|  |  |  |  |
| --- | --- | --- | --- |
| **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:** U.S. EPA OCSPP Guideline: 885.4100–Avian Inhalation Test, Tier I

U.S. EPA OCSPP Guideline: 885.4050–Avian Oral (for Pulmonary injection, Tier I) PMRA Data Code: M9.2.2–Avian pulmonary, inhalation or injection

OECD Data Code: IIM 8.1, IIIM 10.1

**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 Unpublished *[OR if published, list Journal name, vol.: pages]*.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.]*

The acute *[inhalation or pulmonary injection]* toxicity and pathogenicity of *[test material]* study to *[#]*-day-old *[common name of species (scientific name), enter the number of birds per treatment]* were exposed to a *[single OR #] [indicate exposure method]* dose of *[indicate doses used, e.g., mg a.i./kg bw and/or CFU/kg bw]* of *[formulation, note its potency, biological activity or concentration per unit weight or volume]* (containing % *a.i. name*). *[Include other pertinent details such as the controls used.]*

##### [Describe toxicity or pathogenicity briefly including mortality, behavioral abnormalities, and other clinical signs. If there was no toxicity, state that there was no test material-related toxic or pathogenic effect. Describe microbial clearance, if assessed.]

The *[#]*-day acute *[inhalation or pulmonary injection]* LD50 *[or LC50]* was *[****=, > or <****] [insert LD50 or LC50 if applicable in mg a.i./kg bw and/or cru/kg bw]*. The *[#]*-day NOEL of *[test material]* to the *[species]*, based based on mortality *[and sublethal effects (if applicable)]*, was *[****=, > or <****] [insert NOEL if applicable in mg a.i./kg bw and/or cru/kg bw]*.

This acute *[inhalation or pulmonary injection]* toxicity and pathogenicity study is classified as *[acceptable / unacceptable / supplemental.]* This study was *[not]* conducted in accordance with the guideline recommendations for an acute *[inhalation or pulmonary injection] toxicity [or] infectivity/pathogenicity* study for birds (OCSPP 885.4100; PMRA: M9.2.2 and OECD: IIM 8.1, IIIM 10.1) 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 of the following guidelines were followed most closely in*

##### testing. Such as:

*U.S. EPA OCSPP 885.4100–Avian inhalation test, Tier I1*

*U.S. EPA OCSPP 885.4050–Avian oral- if conducting avian pulmonary injection study]*

*PMRA 2001-02 Part 9.21*

*Environnent Canada EPS 1/RM/44 Section 14.11*

###### 1 Guideline designed to test acute oral infectivity and pathogenicity of microbial 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 nominal potency and/or concentration per unit weight or volume.]

**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 885.4100*** *One avian species, preferably bobwhite quail.*

**U.S. EPA OCSPP 885.4050** The use of two avian species is recommended, one insectivorous, and one herbivorous (preferably bobwhite). **PMRA DIR 2001-02** Preferably bobwhite quail (Colinus virginianus) or mallard duck (Anas platyrchynchos). Others acceptable, especially altricial species, with justification based on susceptibility to the MPCA or ecological considerations.

***Environment Canada EPS 1/RM/44*** *Mallard duck or northern bobwhite quail.*

**Age at study initiation:** *[Insert the ages (mean and range) of birds used in the test.]*

***U.S. EPA OCSPP 885.4100*** *Birds should be 14 to 28 days old at time of testing, and as near the same age as possible.*

***U.S. EPA OCSPP 885.4050*** *Birds should be 14 to 24 days old at time of testing, and as near the same age as possible.*

**PMRA DIR 2001-02** Young birds of approximately 14 days of age are recommended at the beginning of the test. Within a given test, all birds should be as near the same age as possible.

***Environment Canada EPS 1/RM/44*** *Young birds, 14–28 days old at start of test. Birds should be as similar in age as possible.*

## Number of animals/Sex:

***U.S. EPA OCSPP 885.4100*** *No specific recommendations.*

***U.S. EPA OCSPP 885.4050*** *No specific recommendations.*

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

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

**Weight at study initiation:** *[Insert weight (mean and range) of birds used in the test.]*

***U.S. EPA OCSPP 885.4100*** *Must be reported, but no specific recommendations given.*

***U.S. EPA OCSPP 885.4050*** *Must be reported, but no specific recommendations given.*

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

***Environment Canada EPS 1/RM/44*** *Birds should be as similar in weight as possible.*

##### **Strain/Source:** [Identify the strain, supplier, and/or describe the source of birds used in testing.]

***U.S. EPA OCSPP 885.4100*** *Must be reported, but no specific recommendations given.*

***U.S. EPA OCSPP 885.4050*** *Must be reported, but no specific recommendations given.*

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

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

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

1. **STUDY DESIGN AND METHODS:**

*[Briefly describe the experimental design.]*

**U.S. EPA OCSPP 885.4100** A single-concentration test is done in 30 birds at the maximum hazard dose or a multi-concentration test is done in 10 birds per concentration. The dose is administered intranasal or intratracheal instillation for 5 consecutive days. Birds are observed for 30 days for mortality and clinical or behavioral signs of toxicity or pathogenicity.

**U.S. EPA OCSPP 885.4050** A single-concentration test is done in 30 birds at the maximum hazard dose or a multi-concentration test is done in 10 birds per concentration. The dose is administered by intravenous or intraperitoneal injection. Birds are observed for 30 days for mortality and clinical or behavioral signs of toxicity or pathogenicity.

**PMRA DIR 2001-02** The maximum challenge concentration is administered once daily by inhalation, by intratracheal instillation for 5 consecutive days or by a single injection (intravenous or intraperitoneal; dose given once only). Birds are observed for approximately 30 days for mortality and clinical or behavioral signs of toxicity or pathogenicity.

**Environment Canada EPS 1/RM/44** A single-concentration test is done in 30 birds at the maximum hazard dose or a multi-concentration test of at least 5 concentrations of the test substance is done in 10 birds per concentration. The dose is administered daily by inhalation for 5 consecutive days. Birds are observed for 30 days for mortality and clinical or behavioral signs of toxicity or pathogenicity.

## Experimental Methods and Conditions:

**Acclimation:**

#### Duration: Conditions: Feeding: Water: Temperature:

Health *(any mortality observed?)*:

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

***U.S. EPA OCSPP 885.4100*** *Acclimation conditions should be reported, but no specific recommendations are given.*

***U.S. EPA OCSPP 885.4050*** *Acclimation conditions should be reported, but no specific recommendations are given.*

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

***Environment Canada EPS 1/RM/44*** *Acclimation to test chambers and test conditions for ≥7 days before start of test.*

## Pen size and construction materials:

##### [Insert details of pen size and construction.]

***U.S. EPA OCSPP 885.4100*** *Should be reported, but no specific size or construction material is recommended.*

***U.S. EPA OCSPP 885.4050*** *Acclimation conditions should be reported, but no specific recommendations are given.*

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

***Environment Canada EPS 1/RM/44*** *Cages (e.g., commercial brooder pens) with a floor area of ≥800 cm2/bird if ducks, or ≥600 cm2/bird if quail.*

## Method of administration:

##### [Describe the dosing regimen and method of dose administration.]

***U.S. EPA OCSPP 885.4100*** *Intratracheal or intranasal instillation of maximum challenge dose daily for five consecutive days.*

***U.S. EPA OCSPP 885.4050*** *Intravenous or intraperitoneal injection.*

**PMRA DIR 2001-02** Intratracheal instillation, inhalation of aerosolized test substance, or injection (intraperitoneal for fungi or protozoa, intravenous for viruses or bacteria).

***Environment Canada EPS 1/RM/44*** *Intratracheal or intranasal instillation of the test material daily for five consecutive days is preferred.*

## Dose levels:

#### Nominal:

Measured: *(from confirmation of dose viability)*

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

**U.S. EPA OCSPP 885.4100** Single concentration test at the maximum hazard dose (i.e., [MPCA] in test substance × 0.2 mL/kg bw × weight of bird) or multi-concentration test to establish a definitive LC50 or IC50.

**U.S. EPA OCSPP 885.4050** Single concentration test at the maximum hazard dose (i.e., 0.5 mL/kg bw for intravenous and 2 mL/kg bw for intraperitoneal). If toxicity or pathogenicity is observed, sequentially lower doses should be tested to establish an LD50 or ID50.

**PMRA DIR 2001-02** Maximum hazard dose: Pulmonary 0.2 mL TGAI/kg bw (doses given daily for 5 consecutive days); Intravenous 0.5 mL TGAI/kg bw; or Intraperitoneal 2.0 mL TGAI/kg bw (single dose administration).

**Environment Canada EPS 1/RM/44** Single-concentration test at the maximum hazard dose (i.e., [MPCA] in test substance × 0.2 mL/kg bw × weight of bird), or multi-concentration test using ≥5 doses, one being the maximum hazard dose.

## Dose preparation:

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

***U.S. EPA OCSPP 885. 4100 & 885.4050*** *Methods must be reported and 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*** *Doses should be prepared such that the dosing volume should not exceed 0.2 mL/kg bw.*

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

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

**U.S. EPA OCSPP 885. 4100** Methods must be reported, but no specific recommendations are given. The actual form of the material to be regarded as the test substance is discussed in OCSPP 885.0001.

**U.S. EPA OCSPP 885.4050** Methods must be reported, but no specific recommendations are given. The actual form of the material to be regarded as the test substance is discussed in OCSPP 885.0001.

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

***Environment Canada EPS 1/RM/44*** *If required, the use of isotonic saline to dilute the test material is recommended. No other solvent should be used.*

## Confirmation of MPCA viability:

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

***U.S. EPA OCSPP 885.4100 & 885.4050*** *No specific recommendations. The actual form of the material to be regarded as the test substance is discussed in OCSPP 885.0001.* ***From U.S. EPA OCSPP 885.4000 Background for Nontarget Organism Testing of Microbial Pesticide Control Agents*** *The concentration of MPCA in the water or food must be monitored to ensure that the test organisms are exposed to a sufficient MPCA level throughout the test period.*

**PMRA DIR 2001-02** Viability or potency of the MPCA in the dosing suspension should be confirmed. No specific methods are recommended. **Environment Canada EPS 1/RM/44** Analytical techniques permitting, the concentration of the MPCA in the test suspension administered to each treatment (including controls) should be determined daily for five days.

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

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

**U.S. EPA OCSPP 885.4100 & 885.4050** Any substances used to enhance virulence should be tested along with the test substance. **From U.S. EPA OCSPP 885.0001 Overview for Microbial Pest Control Agents** Positive controls generally are not required unless to serve as internal quality controls, demonstrate known test organism sensitivity and respond to known toxic or infective agents, and/or to ascertain if a strain or species reacts similarly to another strain or species when exposed to the same known or standard toxicant or infective agent.

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

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

## Other controls:

##### [Insert description of each control group included in the test.]

**U.S. EPA OCSPP 885.4100** A negative, nondosed control group should be performed. An infectivity control group should be treated with the MPCA inactivated in such a way as to retain the structural integrity of the cell. A control group in which the birds are dosed with sterile filtrate from production cultures should be performed 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.

**U.S. EPA OCSPP 885.4050** A negative, nondosed control group should be performed. An infectivity control group should be treated with the MPCA inactivated in such a way as to retain the structural integrity of the cell. A control group in which the birds are dosed with sterile filtrate from production cultures should be performed concurrently with the test groups.

**PMRA DIR 2001-02** For all tests, a negative, no-dosed control group of the non-target organism should also be run concurrently with the test group and the positive control. A concurrent control group is required consisting of the active ingredient that has been inactivated in such a way as to preserve cellular integrity. For birds dosed by the pulmonary route of administration, a contact control is required in which two no-dosed birds are placed in the same pen as dosed birds.

**Environment Canada EPS 1/RM/44** Each test must include a negative control. A non-infectious control is strongly recommended. A sterile filtrate control is optional but recommended.

## Number of birds per groups/treatment:

#### For negative control:

For solvent/vehicle control: For non-infective control: For sterile filtrate control: For treated birds:

**U.S. EPA OCSPP 885.4100** 10 birds are required per treatment group for multiple-dose testing, and 10 birds for each control and vehicle group. When only one treatment group is tested, at least 30 birds are required. Three control groups: 1) an untreated negative control, 2) an infectivity control group (inactivated MPCA), and 3) two untreated contact control birds placed in with the treatment group receiving maximum challenge concentration are required.

**U.S. EPA OCSPP 885.4050** For multiple-dose testing, 10 birds per treatment group and 10 birds for each control and vehicle group are required. For single-dose testing, at least 30 birds for the treated group.

**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** For a single-concentration test, 30 birds should be treated. For a multi-concentration test, 10 birds should be treated per dose level.

## Recovery of MPCA from tissues:

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

**U.S. EPA OCSPP 885.4100** Attempts should be made, using appropriate techniques, to re-isolate the MPCA from examined tissues at necropsy.

**U.S. EPA OCSPP 885.4050** Attempts should be made, using appropriate techniques, to re-isolate the MPCA from examined tissues at necropsy. **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 selected organs (e.g., heart, brain, kidney, liver), tissues, or body fluids (e.g., blood or urine) of birds from each treatment is required at test end. The recovery of the MPCA is optional during the test.

## Feeding:

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

**U.S. EPA OCSPP 885.4100** No specific recommendations but total feed consumption must be reported for each test and control group at weekly intervals.

**U.S. EPA OCSPP 885.4050** No specific recommendations but total feed consumption must be reported for each test and control group at weekly intervals.

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

***Environment Canada EPS 1/RM/44*** *Birds should be fed commercial feed of a suitable size, ad libitum.*

## Test conditions:

#### Temperature:

Ventilation:

Relative humidity:

Lighting:

Photoperiod:

***U.S. EPA OCSPP 885.4100*** *Test conditions must be reported, but no specific recommendations are given.*

***U.S. EPA OCSPP 885.4050*** *Test conditions must be reported, but no specific recommendations are given.*

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

**Environment Canada EPS 1/RM/44** Daily mean temperature 25 ± 5°C, relative humidity 45 to 70%,, lighting may be incandescent or fluorescent, 500 to 1000 lux, with a photoperiod of 14 ± 1 h light/10 ± 1 h dark (gradual transition from light to dark and dark to light.

## Duration of study:

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

**U.S. EPA OCSPP 885.4100** Observation period of at least 30 days after dosing initiation. If symptomatology or toxic signs are manifested at the 30th day, observation should continue until recovery, mortality, or unequivocal moribundity is established.

**U.S. EPA OCSPP 885.4050** Observation period of at least 30 days after dosing initiation. If symptomatology or toxic signs are manifested 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 30 days.*

## Other methods or conditions, if any:

* 1. **Observations:**

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

##### [List the parameters measured during the experiment, e.g., survival, abnormal behavior or appearance, temperature, relative humidity, individual body weights, concentration of the MPCA in the dosing suspension. Provide references to data summary tables, if used.]

**U.S. EPA OCSPP 885.4100** Measurements of body weight, ambient temperature, and ambient humidity are required. Observations for signs of intoxication, abnormal behavior and regurgitation, pathogenic symptomatology or pathological changes.

**U.S. EPA OCSPP 885.4050** Measurements of body weight, ambient temperature, and ambient humidity are required. Observations for signs of intoxication, abnormal behavior and regurgitation, pathogenic symptomatology or pathological changes.

**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 and relative humidity in the test facility, individual body weights of birds, and concentration of the MPCA in each dosing suspension is required. Observations for survival, abnormal behavior (e.g., lethargy, excessive aggression) and appearance (including external lesions) of birds in each test cage.

## Observation/measurement intervals:

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

***U.S. EPA OCSPP 885.4100*** *Total feed consumption for each test and control group must be determined at weekly intervals.* ***From U.S. EPA OCSPP 885.0001 Overview for Microbial Pest Control Agents*** *Method, frequency, and duration of observations made during the study are to be reported.* ***U.S. EPA OCSPP 885.4050*** *Total feed consumption for each test and control group must be determined at weekly intervals. Observation intervals not specified..*

**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 measured daily (min/max) or continuously; relative humidity measured weekly, body weight measured weekly and concentration of the MPCA in dosing suspension daily for 5 days. Birds should also be observed daily for survival, appearance and behavior.

## Testing for infectivity:

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

**U.S. EPA OCSPP 885.4100** Infectivity testing should be performed, using appropriate techniques, to re-isolate the MPCA from examined tissues at necropsy.

**U.S. EPA OCSPP 885.4050** Infectivity testing should be performed, using appropriate techniques, to re-isolate the MPCA from examined tissues at necropsy.

**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 required at test end based on measured concentrations of new microbial substance in selected organs, tissues and body fluids. Infectivity testing is optional during the test.

## Necropsy:

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

***U.S. EPA OCSPP 885.4100*** *Gross necropsy and histopathology on enough birds to characterize any gross lesions.*

***U.S. EPA OCSPP 885.4050*** *Gross necropsy and histopathology on enough birds to characterize any gross lesions.*

**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** Necropsies should be performed on each bird dying during test as well as those surviving until the end of the test period; animals examined for lesions evident grossly, and selected tissues collected for processing and future microscopic examination where deemed necessary.

## Were raw data included?

##### [Comment on the acceptability of the raw data provided.]

**Other observations, if any:**

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

**TABLE *[#]*.** Viability and potency of *[test substance]* in *[dosing suspension/diet]* administered to *[test organism]* over *[#]* days.

|  |  |  |  |
| --- | --- | --- | --- |
| **Dose Group** | **Dosing Day** | **Nominal Dose *[count or potency]*****(*insert units*)** | **Measured Dose *[count or potency]*****(*insert units*)** |
| Negative control | 1 |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| *Test dose 1* | 1 |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| *Test dose 2* | 1 |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Dose Group** | **Dosing Day** | **Nominal Dose *[count or potency]*****(*insert units*)** | **Measured Dose *[count or potency]*****(*insert units*)** |
|  | 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |
| *Test dose n* | 1 |  |  |
| 2 |  |  |
| 3 |  |  |
| 4 |  |  |
| 5 |  |  |

###### [Table suitable for microbial infectivity/pathogenicity (MHD) testing. Modify as appropriate to accommodate differences in experimental design.]

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

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

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

days.

|  |  |
| --- | --- |
| **Treatment** | **Cumulative Mortality/Total Number of Birds** |
| **Negative Control** | **Killed *[test substance]* Control** | ***[Test substance]*** |
| Cumulative mortality | *Day 0* |  |  |  |
| *Day x1* |  |  |  |
| *Day x2* |  |  |  |
| *Day n* |  |  |  |
| *LD50 or ID50 (if applicable)* | *[insert [***>***] if greater than]* |
| *NOEL* | *[insert [***>***] if greater than]* |

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

**TABLE *[#]***. Effect of *[test material]* on mortality of *[test organism]* exposed by *[dosing method]* over *[#]*

#### days.

|  |  |  |
| --- | --- | --- |
| **Treatment (mg a.i./kg bw)** | **No. of Birds** | **Cumulative Mortality** |
| ***Day 0*** | ***Day x1*** | ***Day x2*** | ***Day x3*** | ***Day n*** |
| Negative control |  |  |  |  |  |  |
| *Test dose 1* |  |  |  |  |  |  |
| *Test dose 2* |  |  |  |  |  |  |
| *Test dose 3* |  |  |  |  |  |  |
| *Test dose n* |  |  |  |  |  |  |
| LD50 or ID50 | *[insert [***>***] if greater than]* |
| NOEL | *[insert [***>***] if greater than]* |

###### [Table suitable multiple-dose testing. Modify as appropriate to accommodate differences in experimental design.]

1. **SUBLETHAL TOXICITY ENDPOINTS:** *[Briefly summarize behavioral abnormalities; other signs of toxicity (body weight loss, decreased food consumption, organ effects, etc.). Indicate effects that appear to be related to the chemical or biological properties of the test material. Compare the sublethal effects to that of the reference chemical, if used.]*

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

measured *[frequency of weighing]*.

|  |  |
| --- | --- |
| **Day** | **Body Weight (g)** |
| **Negative Control** | **Killed *[test substance]*****Control** | ***[test substance]*** |
| *Initiation* |  |  |  |
| *Day 7* |  |  |  |
| *Day 14* |  |  |  |
| *Day 21* |  |  |  |
| *Day 28* |  |  |  |
| Termination |  |  |  |

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

**TABLE *[#]*.** Sublethal effect of *[test material]* on *[test organism]* exposed by *[dosing method]* over *[#]* days.

|  |  |
| --- | --- |
| **Treatment** | **Observation** |

|  |  |  |  |
| --- | --- | --- | --- |
| **(mg a.i./kg bw)** | **Body Weight** | **Food Consumption** | **Other Endpoint (% Affected)** |
| ***Day 0*** | ***Day x1*** | ***Day n*** | ***Day 0*** | ***Day x1*** | ***Day n*** |
| Negative control |  |  |  |  |  |  |  |
| *Test dose 1* |  |  |  |  |  |  |  |
| *Test dose 2* |  |  |  |  |  |  |  |
| *Test dose 3* |  |  |  |  |  |  |  |
| *Test dose n* |  |  |  |  |  |  |  |
| EC50 or ID50 |  |
| NOEL |  |

###### [Table suitable multiple-dose testing. Modify as appropriate to accommodate differences in experimental design.]

**TABLE *[#]*.** Mean daily food consumption of control and *[test material]*-treated *[test organism]* measured

*[frequency of measurement]*.

|  |  |
| --- | --- |
| **Day** | **Food Consumption (g/duck/day)** |
| **Negative Control** | **Killed *[test substance]*****Control** | ***[test substance]*** |
| *Initiation* |  |  |  |
| *Day 7* |  |  |  |
| *Day 14* |  |  |  |
| *Day 21* |  |  |  |
| *Day 28* |  |  |  |
| Termination |  |  |  |

###### [Table suitable for microbial infectivity/pathogenicity (MHD) testing. Modify as appropriate to accommodate differences in experimental design.]

**TABLE *[#]*.** Body weights and weight gain in control and *[test material]*-treated *[test organism]*.

|  |  |
| --- | --- |
| **Test Concentration** | **Body Weight (g)** |
| ***Day 0*** | ***Day 7*** | ***Day 14*** | ***Day 21*** | ***Day 28*** | **Total Change** |
| Negative control |  |  |  |  |  |  |
|  |  |  |  |  |  |
| *[Test dose 1]* |  |  |  |  |  |  |
|  |  |  |  |  |  |

|  |  |
| --- | --- |
| **Test Concentration** | **Body Weight (g)** |
| ***Day 0*** | ***Day 7*** | ***Day 14*** | ***Day 21*** | ***Day 28*** | **Total Change** |
| *[Test dose 2]* |  |  |  |  |  |  |
|  |  |  |  |  |  |
| *[Test dose 3]* |  |  |  |  |  |  |
|  |  |  |  |  |  |
| *[Test dose n]* |  |  |  |  |  |  |
|  |  |  |  |  |  |

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

**TABLE *[#]*.** Microbiological analysis of tissue samples from *[test organism]* challenged by *[dosing method]*

of *[test material]*.

|  |  |  |  |
| --- | --- | --- | --- |
| **Tissue** | **Negative Control** | **Killed *[test substance]* Control** | ***[test substance]*** |
| ***Dose x1*** | ***Dose x2*** | ***Dose x3*** | ***Dose n*** |
| Blood |  |  |  |  |  |  |
| Brain |  |  |  |  |  |  |
| Lung |  |  |  |  |  |  |
| Liver |  |  |  |  |  |  |
| Spleen |  |  |  |  |  |  |
| Kidney |  |  |  |  |  |  |
| GI tract |  |  |  |  |  |  |
| *[other tissues]* |  |  |  |  |  |  |

###### [Table suitable for microbial infectivity/pathogenicity (MHD) 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 885.4100** LD50 or ID50 in appropriate units with 95% confidence limits, if obtained, methods used, and slope of the dose- response line, if obtained. 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.

**U.S. EPA OCSPP 885.4050** LD50 or ID50 in appropriate units with 95% confidence limits, if obtained, methods used, and slope of the dose- response line, if obtained.

***PMRA DIR 2001-02*** *All relevant analyses of results must be provided.*

**Environment Canada EPS 1/RM/44** Single concentration test: percent survival and percentage of surviving birds showing atypical appearance (necropsy) or behavior at test end, comparing MHD 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 30-day LD50, 30-day ED50 for atypical appearance and/or behavior, NOED/LOED.

## VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

##### [If applicable- Report the statistical methods used by the reviewer to verify the applicant’s results; If values for LD50, LC50, LT50, NOEL, NOEC are greater than the MHD level, use **<** symbol.]

Statistical Method:

LD50: 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., values for LD50, ID50 LC50, NOEL, NOEC]*
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 an acute *[inhalation or pulmonary injection] toxicity [or] infectivity/pathogenicity* study for birds (OCSPP 885.4100; 885.4050; PMRA: M9.2.2 and OECD: IIM 8.1, IIIM 10.1) 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 885.4100*** *No specific validity criteria.*

***U.S. EPA OCSPP 885.4050*** *No specific validity criteria.*

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

***Environment Canada EPS 1/RM/44*** *The test is invalid if <90% survival in negative control at test end.*

## 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 avian *[inhalation or pulmonary injection] toxicity [or] infectivity/pathogenicity* study (OCSPP 885.4100 *[or 885.4050]*). 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 birds.

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

# (For a waiver request, otherwise delete)

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

The waiver request is based on the following rationales:

* 1. **Increased environmental exposure to *[name of active ingredient]*, due to use of the end-use product *[product name]*, will be minimal.** *[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) crops/vegetables/fruits? Give 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 birds. [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 reduction of the MPCA to background levels in days/weeks/months, if available.]

* 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 fate and interactions with birds. *[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 avian species?]*

##### [Discuss whether runoff or overspray would result in effects not seen from the naturally occurring MPCA levels. Discuss whether the MPCA does/does not grow at avian body temperatures. Does the MPCA appear on any authoritative list of avian 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)***

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

*e.g., MPCA-based products are widely used in forest management to control forest pests in Canada and the United States ... As noted by Linnaeus (1758), three approaches have been used in Canada to examine the effects of this MPCA on birds. These include acute toxicity testing, dietary toxicity testing, and field testing.*

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

### DIETARY TOXICITY TESTING:

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

### FIELD TESTING:

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

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

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

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

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

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