4280*** *Fish should weigh between 0.5 and 5.0 g.*

***PMRA DIR 2001-02*** *All test fish should weigh between 0.5 and 5.0 grams and be from the same year class.*

***Environment Canada EPS 1/RM/44*** *Individual wet weights should be within ± 10% of mean wet weight, and must be within 25% of mean wet weight.*

***OECD 203 & 204*** *No specific recommendations.*

**Length at test initiation (mean and range):** *[Insert the length of the test organisms.]*

***U.S. EPA OCSPP 885.4280*** *The length of the longest fish no more than twice that of the shortest fish.* ***PMRA DIR 2001-02*** *The length of the longest fish should be no more than twice that of the shortest fish.* ***Environment Canada EPS 1/RM/44*** *Length of the longest fish no more than twice that of the shortest fish.* ***OECD 203 & 204*** *No specific recommendations for estuarine/marine species.*

## Number of animals/Sex:

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

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

***OECD 203 & 204*** *No specific recommendations.*

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

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Test organisms should all be from the same population.*

***OECD 203 & 204*** *No specific recommendations for estuarine/marine species. The fish should be in good health and free from any apparent* malformation.

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

1. **STUDY DESIGN AND METHODS:**

*[Briefly describe the experimental design.]*

***U.S. EPA OCSPP 885.4280*** *A single group of fish is tested at the maximum hazard dose administered as a suspension in the water, and in the* diet. If deleterious effects are seen, a series of test concentrations is tested. After an observation period of at least 30 days, an LC50 and/or IC50 are determined.

***PMRA DIR 2001-02*** *Test fish should be exposed to the maximum hazard concentration of the MPCA for the duration of the study period.* During testing fish should be periodically examined for the incidence of any adverse effects. If any adverse effects are observed, then a period of recovery should be instituted immediately on half of the tank replicates to determine whether the effects are reversible. The dissemination, replication and survival of the MPCA should also be determined in representative tissues organs and fluids.

***Environment Canada EPS 1/RM/44*** *Juvenile fish in exponential growth phase are placed, after being weighed, in test chambers and are* exposed to the maximum hazard concentration (limit test) or a range of concentrations of the test substance dissolved in water preferably under static-renewal conditions. The test duration is 28 days. At the end of the test, the fish are weighed again. Effects on growth rates are used to estimate ECp or LOEC/NOEC.

***OECD 203*** *The fish are exposed to the test substance preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours* and the concentrations which kill 50% of the fish (LC50) are determined where possible.

***OECD 204*** *Threshold levels of lethal and other observed effects and NOEC are determined at intervals during the test period, which is at least* 14 days.

## Experimental Methods and Conditions:

**Acclimation:**

#### Period: Conditions: Feeding: Water:

Health *(any mortality observed?)*:

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

***U.S. EPA OCSPP 885.4280*** *No specific recommendations, but acclimation conditions must be reported.*

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

***Environment Canada EPS 1/RM/44*** *Acclimation for at least two weeks to the test conditions (i.e., temperature, photoperiod, food, dilution water).*

***OECD 203*** *All fish must be obtained and held in the laboratory for at least 12–15 days before testing. For at least 7 days preceding the test,* they must be held in dilution water with 12 to 16 hours light per day, at test temperature and DO 80% saturation. Feeding is 3 times weekly or daily until 24 h before the start of testing. Prophylactic treatments should be avoided but reported when used. The entire batch of fish should be rejected if more than 10% of population dies in seven days following a 48-hour settling-in period. Acclimation should continue for 7 additional days if 5–10% of the population dies following a 48-hour settling-in period.

***OECD 204*** *All fish must be obtained and held in the laboratory for at least 12–15 days before testing. For at least 7 days preceding the test,* they must be held in dilution water with 12 to 16 hours light per day, at test temperature and DO 80% saturation. Feeding is daily. Prophylactic treatments should be avoided but reported when used. The entire batch of fish should be rejected if more than 10% of population dies in seven days following a 48-hour settling-in period. Acclimation should continue for 7 additional days if 5–10% of the population dies following a 48- hour settling-in period.

## Test vessel:

*[Describe the test vessel.]*

Material: Size:

Fill volume:

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Depth of water in test chamber ≥15 cm.*

***OECD 203 & 204*** *Tanks made of chemically inert material and of a suitable capacity in relation to the recommended loading.*

## Test system:

##### *Static/flow through*

*Type of dilution system- for flow through method Flow rate*

*Renewal rate for static renewal*

***U.S. EPA OCSPP 885.4280*** *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. ***Environment Canada EPS 1/RM/44*** *Static renewal, three times per week on non-consecutive days (e.g., Mon., Wed., Fri.)*

***OECD 203*** *Static, static renewal or flow-through tests are acceptable. Static renewal and flow-through testing are preferred to maintain* constant conditions.

***OECD 204*** *Flow-through testing should normally be used to maintain constant conditions, but static renewal may be adopted if adequate to* maintain conditions.

## Source of dilution water:

##### *[Describe the source of dilution water.]*

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Natural (uncontaminated) or artificial seawater. 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 203 & 204*** *No specific recommendations for estuarine/marine fish testing.*

## Water parameters:

#### Dissolved oxygen pH

Temperature Salinity Particulate matter

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

Pesticides Chlorine

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

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

***PMRA DIR 2001-02*** *For marine fish, salinity of the test water should be maintained between 30‰ and 35‰. For estuarine fish, the salinity for* the test water should be maintained between 10‰ and 20‰.

***Environment Canada EPS 1/RM/44*** *Dissolved oxygen ≥60% saturation, pH 7.0–8.5, temperature 5–16°C if salmonid fish or 19–25°C if inland* silverside, sheepshead minnow, topsmelt or threespine stickleback (temperature held within ± 2°C of the mean temperature throughout the test), salinity range: ≤14‰ if rainbow trout, 10–32‰ if chum or pink salmon, 5–32‰ if inland silverside, topsmelt or threespine stickleback, 20–32‰ if sheepshead minnow (salinity held within ±2‰ of mean salinity throughout the test). 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 203 & 204*** *No specific recommendations for estuarine/marine fish testing.*

## Aeration:

##### *[Describe how the test vessels were aerated, if applicable.]*

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Aerate gently in all test chambers only if necessary to maintain a DO ≥60% saturation.*

***OECD 203*** *Aeration can be used provided it does not lead to a significant loss of test substance, to keep the dissolved oxygen concentration* above 60% saturation.

***OECD 204*** *In semi-static tests, aeration can be used provided it does not lead to as significant loss of test substance.*

## Route(s) of exposure:

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

***OCSPP 885.4280*** *The test substance shall be administered as a suspension directly into the water. Additionally the MPCA should be* administered through the oral route of exposure, preferably through incorporation in standard fish food or through the use of infected insects. ***PMRA DIR 2001-02*** *Aquatic exposure and/or diet in the form of diseased target pests or incorporation of the MPCA into standard fish feed.* ***Environment Canada EPS 1/RM/44*** *Aquatic and dietary exposure for single-concentration (MHC) testing; aquatic or dietary exposure for* multiple concentration testing.

***OECD 203 & 204*** *Aquatic exposure.*

## Test concentrations:

#### Nominal: Measured:

***U.S. EPA OCSPP 885.4280*** *Single group tested at the MHC (106 active MPCA units/mL or 1000× the EEC of the MPCA, following application to a 6 inch layer of water). If effects observed, then sequentially lower doses tested to establish LC50 /ID50 with 95% CI.* ***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*** *Aquatic exposure: 106 active MPCA units/mL or 1000× the expected environmental concentration (EEC) of the MPCA,*

*following application to a 15 cm layer of water (whichever is greater). Dietary exposure: the concentration of the MPCA per gram of feed should* be equivalent to the maximum concentration found in target species or fish can be fed the target organism that has been maximally infected with the MPCA.

***Environment Canada EPS 1/RM/44*** *Single group tested at the maximum hazard concentration or 5 test concentrations, including the maximum* hazard concentration. The maximum hazard concentration 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 maximum hazard concentration for dietary exposure is 100× the EEC of the MPCA following application to a 15-cm layer of water.

***OECD 203*** *A limit test (100 mg/L) may be conducted using 7 fish. If any mortalities observed, a full study is required, with 5 concentrations,* geometric series with ratio of 2.2. A range-finding study enables choice of the appropriate concentration range.

***OECD 204*** *The chosen test concentrations must permit the determination of LC50, EC50 and NOEC. Concentrations of the substance in excess of* 100 mg/L need not be tested if a threshold level has not been reached up to this concentration.

## Preparation of test concentrations:

##### *[Describe how the test concentrations were prepared.]*

***U.S. EPA OCSPP 885.4280*** *The test substance shall be administered as a suspension directly into the water (i.e. aquatic exposure) and in feed.* 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*** *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 203*** *Test solutions of the chosen concentrations are prepared by dilution of a stock solution. Stock solutions of substances of low water* solubility may be prepared by ultrasonic dispersion or other suitable physical means. If necessary, vehicles such as organic solvents, emulsifiers or dispersants of low toxicity to fish may be used. When such vehicles are used an additional control should be exposed to the same concentration of the vehicle as that used in the most concentrated solution of the test substance. The concentration of organic solvents, emulsifiers or dispersants should not exceed 100 mg/L.

***OECD 204*** *Stock solutions of the appropriate concentrations are prepared by dissolving the appropriate amount of the test substance in the* required volume of dilution water. Stock solutions of test substances of low water solubility may be prepared by mechanical dispersion or, if necessary, by use of vehicles, such as organic solvents, emulsifiers or dispersants of low toxicity to fish. The concentration of organic solvents, emulsifiers or dispersants should preferably not exceed 100 mg/L in the test solution. Test solutions of chosen concentrations are prepared by dilution of the stock solution.

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

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

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

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

***OECD 203 & 204*** *If necessary, vehicles such as organic solvents, emulsifiers or dispersants of low toxicity to fish can be used but the* concentrations should not exceed 100 mg/L.

## Confirmation of MPCA viability:

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

***U.S. EPA OCSPP 885.4280*** *No method specified, but must be reported.* ***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 test suspension(s) 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.

***OECD 203 and 204*** *No specific recommendation for viability testing as the guidelines were developed for chemical toxicity testing. However,* the concentration of test substance should be determined.

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

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

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.* ***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. A positive chemical control is not required.

***OECD 203*** *No specific recommendations.*

***OECD 204*** *No reference substances are recommended for this test. However, if a reference substance has been tested, the results should be* given.

## Other controls:

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

***U.S. EPA OCSPP 885.4280*** *A concurrent, negative, non-dosed control group and a group in which fish are exposed to sterile culture filtrate* from production cultures are run concurrently with the test groups. ***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 should be run concurrently with the test group and positive control.*

***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 203 and 204*** *One blank and, if relevant, a control containing the solubilizing agent (solvent control) is required at the highest level used* in treatments.

## Number of replicates/groups:

#### Control(s): Treatments:

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *3 replicates/test concentration or control for single concentration test, 1 replicate/test concentration or* control for multiple concentration tests.

***OECD 203 & 204*** *No specific recommendations.*

## Number of organisms per replicate /groups:

#### Control(s): Treatments:

***U.S. EPA OCSPP 885.4280*** *10 fish per test concentration.*

***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*** *Ten fish per replicate (i.e., 30 fish/test concentration for single-concentration test, 10 fish/test* concentration for multiple-concentration testing.)

***OECD 203*** *At least 7 fish per treatment concentration or control.*

***OECD 204*** *At least 10 fish per treatment concentration or control.*

## Biomass loading rate:

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.* ***PMRA DIR 2001-02*** *No specific recommendations.* ***Environment Canada EPS 1/RM/44*** *≤0.5 g/L*

***OECD 203 and 204*** *Maximum loading of 1.0 g fish/L for static/semi-static tests. Loading can be higher for flow-through systems.*

## Recovery of MPCA from tissues :

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

***U.S. EPA OCSPP 885.4280*** *A detailed description of steps taken to determine microorganism dissemination, replication and survival in test* animal tissues, organs and fluids is required.

***PMRA DIR 2001-02*** *Recovery of MPCA from tissues is 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. The dissemination, replication, and survival of the MPCA should also be determined in representative tissues and organs of fish if any adverse effect is observed.

***Environment Canada EPS 1/RM/44*** *Analytical techniques permitting, the recovery of the MPCA in selected organs (e.g., heart, brain, kidney,* liver), tissues, body fluids (e.g., blood or urine), or whole-body homogenate of fish from each treatment is optional during and/or at test end.

***OECD 203 and 204*** *No specific recommendations (guideline designed for chemical toxicity testing).*

## Feeding:

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

***U.S. EPA OCSPP 885.4280*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *At least 1/day, throughout test with commercial fish food pellets; daily ration, 4% of wet body weight;* withhold feeding 24 h prior to weighing.

***OECD 203*** *No feeding during the test or for 24 hours prior to test initiation.*

***OECD 204*** *Either several times daily (the quantity of feed administered should not exceed the amount ingested immediately by the fish) or daily* (the quantity of food being kept constant, e.g., 2% of dry weight related to the initial fish weight).

## Lighting:

##### *[Describe the lighting used during the experiment.]*

***U.S. EPA OCSPP 885.4280*** *No specific recommendations, but lighting must be reported.*

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

***Environment Canada EPS 1/RM/44*** *Full spectrum light at an intensity of 100–500 lux at the water surface. 16 ± 1 h light:8 ± 1 h dark. A* gradual transition period between dark and light is preferred.

***OECD 203 and 204*** *12–16-h light per day is recommended.*

## Duration of study:

***U.S. EPA OCSPP 885.4280*** *The test duration should be at least 30 days. If pathogenicity and/or toxicity are apparent at the 30th day,* observation should continue until recovery, mortality or unequivocal moribundity is established.

***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*** *The test duration is at least 28 days.*

***OECD 203*** *The test duration is 96 hours.*

***OECD 204*** *The test duration is normally 14 days, but can be extended by one or two weeks.*

## Other methods or conditions, if any:

* 1. **Observations:**

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

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

***U.S. EPA OCSPP 885.4280*** *Measurements of temperature, dissolved oxygen content, pH, lighting, and concentrations of MPCA are required.* Observations for mortality, and abnormal behavior or appearance are required during the test.

***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, pH, salinity, hardness, dissolved oxygen concentration, wet weight of* individual fish and concentration of the MPCA in each treatment group is required. Observations for survival, abnormal behavior and appearance of fish in each test vessel.

***OECD 203*** *Measurements of temperature, pH, dissolved oxygen concentration and concentration of the test substance are required.* Observations for survival and visible abnormalities (e.g., loss of equilibrium, swimming behavior, respiratory function, pigmentation, etc.). Fish are considered dead if there is no visible movement (e.g., gill movements) and if touching the caudal peduncle produces no reaction. Dead fish are removed when observed and mortalities recorded.

***OECD 204*** *Measurements of temperature, pH, dissolved oxygen concentration, fish length and weight, and concentration of the test substance* are required. Observations for survival and visible abnormalities (e.g., loss of equilibrium, swimming behavior, respiratory function, pigmentation, etc.). Fish are considered dead if there is no visible movement (e.g., gill movements) and if touching the caudal peduncle produces no reaction. Dead fish are removed when observed and mortalities recorded.

## Observation/measurement intervals:

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

***U.S. EPA OCSPP 885.4280*** *No specific recommendations, presumably daily observation.* ***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 observation intervals are required to record mortalities and note any behavioral, pathogenic or toxic adverse* effects.

***Environment Canada EPS 1/RM/44*** *Temperature, pH, salinity hardness and DO measured weekly in fresh and old test suspensions of each* control and the highest test concentration; analyses permitting, concentration of the MPCA in each treatment (including controls) at beginning and end of test and at beginning and end of 1 renewal cycle/week. Daily observation for fish survival, appearance and behavior.

***OECD 203*** *Dissolved oxygen concentration, pH and temperatures of test solutions should be determined every 24 hours. No specific intervals* were given for measuring test concentrations. The fish must be inspected at least after 24, 48, 72 and 96 hours. Observations at three and six hours after the start of the test are desirable.

***OECD 204*** *Measurements of pH, dissolved oxygen and temperature must be carried out at least twice a week. In the flow-through tests, the* concentration of the substance in the test solution may be determined at the beginning of the test; in the semi-static test at the beginning, immediately prior to the first renewal of the test solution and at the termination of the test. All survivors should be weighed and measured at the termination of the test. Fish must be inspected once/day for mortality. It is desirable that daily records be kept of all observed effects, but a minimum of three observation sessions per week must be conducted.

## Testing for infectivity:

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

***U.S. EPA OCSPP 885.4280*** *Infectivity testing (i.e., dissemination, replication or survival in any test animal tissues, organs or fluid) is required* and must be reported.

***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. The dissemination, replication, and survival of the MPCA should also be determined in representative tissues and organs of fish if any adverse effect is observed.

***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 selected organs, tissues, body fluids or whole-body homogenates.

***OECD 203 & 204*** *No specific recommendations (guideline designed for chemical toxicity testing).*

## Necropsy:

##### *[Indicate on which groups necropsies were performed, and list observations made at necropsy (gross* lesions, histological examination, attempts to recover the MPCA).]

***U.S. EPA OCSPP 885.4280*** *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 fish 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 203 & 204*** *No specific recommendations.*

## Water quality was acceptable? (Results and Discussion Part A):

**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 |  |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Replicate** | ***Day 0*** | ***Day 2*** | | ***Day 4*** | | ***Day –2*** | | ***Day n*** |
| **New** | **Old** | **New** | **Old** | **New** | **Old** | **New** | **Old** |
| *n* |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |
| Salinity (‰) | 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 |  |  |  |  |  |  |
| 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.]

**TABLE *[#]*.** Water quality parameters in test suspensions during the *[X]*-hour exposure of *[test organism]* to

##### *[test substance]*.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Replicate** | ***Treatment 1*** | | ***Treatment 2*** | | ***Treatment 3*** | | ***Treatment n*** | | **Negative Control** | |
| **0 h** | **48 h** | **0 h** | **48 h** | **0 h** | **48 h** | **0 h** | **48 h** | **0 h** | **48 h** |
| Dissolved oxygen (% saturation or mg/L) | 1 |  |  |  |  |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |  |  |
| Temperature (̊C) | 1 |  |  |  |  |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |  |  |
| Salinity (‰) | 1 |  |  |  |  |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |  |  |
| pH | 1 |  |  |  |  |  |  |  |  |  |  |
| *n* |  |  |  |  |  |  |  |  |  |  |
| Mean |  |  |  |  |  |  |  |  |  |  |

*[Table suitable for chemical acute toxicity (multiple-dose) testing (e.g., U.S. EPA guideline OCSPP 850.1075). Modify as* appropriate to accommodate differences in experimental design, or delete if infectivity/pathogenicity and toxicity test used.]

1. **VIABILITY OF DOSING 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 results. If values for LC50 and 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 (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 *[OR*  *immobility]* |  | |  | |  | |  | |  | |  | |

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

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

|  |  |
| --- | --- |
| **Mean Measured Concentration (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]* |  |

|  |  |  |
| --- | --- | --- |
| **Mean Measured Concentration (CFU/L)** | | **Mean Percent Survival (%)** |
| *NOEC*  *[insert [***>***] if greater than]* | |  |
| *Reference Material (if used)* | *Mortality (% or No.)* |  |
| *LC50*  *[insert [***>***] if greater than]* |  |
| *NOEC*  *[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 significantly different from control.]*

1. **SUBLETHAL TOXICITY ENDPOINTS:** *[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 effect, (e.g., growth)]* in *[test organism]* during *[test duration] [acute/chronic]*

exposure to *[test substance]* under *[static-renewal/flow-through]* conditions.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Day** | ***[Sublethal effect]* in *[test organism]*** | | | | | |
| **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* |  |  |  |  |  |  |
| *EC50/ or other sublethal endpoint [insert [***>***] if greater than]* |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Day** | ***[Sublethal effect]* in *[test organism]*** | | | | | |
| **Mean Measured Concentration (CFU/L)** | | | | | |
| ***X.XX × 10X*** | ***X.XX × 10X*** | ***X.XX × 10X*** | ***X.XX × 10X*** | ***X.XX × 10X*** | **Negative Control** |
| *NOEC*  *[insert [***>***] if greater than]* |  |  |  |  |  |  |

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

**TABLE *[#]*.** Mean body weight and weight gain for control and *[test material]*-treated *[test organism]*

measured *[frequency of weighing]*.

|  |  |  |  |
| --- | --- | --- | --- |
| **Day** | **Mean Body Weight (g)** | | |
| **Negative Control** | **Killed *[test substance]***  **Control** | ***[test substance]*** |
| *Initiation* |  |  |  |
| *Termination* |  |  |  |

*[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.]

**TABLE *[#]*.** *[Sublethal effects (e.g., growth,, 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 x*** | ***endpoint 2* at *Day x*** | ***endpoint 3* at *Day x*** |
| **% Affected** | **% Affected** | **% Affected** |
| *Test concentration 1* |  |  |  |
| *Test concentration 2* |  |  |  |
| *Test concentration 3* |  |  |  |
| *Test concentration n* |  |  |  |
| Control |  |  |  |
| EC50  *[insert [***>***] if greater than]* |  |  |  |
| NOEC  *[insert [***>***] if greater than]* |  |  |  |
| LOEC  *[insert [***>***] if greater than]* |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Mean Measured Concentration** | | **Observation Period** | | |
| ***endpoint 1* at *Day x*** | ***endpoint 2* at *Day x*** | ***endpoint 3* at *Day x*** |
| **% Affected** | **% Affected** | **% Affected** |
| *Reference chemical (if used)* | % sublethal effect: |  |  |  |
| EC50:  *[insert [***>***] if greater than]* |  |  |  |
| NOEC  *[insert [***>***] if greater than]* |  |  |  |

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

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

***U.S. EPA OCSPP 885.4280*** *The data should establish that the estuarine or marine fish LC50 or IC50 is greater than the maximum hazard* concentration level. If the LC50 or IC50 is lower than the hazard dose, an LC50 or IC50 with confidence intervals should be established. 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, wet weight of surviving fish, percent surviving fish showing* atypical appearance (necropsy) or behavior at test end, comparing MHC to controls; Multiple concentration test: percent survival and percentage of surviving birds showing atypical appearance (necropsy) or behavior at test end, comparing each test chamber and treatment. Data permitting, calculation of 28-day LC50, 28-day IC50 for wet of surviving fish, 28-day EC50 for atypical appearance and/or behavior, NOEC/LOEC. ***OECD 203*** *The cumulative percent mortality for each exposure period is plotted against concentration on logarithmic probability paper.*

*Normal statistical procedures are then employed to calculate the LC50 for the appropriate exposure period. Confidence limits for the calculated* LC50 values are determined using standard procedures. Where the data obtained are inadequate for the use of standard methods of calculating the LC50, the highest concentration causing no mortality and the lowest concentration producing 100% mortality should be used as an approximation for the LC50 (this being considered the geometric mean of these two concentrations.

***OECD 204*** *The threshold level of lethal effect, threshold level of observed effects, NOEC are calculated.*

## VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

##### *[Report the statistical methods used by the reviewer to verify the applicant’s results, if applicable]:* Statistical Method:

|  |  |  |
| --- | --- | --- |
|  | LC50: | 95% C.I.: |
| *[If applicable:* |  |
| *EC50:* | *95% C.I.:]* |
| NOEC: |  |
| **III.** | Probit Slope:  **CONCLUSION:** | 95% C.I.: |
| **A.** | **STUDY AUTHOR CONCLUSION:** | *[Summarize the study author’s conclusions- Provide the major* |

*conclusions e.g., values for LC50, EC50 and NOEC were [****=, > or <****] in appropriate units]*

1. **REVIEWER’S COMMENTS:** The reviewer agrees *[does not agree]* with the study author’s conclusion. *[Provide additional comments that do not appear under other sections of the template. Discuss the specific methods/ results/findings that may affect the validity of the study and overall acceptability of the study.]* The study was *[not]* conducted in accordance with the guideline recommendations for a *[contact, oral or dietary]* toxicity and pathogenicity study for estuarine and marine animal testing (OCSPP 885.4280; PMRA: M9.6 and OECD: IIM 8.2, IIIM 10.2) 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 885.4280*** *No specific validity criteria.*

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

***Environment Canada EPS 1/RM/44*** *The test is invalid if <80% survival in negative control at test end.*

***OECD 203 and 204*** *For a test to be valid, the mortality in controls should not exceed 10% or (one fish, if less than 10) at the end of the test.* Constant conditions should be maintained as far as possible throughout the test. The dissolved oxygen concentration must have been at least 60^ of the air saturation value throughout the test. There must be evidence that the concentration of the substance being tested has been satisfactorily maintained, and preferably it should be at least 80% of the nominal concentration throughout the test. If the deviation from the nominal concentration is >20%, results should be based on the measured concentration.

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

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

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

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

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

**EXECUTIVE SUMMARY *[FOR EXAMPLE]:*** *[Applicant]* is submitting a justification for a data waiver from estuarine and marine animal testing studies (OCSPP 885.4280). 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 estuarine and marine animals.

The proposed uses of *[product name]* on *[identify use sites/crops]* is not expected to result in increased exposure or adverse effects to estuarine and marine animals. *[If environmental concentration will show a substantial increase, give the rate of environmental reduction to background levels in days/weeks/months].* Any *[name of MPCA]* that reaches aquatic systems, in the form of run-off, is expected to behave as it would in the wild. *[If aquatic concentration will show a substantial increase, give the rate of reduction to background levels in days/weeks/months.]* Therefore, additional testing is not considered necessary to assess the risks of the *[product name]* to estuarine and marine animals. The *[applicant]* requests a waiver of estuarine and marine animal 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/MPCA]*, due to use of the end-use product *[product name]*, will be minimal.** *[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/estuarine/marine systems and a variety of plant surfaces including (identify) 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 freshwater and estuarine/marine fish. *[Does timing of application preclude direct exposure? Discuss crop use sites and application methods and its effects on limiting runoff, if applicable. Provide the rate in*

##### *environmental, including aquatic, reduction of the MPCA to background levels in days/weeks/months, if* available. Include any other factors that would limit exposure to freshwater and estuarine/marine fish. 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, (Hazardous and Regulated Chemicals Database), PUBMED, 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 fish. *[Identify 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 freshwater or estuarine/marine fish 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 freshwater or estuarine/marine fish pathogens? Identify the lists examined.]

**[*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 IN TISSUES, ORGANS:]***

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 Cope (1867), three approaches have been used in Canada to examine the effects of this MPCA on non-target estuarine/marine fish. These include acute toxicity testing, acute infectivity testing, and field testing.*

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

### *ACUTE INFECTIVITY 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:** *[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.*]**