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

**DATA EVALUATION RECORD**

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

|  |  |  |
| --- | --- | --- |
| **REQUIREMENT:** | Nontarget Plant Testing, Tier I |  |
|  | U.S. EPA OCSPP Guideline: | 885.4300 |
|  | PMRA Data Code: | M9.8.1–Terrestrial plants |
|  | PMRA Data Code: | M9.8.2–Aquatic plants and Algae |
|  | OECD Data Code: | IIM 8.6– Terrestrial plants |
|  | OECD Data Code: | IIM 8.4, IIM 8.5, IIIM 10.2– Aquatic plants and Algae |

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

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

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

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 Unpublished *[OR if published, list Journal name, vol.: pages]*.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

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

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

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

In a *[#]*-*[day]* toxicity and pathogenicity study, the effect of *[test material/formulation- note its potency, biological activity and/or concentration per unit weight or volume]* (containing % *a.i. name*) on the *[seed germination, seedling emergence, and/or vegetative vigor]* of terrestrial plant, *(insert scientific and common names of tested species]* was studied at *[nominal/measured]* concentrations of *[0, x1, x2, ... xn biological or*

##### *bioactivity units/kg soil or ha]*. *[Include other pertinent details such as the growth medium, use of heat-killed* controls, etc.]

***[OR]*** *If aquatic plant study was conducted-* In a *[#]*-*[day]* toxicity and pathogenicity study, the effect of *[test material/formulation- note its potency, biological activity and/or concentration per unit weight or volume]* (containing % *a.i. name*) on the *[freshwater/marine]* aquatic vascular *[plant/algae], (insert scientific and common names of tested species]* was studied at *[nominal/measured]* concentrations of *[0, x1, x2, ... xn biological or bioactivity units/L]* under *[static, static-renewal or flow-through]* conditions. *[Include other pertinent detail use of heat-killed controls, etc ...]*

##### *[Describe toxicity briefly including mortality, seedling emergence, growth inhibition, vegetative vigor, and other* signs of toxicity or abnormalities; ***[OR]*** *if aquatic plant study was conducted - include any effects on number of* fronds, dry weight, and growth rate (if algae was tested). If there were no effects, state that there was no test- material related phytotoxic or pathogenic effect.]

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This study is classified as *[acceptable, unacceptable, supplemental].* This study was *[not]* conducted in accordance with the guideline recommendations for a toxicity and pathogenicity study for nontarget plant testing (OCSPP 885.4300, PMRA: M9.8.1, M9.8.2 and OECD: IIM 8.6, IIM 8.4, IIM 8.5, IIIM 10.2). *[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 885.4300– Nontarget Plant Testing, Tier I1*

*PMRA DIR 2001-02 Part 9.81Terrestiral and Aquatic Plants and Algae Environment Canada EPS 1/RM/44 Section 12.2- Terrestrial Plants Environment Canada EPS 1/RM/44 Sections 9.11 and 9.21-Aquatic Plants and Algae3*

*OECD 208–Terrestrial plants, growth test2 OECD 201–Alga, growth inhibition test, 2, 3]*

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

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

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

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

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

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

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

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

**Purity:** *[Insert the test material’s 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 if the MPCA is stable under these conditions.]*

* 1. **Test Organism:**

**Species:** *[include common and scientific names, family, number]*

***U.S. EPA OCSPP 885.4300*** *For animal-controlling MPCAs, six species of dicotyledons from ≥4 families and 4 species of monocotyledons from*

*≥2 families. For plant-controlling MPCAs, or those closely related to a plant pathogen, in addition to animal-controlling MPCA requirements,* testing must be performed on all plants of economic importance (horticultural/agronomic; see Table 1 of guideline for list) or known to be beneficial to maintenance of the ecosystem that have any reasonable likelihood of serving as hosts.

***PMRA DIR 2001-02*** *Selection of test plants should take into account such factors as the purpose of the MPCA, the ecozone of intended use, the* likelihood of exposure to the MPCA, as determined by the use pattern and routes of dissemination in the environment, and the phylogenetic proximity of the test species to target pest species. The following families are suggested because of their environmental and economic importance in Canada: Apiaceae (Umbelliferae), Asteraceae (Compositae), Brassicaceae (Cruciferae), Chenopodiaceae, Cucurbitaceae, Fabaceae (Leguminosae), Liliaceae, Malvaceae, Poaceae (Gramineae), Polygonaceae, Rosaceae, Solanaceae. Applicants should also endeavor to test some weed and wild plant species.

***Environment Canada EPS 1/RM/44*** *One or more monocotyledons, barley (Hordeum vulgare var. Chapais), blue grama grass (Bouteloua* gracilis), northern wheatgrass (Elymus lanceolatus; formerly Agropyron dasystachyum), red fescue (Festuca rubra var. creeping), or Durum wheat (Triticum durum var. Durum); or dicotyledons alfalfa (Medicago sativa var. greencrop), carrot (Daucus carota var. Royal Chantenay),

*cucumber (Cucumis sativa var. Marketmore76), lettuce (Lactuca sativa var. Buttercrunch), radish (Raphanus sativus var. Champion or Cherry* Belle), red clover (Trifolium pratense var. greencrop), or tomato (Lycopersicon esculentum var. Heinz 1439).

***OECD 208*** *The test species and the number of species to be tested are not specified. The following characteristics of the possible test species* should be considered in the selection of a test species:

*– the species must have uniform seeds that are readily available from reliable standard seed source(s) and that produce consistent,* reliable and even germination, as well as uniform seedling growth;

*–plant is amenable to testing in the laboratory, and can give reliable and reproducible results within and across testing facilities;*

*–the sensitivity of the species tested should be consistent with the responses of plants found in the environment exposed to the* substance;

*–they have been used to some extent in previous toxicity tests and their use in, for example, herbicide bioassays, heavy metal* screening, salinity or mineral stress tests or allelopathy studies indicates sensitivity to a wide variety of stressors;

*–they are compatible with the growth conditions of the test method; and*

*–they meet the validity criteria of the test.*

*Lists of potential test species are provided in Annex 2 and Annex 3.*

###### *For Aquatic Plants and Algae:*

***U.S. EPA OCSPP 885.4300*** *Selenastrum capricornutum (freshwater green alga), lemna gibba (duckweed), Skeletonema costatum (marine* diatom), and Anabaena flos-aquae (blue-green bacterium) are recommended test species.

***PMRA DIR 2001-02*** *Test species should be selected from up to 6 representative aquatic vascular plant families (Lemnaceae, Potomogetonaceae,* Haloragaceae, Typhaceae, Cyperaceae, Alismaceae) using the centrifugal taxonomic approach. Additional effects testing is also required on at least one representative species from each of the algal families (Chlorophycaea (green), Cyanophyceae (blue-green), and Bacillariophyceae (diatom).

***Environment Canada EPS 1/RM/44*** *Lemna minor is the freshwater test species. For marine studies, Champia parvula is the test species.* ***OECD 201*** *The green algae, Pseudokirchneriella cubcapitata (formerly known as Selebastrum capricornutum) and Desmodesmus subspicatus* (formerly known as Scenedesmus subspicatus), the diatom, Navicula pelliculosa, and the blue-green bacteria, Anabaena flos-aquae and Synechoccus leoppoliensis, are the recommended species.

## Seed source:

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

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

***OECD 208*** *Within a given test, all plants including the controls should be from the same source.*

###### *For Aquatic Plants and Algae:*

***OECD 201*** *Pseudokirchneriella cubcapitata strains ATCC 22662, CCAP 278/4, 61.81 SAG; Desmodesmus subspicatus strain 86.81 SAG;* Navicula pelliculosa UTEX 664; Anabaena flos-aquae strains UTEX 1444, ATCC 29413, CCAP 1403/13A; and Synechoccus leoppoliensis strains UTEX 625, CCAP 1405/1.

**Prior seed treatment/sterilization:** *[Report only for terrestrial plant testing]*

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

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

***OECD 208*** *Seeds coated with an insecticide or fungicide should be avoided. If seed-borne pathogens are a concern, the seeds may be soaked* briefly in a weak 5 % hypochlorite solution, then rinsed extensively in running water and dried. No remedial treatment with other crop protection product is allowed.

**Historical % germination of seed:** *[Report only for terrestrial plant testing]*

**Seed storage, if any:** *[Report only for terrestrial plant testing]*

## Number of plants:

**Age of inoculum (at test initiation):** *[Report only for aquatic plant testing] [Insert the age of the organisms.]*

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

***Environment Canada EPS 1/RM/44*** *Lemna minor: 7- to 10-day-old culture; Champia parvula: sexually mature females, comprised of branch* tips 7–10 mm in length; sexually mature males, comprised of a branch 2–3 cm in length.

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *3- to 7-day-old stock cultures*

***OECD 201*** *No specific recommendations.*

**Method of cultivation:** *[Report only for aquatic plant testing- Insert details of the method of cultivation.]*

##### **Rationale:** *[Insert rationale for using the host plant(s), if applicable]*

1. **STUDY DESIGN AND METHODS:**

*[Insert a brief overview of the experimental protocol (details to follow in Tables 1 and 2, below).]*

***U.S. EPA OCSPP 885.4300*** *Test plants are exposed to the MPCA at the maximum hazard concentration (MHC) via the exposure route* predicted by the proposed use pattern, and/or that expected to result in greatest susceptibility. Adverse effects on growth and development are monitored, and attempts are made to recover the MPCA from plant tissues.

***PMRA DIR 2001-02*** *Test plants are exposed to the MPCA at the maximum hazard concentration (MHC) via the exposure route predicted by* the proposed use pattern, and/or that expected to result in greatest susceptibility. Adverse effects on growth and development are monitored, and attempts are made to recover the MPCA from plant tissues.

***Environment Canada EPS 1/RM/44*** *Test for adverse effects on seeds/developing plants in soil containing the MPCA. Emergence, growth and* abnormal appearance are monitored over 14 days or 21 days depending on the species of plant.

***OECD 208*** *The test substance is incorporated at various concentrations into soil in which the seeds are sown. The number of seedlings that* emerge is recorded. At least two weeks after 50% of the seedlings have emerged in the control, the plants are harvested and weighed.

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *Test for adverse effects on growth in Lemna minor growing in a suspension of the MPCA. Weight, and* frond number are monitored over a period of 7 days. In marine tests, sexually mature male and female Champia parvulaares exposed to the MPCA over a period of 48 hours followed by a 7-day period of recovery in seawater for observations and for the development of cystocarps. ***OECD 201*** *Exponentially-growing cultures of selected green algae, diatoms and blue-green bacteria are exposed to various concentrations of* the test substance over several generations under defined conditions. The inhibition of growth in relation to a control culture is determined over a fixed period.

# *(Use the following section for terrestrial plant testing. If testing aquatic* plant/algae- delete this section and proceed to “Experimental Methods and Conditions “for aquatic plant/algae testing. )

## Experimental Methods and Conditions:

**Test chamber - description and size:** *[if applicable] [Describe the test container.]*

#### Type: (e.g., petri dish) Material: (glass/polystyrene) Size:

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *1-L polystyrene cups, covered until plants reach top of container.*

***OECD 208*** *Non-porous plastic or glazed pots with a tray or saucer under the pot. The size of pots/containers must be adequate to allow* unrestricted growth.

## Soil parameters:

Geographic location: Depth of soil collection: Soil texture:

% sand

% silt

% clay

pH:

% organic carbon: CEC:

Moisture at 1/3 atm (%):

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *500 g (wet wt.) natural (uncontaminated) or artificial (laboratory formulated) soil. If field collected,* hydrate if and as necessary until a homogeneous crumbly texture is achieved. If artificial soil, hydrate to ~70% of water-holding capacity. The soil must enable the negative control groups included in the test to meet the validity criteria and must not cause any discernible adverse effects to test organisms. The testing facility should know the basic physicochemical characteristics of the soil (e.g., particle size distribution, percent water content, percent organic carbon, pH, metals, pesticides, petroleum hydrocarbons). The use of sterile soil is not recommended.

***OECD 208*** *Plants may be grown in pots using a sandy loam, loamy sand, or sandy clay loam that contains up to 1.5 percent organic carbon* (approx. 3 percent organic matter). Commercial potting soil or synthetic soil mix that contains up to 1.5 percent organic carbon may also be used. Clay soils should not be used if the test substance is known to have a high affinity for clays. Field soil should be sieved to 2 mm particle size in order to homogenize it and remove coarse particles. Artificial soils are not typically used but they may be used. The type and texture, % organic carbon, pH and salt content as electronic conductivity of the final prepared soil should be reported. The soil should be classified according to a standard classification scheme. The soil could be pasteurized or heat treated in order to reduce the effect of soil pathogens.

**Details of nutrient medium:** *[if used] [Describe nutrient medium, if used.]*

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

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

***OECD 208*** *No specific recommendations.*

## Number of seeds/species/replicate:

#### Control(s): Treatments:

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *5 seeds per test chamber for barley, northern wheat grass, red fescue, Durum wheat, cucumber, lettuce,* radish, red clover or tomato; 10 for blue grama grass, alfalfa or carrot.

***OECD 208*** *Only seeds of the same species are planted in same pot. The number of seeds planted per pot will depend upon the species, pot size* and test duration. The number of plants per pot should provide adequate growth conditions and avoid overcrowding for the duration of the test. The maximum plant density would be around 3–10 seeds per 100 cm2 depending to the size of the seeds. There should be at least four replicates and at least 20 seeds per test group. More replicates may be required for plant species with low germination rates.

## Method and depth of seeding:

##### *[Describe seeding method.]*

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

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

***OECD 208*** *Species-specific guidance is provided in Annex 3.*

## Watering regime and schedules:

#### Water source/type:

Volume applied:

Interval of application:

Method of application:

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Test water sprayed over plants and soil surface until saturation of soil, every 3 days when test chambers* are covered and 1–2 times per day once covers are removed.

***OECD 208*** *Bottom watering of test containers (e.g. by using glass fiber wicks) is recommended. However, initial top watering can be used to* stimulate seed germination and, for soil surface application it facilitates movement of the chemical into the soil. The watering schedule must be reported.

**Pest control method/fertilization:** *[if used]*

##### *[Describe any pest control method and/or fertilization regimens.]*

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

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

***OECD 208*** *Nutrients for plant growth should be provided to ensure that plants are not stressed through nutrient deficiencies, and where* possible this should be assessed via chemical analysis or by visual assessment of control plants.

**Number of plants retained after thinning:** *[if applicable]*

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

***PMRA DIR 2001-01*** *No specific recommendations.*

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

***OECD 208*** *No specific recommendations.*

# *(Use the following section for Aquatic plant testing. If testing terrestrial plants-* delete this section and proceed to ‘Route of Exposure’ test parameter. )

## Experimental Methods and Conditions:

**Acclimation:**

#### Period:

Culturing media and conditions: *(same as test or not)*

Health: *(any toxicity observed)*

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

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

***Environment Canada EPS 1/RM/44*** *Lemna minor must be acclimated for a minimum of 18–24 hours to the control/dilution water. Unialgal* stock cultures of male and female Champia parvula must be maintained in separate 1000 mL Erlenmeyer flasks containing aerated culture medium.

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *Inocula from 3- to 7-day-old stock cultures should be used.*

***OECD 201*** *A pre-culture is intended to give an amount of algae suitable for the inoculation of test cultures. It is incubated under conditions of* the test and used when still exponentially growing, normally after an incubation period of 2–4 days. When algal cultures contain deformed or abnormal cells, they must be discarded.

## Test vessel:

#### Material: (glass/polystyrene) Size:

Fill volume:

##### *[Insert details of chamber size and construction, and fill volume]*

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

***Environment Canada EPS 1-RM/44*** *Lemna minor: a 150-mL beaker is the test vessel. Champia parvula: 200-mL polystyrene cup or a 250-mL Erlenmeyer flask.*

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *The test should be conducted in Erlenmeyer flasks. Volumes should be the same for each test group, but may be* between 125 and 500 mL if the fill volume does not exceed 50% of the flask volume.

***OECD 201*** *Test vessels should be made entirely of glass or other chemically inert material. Test flasks should have a suitable volume for* sufficient mass transfer of CO2 from the atmosphere.

## Test system:

##### *Static/static renewal/*

*Renewal rate for static renewal:*

***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*** *Lemna minor: static-renewal test with a renewal rate of at least twice (on Days 3 and 5 of the test).*

*Champia parvula: static-renewal test with a renewal after 24 hours.*

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *Static test.*

***OECD 201*** *Static test.*

## Sediment parameters (for rooted aquatic vascular plants):

#### Origin:

Textural classification (% sand, silt and clay): Organic carbon (%):

##### *[Describe the sediment and source.]*

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

***Environment Canada EPS 1/RM/44*** *Not required.*

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *Not required.*

***OECD 201*** *Not required.*

**Details of growth medium:** *[if used]*

#### Name:

pH at test initiation: pH at test termination: Chelator used: Carbon source:

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

***Environment Canada EPS 1/RM/44*** *Lemna minor: SIS (Swedish Standard) growth medium; dissolved oxygen 90–100% saturation when added* to test chambers. Champia parvula: not applicable.

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *Nutrient media should conform to ASTM standards (ASTM 1991). The pH of nutrient medium in which algae are* subcultured is to be 7.5 (± 0.1) for Selenastrum, 8.1 (± 0.1) for Skeletonema, 7.5 (± 0.1) for Navicula, and 7.5 (± 0.1) for Anabaena, and is not adjusted after the addition of the algae.

***OECD 201*** *The following medium is recommended: NH4Cl 15 mg/L; MgCl2**6H20 12 mg/L; CaCl2**2H2O 18 mg/L; MgSO4**7H2O 15 mg/L;* KH2PO 1.6 mg/L; FeCl3*5H2O 0.08 mg/L; Na2EDTA**2H2O 0.1 mg/L; H3BO3 0.185 mg/L; MnCl2**4H2O 0.415 mg/L; ZnCl2 3 × 10-3 mg/L;*

*CoCl2**6H2O 1.5 × 10-3 mg/L; CuCl2**2H2O 10-5 mg/L; Na2MoO4**2H2O 7 × 10-3 mg/L; NaHCO3 50 mg/L; pH ~8. The use of other media is not* precluded (e.g., U.S. EPA AAP-medium) provided that the following limits are respected: P≤ 0.7 mg/L; N ≤ 10 mg/L; chelators ≤ 10-3 mmol/L; hardness (Ca + Mg) ≤ 0.6 mmol/L. The U.S. EPA medium AAP is also acceptable (ASTM medium). The pH of the control medium should not increase by more than 1.5 units during the test (indicator of insufficient mass transfer of CO2)

**If a non-standard nutrient medium was used, was a detailed composition provided?** *[Yes/No] [Insert composition of nutrient medium, if applicable.]*

**Source of dilution water:** *[if applicable] [Describe source of dilution water, if applicable.]*

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

***Environment Canada EPS 1/RM/44*** *Champia parvula: natural (uncontaminated) seawater at 30‰ or a mixture of ≥50% natural* (uncontaminated) seawater and ≤50% artificial seawater adjusted to a salinity of 30‰. The dissolved oxygen concentration when added to test chambers should be 90–100% saturation. 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.

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *Dilution water used for the preparation of nutrient medium and test solutions should be of a sufficient quality (e.g.,* ASTM Type I water).

***OECD 201*** *When preparing media, deionized or distilled water should be used.*

**Dilution water parameters:** *[if applicable]*

Source/type: pH:

Total Organic Carbon:

Particulate matter:

Metals:

Pesticides: Chlorine:

Water pretreatment (if any):

Intervals of water quality measurement:

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

***Environment Canada EPS 1/RM/44*** *Nutrient medium is recommended for Lemna minor(see above). Champia parvula: Dissolved oxygen 90–* 100% saturation (when added to test chambers), pH 7.0–8.5, temperature 23± 1°C, salinity 30±2‰ 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.

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *Nutrient medium is recommended.*

***OECD 201*** *Nutrient medium is recommended.*

## Aeration:

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

***Environment Canada EPS 1/RM/44*** *No aeration required during the test for Lemna minor. For Champia parvula, aerate gently in all test* chambers only if necessary to maintain a dissolved oxygen concentration of ≥4 mg/L.

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***U.S. EPA OPPTS 850.5400*** *Test containers should be placed on a rotary shaking apparatus. For Selenastrum, the oscillation rate should be* 100 cycles/minute and at approximately 60 cycles/min for Skeletonema. If clumping of Skeletonema occurs, hand shaking once or twice a day is acceptable.

***OECD 201*** *During the test, it is necessary to keep the algae in suspension and to facilitate transfer of CO2. To this end, constant shaking or* stirring should be used.

# *(The following section is applicable to both terrestrial and aquatic plant testing-* Resume input of data)

## Route(s) of exposure:

##### *[Describe the application of the test material to test plant species.]*

Application time including the plant growth stage: Number of applications:

Application interval: Method of application:

***U.S. EPA OCSPP 885.4300*** *Test plants should be exposed to the MPCA by whatever route of exposure would be expected by the proposed use* pattern. This route of exposure should be supplemented by other routes of exposure if indicated by the transmission of typical pathogens of the test plant or for microbial herbicides, if indicated by the mode of transmission of similar plant pathogens. In some cases, wounding of plants or simulation of (or actual) insect vectors may be appropriate. In other cases, seed treatment, root (soil) application, or foliar spray might be the most appropriate method.

***PMRA DIR 2001-02*** *Test plants should be exposed to the MPCA by whatever route of exposure would be expected by the proposed use pattern.* This route of exposure should be supplemented by other routes of exposure if indicated by the transmission of typical pathogens of the test plant or for herbicidal MPCAs, if indicated by the route of transmission of similar plant pathogens. In some cases, wounding of plants or simulation of actual insect vectors may be appropriate. In other cases, seed treatment, root or soil application, foliar spraying, or direct application to water might be the most appropriate method.

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

***OECD 208*** *Depending on the expected route of exposure, the test substance is either incorporated into the soil (or possibly into artificial soil* matrix) or applied to the soil surface, which properly represents the potential route of exposure to the chemical.

***For Aquatic Plants and Algae: Environment Canada EPS 1/RM/44*** *Aquatic exposure.* ***OECD 201*** *Aquatic exposure.*

## Test concentrations:

##### *[Single concentration test or multi-concentration test? List test concentrations. Insert method for* calculation of the MHC, if applicable.]

Nominal: Measured:

***U.S. EPA OCSPP 885.4300*** *A single concentration equal to no less than the maximum label rate, i.e., the amount of active ingredient in the* recommended volume of carrier per land area or applied directly to the surface of a 15-cm column of water.

***PMRA DIR 2001-02*** *The maximum challenge concentration is equivalent to spraying the plant to runoff with a concentration of the MPCA that* is equivalent to the maximum rate of application proposed on the product label. For seed treatments, seeds should be thoroughly drenched with the MPCA. For applications directly to soil, test plants should be exposed to at least 106 active units of the MPCA/g or /mL; or 1000× the expected environmental concentration of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water, whichever is greater or achievable.

***Environment Canada EPS 1/RM/44*** *A single group tested at the MHC or ≥ 9 test concentrations, including the MHC. The MHC for water is* no less than the maximum concentration specified by the notifier for the final tank mix of a microbial product, when it is applied at the “maximum label rate.” MHC for soil is 106 microbial units/g soil (dry wt), or 1000 × the EEC in soil within the terrestrial environment, whichever is greater and readily attainable.

***OECD 208*** *The recommended number of test concentrations/rates is at least five in a geometric series plus untreated control, and spaced by a* factor not exceeding three. A limit test may be conducted at a maximum level of 1000 mg/kg dry soil.

###### *For Aquatic Plants and Algae:*

***PMRA DIR 2001-02*** *Plants should be treated at the maximum hazard concentration (MHC) by directly applying the product to the dilution* water at a concentration of 106 active units of the MPCA per mL; or 1000 times the expected environmental concentration (EEC) of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of water, whichever is greater or achievable (i.e., MHC for water).

***Environment Canada EPS 1/RM/44*** *Single concentration test: plants treated at the MHC for water. Multi-concentration test: at least 5 test* concentrations (including MHC). 7 test concentrations are recommended.

***U.S. EPA OPPTS 885.4300*** *A single concentration no less than the maximum label rate, i.e., the amount of active ingredient in the* recommended volume of carrier applied directly to the surface of a 15-cm column of water.

***OECD 201*** *The concentration range in which effects are likely to occur may be determined on the basis of results from range-finding tests. For* the final definitive test at least five concentrations, arranged in a geometric series with a factor not exceeding 3.2, should be selected. For test substances showing a flat concentration response curve a higher factor may be justified. The concentration series should preferably cover the range causing 5-75 % inhibition of algal growth rate.

## Preparation of test concentrations:

##### *[Briefly describe methods for preparation of test concentrations.]*

***U.S. EPA OCSPP 885.4300*** *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 (OCSPP 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*** *Soil: for a single concentration test, a measured quantity of the MPCA representing the maximum hazard* concentration should be mixed to homogeneity in a suitable quantity of soil. In multi-concentration testing, the maximum hazard concentration and each lower test concentration should be prepared in the same manner. Mixing must be performed in a standardized manner (i.e., temperature and time). Water: 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.

***OECD 208*** *Surface application: All equipment used in conducting the tests, including equipment used to prepare and administer the test* substance, should be of such design and capacity that the tests involving this equipment can be conducted in an accurate way and it will give a reproducible coverage. The coverage should be uniform across the soil surfaces. Care should be taken to avoid the possibilities of chemicals being adsorbed to or reacting with the equipment (e.g. plastic tubing and lipophilic chemicals or steel parts and elements). The test substance is sprayed onto the soil surface simulating typical spray tank applications. Generally, spray volumes should be in the range of normal agricultural practice and the volumes (amount of water etc. should be reported). Nozzle type should be selected to provide uniform coverage of the soil surface. If solvents and carriers are applied, a second group of control plants should be established receiving only the solvent/carrier. Soil incorporation: Substances which are water soluble or suspended in water can be added to water, and then the solution is mixed with soil with an appropriate mixing device. The water-holding capacity of the soil should not be exceeded by the addition of the test substance. The volume of water added should be the same for each test concentration, but should be limited to prevent soil agglomerate clumping. Substances with low water solubility should be dissolved in a suitable volatile solvent (e.g. acetone, ethanol) and mixed with sand. The solvent can then be removed from the sand using a stream of air while continuously mixing the sand. The treated sand is mixed with the experimental soil. A second control is established which receives only sand and solvent. Equal amounts of sand, with solvent mixed and removed, are added to all treatment levels and the second control. For solid, insoluble test substances, dry soil and the chemical are mixed in a suitable mixing device. Hereafter, the soil is added to the pots and seeds are sown immediately. When an artificial substrate is used instead of soil, chemicals that are soluble in water can be dissolved in the nutrient solution just prior to the beginning of the test. Chemicals that are insoluble in water, but which can be suspended in water by using a solvent carrier, should be added with the carrier, to the nutrient solution. Water-insoluble chemicals, for which there is no non- toxic water-soluble carrier available, should be dissolved in an appropriate volatile solvent. The solution is mixed with sand or glass beads, placed in a rotary vacuum apparatus, and evaporated, leaving a uniform coating of chemical on sand or beads. A weighed portion of beads should be extracted with the same organic solvent and the chemical assayed before the potting containers are filled.

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *For a single concentration test, a measured quantity of the MPCA representing the maximum hazard* concentration should be mixed to homogeneity in a suitable quantity of 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.

***OECD 201*** *All test solutions must contain the same concentrations of growth medium and initial biomass of test alga. Test solutions of the* chosen concentrations are usually prepared by mixing a stock solution of the test substance with growth medium and inoculum culture. Stock solutions are normally prepared by dissolving the substance in test medium. Solvents may be used as carriers to add substances of low water solubility to the test medium, but the concentration of solvent should not exceed 100 µL/L.

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

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

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

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

***OECD 208*** *A volatile solvent can be used (to be evaporated before seeds are sown).*

###### *For Aquatic Plants and Algae:*

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

***OECD 201*** *Solvents, e.g. acetone, t-butyl alcohol and dimethyl formamide, may be used as carriers to add substances of low water solubility to* the test medium. The concentration of the solvent should not exceed 100 µL/L, and the same concentration should be added to all cultures (including controls).

## Confirmation of MPCA viability:

##### *[Describe methods used to confirm the concentration and viability of the MPCA in the test soil.]*

***U.S. EPA OCSPP 885.4300*** *No specific recommendations.*

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

***OECD 208*** *No specific recommendations for viability testing (guideline designed for chemical toxicity testing). The concentrations/rates of* application must be confirmed by an appropriate analytical verification. For soluble substances, verification of all test concentrations/rates can be confirmed by analysis of the highest concentration test solution used for the test with documentation on subsequent dilution and use of calibrated application equipment (e.g., calibrated analytical glassware, calibration of sprayer application equipment). For insoluble substances, verification of compound material must be provided with weights of the test substance added to the soil.

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *Lemna minor: 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 the test. Champia parvula: Analytical techniques permitting, the concentration of the MPCA in each test suspension (including controls) should be determined at the beginning and end of each renewal during the initial 48 hours.

***OECD 201*** *No specific recommendations for viability testing (guideline designed for chemical toxicity testing). The concentration of the test* substance, however, must be determined.

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

##### *[Describe test methods, and give concentration of reference material, if used.]*

***U.S. EPA OCSPP 885.4300*** *Positive controls are required for microbial herbicides, or for MPCAs similar to known plant pathogens, in order* to ascertain that environmental conditions are such that penetration, infection and disease development are likely to occur in a susceptible host. The positive control should be selected to closely resemble the subject MPCA in terms of taxonomy and optimal conditions for infection and disease development, if known. In the case of a MPCA not intended for herbicidal use, the positive control may consist of a known plant pathogen, with taxonomic characteristics similar to the MPCA and its susceptible host. In the case of a microbial herbicide, however, the positive control should consist of the target pest weed and the microbial herbicide.

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

***Environment Canada EPS 1/RM/44*** *The inclusion of a positive microbial control is not required and is not recommended for most* applications. In instances where a suitable pathogen is available (i.e., genetically related with known toxic/pathogenic effects), a positive microbial control might be warranted. Sensitivity of test organisms to a reference toxicant (i.e., a positive chemical control) must be determined. ***OECD 208*** *A reference substance may be tested at regular intervals, to verify that performance of the test and the response of the particular test* plants and the test conditions have not changed significantly over time. Alternatively, historical biomass or growth measurement of controls could be used to evaluate the performance of the test system in particular laboratories, and can serve as an intra-laboratory quality control measure.

## Other controls:

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

***U.S. EPA OCSPP 885.4300*** *Untreated controls should be as pest-free as reasonably possible. In addition, in the cases of MPCAs that are* readily disseminated (wind, insect, etc.), it may be necessary to conduct tests such that negative controls and treated plants are grown in separate

*geographical locations in separate contained greenhouses under identical conditions. Alternatively, the negative control may be treated with a* non-phytotoxic chemical pesticide known to provide effective control of the MPCA. ***From U.S. EPA OCSPP 885.0001 Overview for Microbial* Pest Control Agents** *All controls shall, to the extent possible, be from the same source, be of the same age, receive the same care, and receive the* same nutrients as the animals or plants receiving the test substance. To prevent bias, a method to assign organisms to treatment and control groups randomly is required and must be referenced in the report.

***PMRA DIR 2001-02*** *A negative, no-dosed control group of the non-target organism should also be run concurrently with the test group. For* herbicidal MPCAs, additional no-dosed control group of the target, i.e., pest, is also required.

***Environment Canada EPS 1/RM/44*** *A negative control is required. The use of a non-infectious control is strongly recommended. A sterile* filtrate control is optional but recommended.

***OECD 208*** *&* ***OECD 201*** *A negative control is required. If a solvent is used, additional controls containing the solvent at the same* concentration as used in the test must be included.

**Number of replicates:** *[Report only for aquatic plant testing]*

Control(s): Vehicle/Solvent control: Treatments:

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Lemna minor: At least three replicates per treatment or control. Four replicates are required when* establishing a NOEC/LOEC. Champia parvula: 4 replicates per treatment or control are required for single concentration tests whereas at least 3 replicates per treatment or control are required for multi-concentration testing. Four replicates are required when establishing a NOEC/LOEC.

***OECD 201*** *The test design should include three replicates at each test concentration. If determination of the NOEC is not required, the test* design may be altered to increase the number of concentrations and reduce the number of replicates per concentration. The number of control replicates must be at least three, and ideally should be twice the number of replicates used for each test concentration.

**Number of plants/replicate or Algal concentration:** *[Report only for aquatic plant testing] [Insert the number of plants per replicate or algal concentration.]*

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

***PMRA DIR 2001-02*** *A sufficient number of test organisms must be treated to allow for adequate controls, statistical analysis, interpretation of* data and for interim sacrifice, if applicable.

***Environment Canada EPS 1/RM/44*** *A minimum of two plants per replicate are required for Lemna minor. For Champia parvula, a minimum* of 5 female plants and one male plant are required per replicate.

***OECD 201*** *The initial cell concentration must be sufficiently low to allow exponential growth throughout the study period (i.e., 0.5 mg/L). The* following initial cell concentrations are recommended: Pseudokirchnerieriella subcapitata 5×103–104 cells/mL; Desmodesmus subspicatus 2– 5×103 cells/mL; Navicula pelliculosa & Anabaena flos-aquae 104 cells/mL; and Synechoccus leopoliensis 5×104–105.

**Number of fronds/plant (for vascular plant studies):** *[Report only for aquatic plant testing] [Insert the number of fronds per plant.]*

***U.S. EPA OPPTS 885.4300*** *No specific recommendations.*

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

***Environment Canada EPS 1/RM/44*** *Lemna minor: 7- to 10-day old cultures, each plant with 3 fronds.*

***OECD 201*** *Not applicable (alga/cyanobacteria study)*

## Recovery of MPCA from plant:

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

***U.S. EPA OCSPP 885.4300*** *If no obvious adverse effects are evident after these observation periods, the roots, foliage, fruit, vascular tissues,* etc. should be analyzed for the presence of the organism using sensitive, specific methods.

***PMRA DIR 2001-01*** *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. If no adverse effects are evident at study determination, plant tissues should be analyzed for the presence of the MPCA using sensitive, specific methods.

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

***OECD 208& 201*** *No specific recommendations (guideline designed for chemical toxicity testing).*

###### *For Aquatic Plants and Algae:*

***U.S. EPA OPPTS 885.4300*** *No specific recommendations for small aquatic organisms such as algae. However, roots, foliage, fruit, vascular* tissues, etc. of plants should be analyzed for the presence of the organism using sensitive, specific methods if no obvious adverse effects are evident at end of test.

## Test conditions:

#### Temperature:

Photoperiod:

Light intensity and quality: Relative humidity:

***U.S. EPA OCSPP 885.4300*** *When the optimum conditions for penetration, infection, and disease development are known or suspected, it is* important, particularly for microbial herbicides, to simulate these conditions rather than those known to be optimum for plant growth and development. In many cases, however the optimum environment may be similar.

***PMRA DIR 2001-02*** *When the optimum conditions for penetration, infection and disease development are known or suspected, it is important,* particularly for herbicidal MPCAs to simulate these conditions rather than those known to be optimum for plant growth and development.

***Environment Canada EPS 1/RM/44*** *Temperature 24±2°C (day) and 15±2°C (night); Lighting 16±1 h full spectrum light (~400 mol/(m2s)).*

***OECD 208*** *Temperature: 22±10°C; , humidity: 70%±25%; light: minimum 16 hours light, 350±50 µE/m2/s, wavelength 400–700 nm.* Conditions must be suitable for maintaining normal growth of each species for the test period.

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *Lemna minor: daily mean water temperature of 25±2°C throughout the test and continuous full spectrum* light, pH 6.5–9.5. Champia parvula: daily mean water temperature of 23±1°C throughout the test and full spectrum light 16 hours light / 8 hours dark.

***OECD 201*** *Temperature in the range of 21–24°C (controlled at ±2°C). Higher temperatures may be required for other species. Continuous* uniform illumination. Light intensity should be selected to suit the test organisms used. For the recommended species of green algae 60–120

*µE/m2/sec when measured in the phtotosynthetically effective wavelength range of 400–700 nm (4400–8880 lux for cool white light), for* Anabaena flos-aquae 40–60 µE/m2/sec.

## Growth facility:

##### *[Describe the growth facility.]*

***U.S. EPA OCSPP 885.4300*** *No specific recommendations, but a description of the growth chambers, greenhouse or other type of test facility is* required.

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

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

***OECD 208*** *Suitable facilities (e.g., phytotrons, glass houses or plant growth chambers) are required.*

###### *For Aquatic Plants and Algae:*

***OECD 201*** *A temperature-controlled cabinet or chamber with suitable lighting is required.*

## Duration of study:

***U.S. EPA OCSPP 885.4300*** *Plants are observed until normal harvest or death or, as in the case of perennials, at periodic intervals for at least 2 years. No specific recommendations for algae.* ***From U.S. EPA OCSPP 885.4000 Background for Nontarget Organism Testing of Microbial Pesticide Control Agents*** *In general- the duration of the test should be sufficient to allow for manifestation of a delayed pathogenic response.* ***PMRA DIR 2001-02*** *Test plants observed until normal harvest or death or, as in the case of perennials, at periodic intervals for at least the time required to adversely affect the target plant pest.*

***Environment Canada EPS 1/RM/44*** *14 days for barley, Durum wheat, alfalfa, cucumber, lettuce radish, red clover or tomato; 21 days for* carrot, blue grama grass, northern wheatgrass or red fescue.

***OECD 208*** *The test is usually terminated 14 to 21 days after 50% of control seedlings have emerged.*

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *Lemna minor: 7-day test. Champia parvula: 9-day test.*

***OECD 201*** *At least 72 hours.*

## 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, appearance, temperature, relative* humidity, individual plant weights, concentration of the MCPA in the test media. Provide references to data summary tables, if used.]

***U.S. EPA OCSPP 885.4300*** *Adverse effects (unspecified). If no adverse effects are evident, the roots, foliage, fruit, vascular tissues, etc. should* be analyzed for the presence of the organism using sensitive, specific methods.

***PMRA DIR 2001-02*** *Regular observations are required to record mortalities and note any phytotoxic effects. If no adverse effects are evident,* the plant tissues should be analyzed for the presence of the MPCA using sensitive, specific method, as asymptomatic plants may serve as sites for proliferation and survival of the MPCA in the environment.

***Environment Canada EPS 1/RM/44*** *Measurement of the temperature in the test facility, soil moisture content (%), soil conductivity, soil pH,* light intensity, and concentration of the MPCA in soil of each treatment group is required. Observations for the number of emerged seedlings in each test chamber, shoot/root length and shoot-root dry weight, and number of surviving plants at test end showing atypical appearance (e.g., chlorosis, lesions).

***OECD 208*** *Measurements of environmental conditions (e.g., temperature, humidity, concentration of carbon dioxide, light intensity and* wavelength), soil parameters (percent organic carbon, pH, salt content, conductivity and redox potential), shoot height (if measured), shoot dry or fresh weight, and concentration of the test substance in the soil are required. Observations for seedling emergence and injury are required.

###### *For Aquatic Plants and Algae:*

***U.S. EPA OPPTS 885.4300*** *No specific recommendations for measurement of adverse effects. If no adverse effect is evident, plant tissues* should be analyzed for the presence of the MPCA using sensitive methods.

***Environment Canada EPS 1/RM/44*** *Lemna minor: measurement of pH, temperature, light intensity, and concentration of the MPCA in the test* suspension of each treatment group including controls is required. Observations for the number of fronds, appearance, and dry weight. Champia parvula: measurement of the dissolved oxygen, temperature, pH, salinity, and concentration of the MPCA in the test suspension of each treatment group including controls is required. Observations for the appearance of test suspensions, aeration rates, appearance of each plant, number of cystocarps per female, and signs of necrotic tissue and/or morphological changes.

***OECD 201*** *Measurements of light intensity, pH, and concentration of the test substance in test suspensions are required. Observations for algal* biomass (e.g., cell/mL, optical density) and microscopic appearance of cultures are required.

## Observation/measurement intervals:

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

***U.S. EPA OCSPP 885.4300*** *Plants should be observed weekly or more frequently until normal harvest or death, or, in the case of perennials, at* regular intervals for at least 2 years. No specific recommendations for algae.

***PMRA DIR 2001-02*** *Plants should be observed regularly for the duration of the study.*

***Environment Canada EPS 1/RM/44*** *Temperature measured daily (max/min) or continuously; soil parameters (moisture content, conductivity* and pH) measured at the beginning and end of the test for at least one replicate of each treatment; concentration of the MPCA in the soil for each treatment including controls at the beginning and end of the test (at minimum); observations for the number of emerged seedlings on Day 7 and at test end; and observations for shoot/root length and shoot-root dry weight, and number of surviving plants showing atypical appearance (e.g., chlorosis, lesions) at test end.

***OECD 208*** *Environmental conditions and soil parameters must be reported but no observation intervals were specified. The concentration of* test substance in soil must be measured but no observation interval was specified. Plants are observed at least weekly for emergence, visual phytotoxicity and mortality. At the end of the study, emergence, injury, shoot height (if required), and shoot dry or fresh weight, injury are observed.

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *Lemna minor: temperature measured daily in representative test chambers; pH measured at start and end* of test, and before and after each renewal in one or more replicates of each treatment including controls; light intensity once during the test; and concentration of MPCA in each treatment including controls at the beginning and end of the test, and at the beginning and end of at least one of the renewal cycles during the test. Observations on the number of fronds and appearance at test start and test end; and observations on dry weight at test end. Counting fronds on two other occasions during the study for growth rate calculation is optional. Champia parvula: during the first 48 hours, temperature, pH, dissolved oxygen concentration and salinity must be measured for one replicate of each treatment for fresh and aged algal suspensions then daily in representative test chambers; concentration of MPCA in each treatment including controls at beginning and

*end of each renewal period during the initial 48 hours; daily observations on appearance of test suspensions, appearance of plants, number of* cystocarps per females; and observations on signs of necrotic tissue and/or morphological changes at test end.

***OECD 201*** *Algal biomass must be determined at least daily. The pH must be determined at the beginning and at the end of the study. The* concentration of the test material should be analyzed to verify initial concentrations and ensure maintenance of exposure concentrations. For stable test materials (i.e., within 20% nominal), the concentration of the low and high test concentrations as well as the concentration around the expected EC50 must be measured at the start and at the end of the study. Analysis of all test concentrations at the beginning and at the end is recommended where concentrations are unlikely to remain within 20% of the nominal concentration. For volatile, unstable or strongly adsorbing test materials, additional assays must be performed at 24-hour intervals. Microscopic analysis of the cell cultures must be determined at the end of the study. Light intensity must be maintained within ±15% from the mean light intensity over the incubation area.

**Phytotoxicity rating system:** *[if used- describe method, any calculations and provide reference]*

## Testing for infectivity:

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

***U.S. EPA OCSPP 885.4300*** *If no obvious adverse effects are evident after these observation periods, the roots, foliage, fruit, vascular tissues,* etc. should be analyzed for the presence of the organism using sensitive, specific methods.

***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. If no adverse effects are evident at study determination, plant tissues should be analyzed for the presence of the MPCA using sensitive, specific methods.

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

***OECD 208& 201*** *No specific recommendations (guideline designed for chemical toxicity testing).*

**Environmental parameters and water quality were acceptable?** *[Report only for aquatic plant testing- indicate as Yes/No]*

***U.S. EPA OCSPP 885.4300*** *Any significant deviations encountered in course of the experiment with temperature, humidity ranges, photoperiod* and lighting etc. must be reported.

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

1. **RESULTS:**
2. **VIABILITY OF TEST MATERIAL:** *[Summarize the dose/concentration verification data and indicate if the tested sample was still viable.]*

**TABLE *[#]*.** Viability *[or potency]* of *[test substance]* in the *[soil/growth medium/ dosing suspension]*

administered to *[test organism]* over *[#]* days.

|  |  |  |
| --- | --- | --- |
| **Test group** | **Nominal concentration *[units]*** | **Measured concentration *[units]*** |
| *x1 Maximum hazard*  *concentration* |  |  |
| *x2*  *(Other measured test treatment)* |  |  |

|  |  |  |
| --- | --- | --- |
| **Test group** | **Nominal concentration *[units]*** | **Measured concentration *[units]*** |
| *x3 Solvent/vehicle control*  *(if used)* |  |  |
| *n Inactivated control (i.e.*  *heat-killed) (if used)* |  |  |
| Negative control |  |  |
| Positive/Reference Control *(if used)* |  |  |

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

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

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

**TABLE *[#]*.** Effect of *[test material]* on cumulative mortality of *[common name (scientific name].*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **No. of Test Organisms** | **Cumulative Mortality** | | | | | |
| ***Day x1*** | ***Day x2*** | ***Day x3*** | ***Day x4*** | ***Day x5*** | ***Day n*** |
| Negative control |  |  |  |  |  |  |  |
| *Solvent control, if used* |  |  |  |  |  |  |  |
| *Measured test concentration 1* |  |  |  |  |  |  |  |
| *Measured test concentration 2* |  |  |  |  |  |  |  |
| *Measured test concentration n* |  |  |  |  |  |  |  |
| *LC50*  *[insert [***>***] if greater than]* |  | | | | | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | | **No. of Test Organisms** | **Cumulative Mortality** | | | | | |
| ***Day x1*** | ***Day x2*** | ***Day x3*** | ***Day x4*** | ***Day x5*** | ***Day n*** |
| *NOEC*  *[insert [***>***] if greater than]* | |  | | | | | | |
| *Reference Material (if used)* | *Mortality (% or No.)* |  |  |  |  |  |  |  |
| *LC50:* | *[insert [***>***] if greater than]* | | | | | | |
| *NOEC* | *[insert [***>***] if greater than]* | | | | | | |

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

***(Use the following section for Terrestrial plant test results. If testing aquatic plants/algae- delete this section and proceed to following section C. for describing “Inhibitory Effects” for aquatic plant test results. )***

1. **INHIBITORY EFFECTS:** *[Briefly describe the phytotoxic inhibition including the effect on percent seed germination, % survival, root length and plant height, discolorations, dry weight and any other observations. Indicate effects that were related to the test-material. Compare inhibitory effects 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. If there was no observed toxicity or inhibitory effects, state “There were no test material-related phytotoxic effects.”]*
   1. **Seed Germination:**

**TABLE *[#]***. Effect of *[test material]* on seed germination and root elongation of *[insert common and scientific names]*.

*e.g., for MHC testing*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Replicate** | **Number of Seeds** | **Percent Germination** | **Root Length (mm)** | | **Plant Injury Index/ Rating\*** |
| **Range** | **Mean Length** |
| Species 1 | 1 |  |  |  |  |  |
| *...* |  |  |  |  |  |
| *x* |  |  |  |  |  |
| ... | 1 |  |  |  |  |  |
| *...* |  |  |  |  |  |
| *x* |  |  |  |  |  |
| Species *x* | 1 |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Replicate** | **Number of Seeds** | **Percent Germination** | **Root Length (mm)** | | **Plant Injury Index/ Rating\*** |
| **Range** | **Mean Length** |
| *...* |  |  |  |  |  |
| *x* |  |  |  |  |  |
| Control | 1 |  |  |  |  |  |
| *...* |  |  |  |  |  |
| *x* |  |  |  |  |  |
| EC50 | *[insert [***>***] if greater than] [biological or bioactivity units/L or ha]* | | | | | |
| NOEC  (if applicable) | *[insert [***>***] if greater than]* | | | | | |

* Description of various ratings used

##### *e.g., for multi-concentration testing*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **NOEC, EC25 and EC50 (*biological or bioactivity units/L or ha*)** | | | | | | | | **Plant Injury Index/ Rating\*** |
| **Germination** | | | | **Root Length (mm)** | | | |
| **%\*\*** | **NOEC** | **EC25** | **EC50** | **Length**  **\*\*** | **NOEC** | **EC25** | **EC50** |
| Control |  |  |  |  |  |  |  |  |  |
| *Species 1* |  |  |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |  |  |
| *Species x* |  |  |  |  |  |  |  |  |  |

* Description of various ratings used

\*\* provide the range

* 1. **Seedling Emergence:**

**TABLE *[#]***. Effect of *[test material]* on seedling emergence of *[insert common and scientific names]*. *e.g., for MHC testing*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Replicate** | **Number of Seeds** | **Percent Seedling Emergence** | **Dry Weight (mg/g)/other** | | **Height (*unit*)** | | **Plant Injury Index/ Rating\*** |
| **Range** | **Mean Dry Weights** | **Range** | **Mean Height** |
| *Species 1* | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |
| *x* |  |  |  |  |  |  |  |
| *...* | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Replicate** | **Number of Seeds** | **Percent Seedling Emergence** | **Dry Weight (mg/g)/other** | | **Height (*unit*)** | | **Plant Injury Index/ Rating\*** |
| **Range** | **Mean Dry Weights** | **Range** | **Mean Height** |
| *x* |  |  |  |  |  |  |  |
| *Species x* | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |
| *x* |  |  |  |  |  |  |  |
| Control | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |
| *x* |  |  |  |  |  |  |  |
| EC50 | *[insert [***>***] if greater than] [biological or bioactivity units/L or ha]* | | | | | | | |
| NOEC (if  applicable) |  | | | | | | | |

* Description of various ratings used

*e.g., for multi-concentration testing*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **NOEC, EC25 and EC50 (*biological or bioactivity units/L or ha*)** | | | | | | | | | | | | **Plant Injury Index/ Rating\*** |
| **Seedling Emergence** | | | | **Dry Weight (mg/g)/other** | | | | **Height** | | | |
| **%\*\*** | **NOEC** | **EC25** | **EC50** | **Dry Weight\*** | **NOEC** | **EC25** | **EC50** | **Height**  **\*** | **NOEC** | **EC25** | **EC50** |
| Control |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Species 1* |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Species x* |  |  |  |  |  |  |  |  |  |  |  |  |  |

* Description of various ratings used

\*\* provide the range

* 1. **Vegetative Vigor:**

**TABLE *[#]***. Effect of *[test material]* on vegetative vigor of *[insert common and scientific names]*. *e.g., for MHC testing*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Replicate** | **Number of Plants** | **Percent Survival** | **Dry Weight**  ***(mg/g)/other*** | | **Height (*unit*)** | | **Plant Injury Index/ Rating\*** |
| **Range** | **Mean Dry Weight** | **Range** | **Mean Height** |
| *Species 1* | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |
| *x* |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Replicate** | **Number of Plants** | **Percent Survival** | **Dry Weight**  ***(mg/g)/other*** | | **Height (*unit*)** | | **Plant Injury Index/ Rating\*** |
| **Range** | **Mean Dry Weight** | **Range** | **Mean Height** |
| *...* | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |
| *x* |  |  |  |  |  |  |  |
| *Species x* | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |
| *x* |  |  |  |  |  |  |  |
| Control | 1 |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |
| *x* |  |  |  |  |  |  |  |
| EC50 | *[insert [***>***] if greater than] [biological or bioactivity units/L or ha]* | | | | | | | |
| NOEC  (if applicable) | *[insert [***>***] if greater than]* | | | | | | | |

* Description of various ratings used.

*e.g., for multi-concentration testing*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **NOEC, EC25 and EC50 (*biological or bioactivity units/L or ha*)** *[insert [***>***] if greater than]* | | | | | | | | | | | | **Plant Injury Index/ Rating\*** |
| **Survival** | | | | **Dry Weight *(mg/g)/other*** | | | | **Height (*unit*)** | | | |
| **% \*\*** | **NOEC** | **EC25** | **EC50** | **Dry Weight\*\*** | **NOEC** | **EC25** | **EC50** | **Height\*\*** | **NOEC** | **EC25** | **EC50** |
| Control |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Species 1* |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *...* |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Species x* |  |  |  |  |  |  |  |  |  |  |  |  |  |

* Description of various ratings used

\*\* provide the range

***(Use the following section for Aquatic plant test results. If testing terrestrial plants- delete this section and proceed to next section D. for describing “Reported Statistics Inhibitory Effects.”)***

1. **INHIBITORY EFFECTS:** *[Briefly describe the phytotoxic inhibition including the effect on number of fronds, % survival, and dry weight. Describe other effects - Any change in frond development or appearance (increase or decrease in size, necrosis, chlorosis, sedimentation of test solutions, sinking of fronds, other abnormalities. For algal studies, describe growth rate effects. Indicate effects that were related to the test-material. Compare inhibitory effects 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. Note if there was [or was not] a major change in pH during the study. If there was no observed toxicity or inhibitory effects, state “There were no test material- related phytotoxic effects.”]*

* 1. **Frond Number:**

**TABLE *[#]*.** Effect of *[test material]* on frond number of *[insert common and scientific names]*.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment *(record measured and nominal concentration)*** | **Initial Frond Number *(or other endpoint)*** | **Number of Fronds** | | | **% Inhibition on Day *[xn]*** |
| **Day *[x1]*** | **Day *[x2]*** | **Day *[xn]*** |
| Negative control |  |  |  |  |  |
| *Treatment 1* |  |  |  |  |  |
| *Treatment 2* |  |  |  |  |  |
| *Treatment n* |  |  |  |  |  |
| *Positive control/Reference chemical (if used)* |  |  |  |  |  |
| EC50 | *[insert [***>***] if greater than] [biological or bioactivity units/L or ha]* | | | | |
| NOEC  *(if applicable)* |  | | | | |

## Dry Weight:

**TABLE *[#]*.** Effect of *[test material]* on the dry weight of *[insert common and scientific names]*.

|  |  |  |
| --- | --- | --- |
| **Treatment**  ***(record measured and nominal concentration)*** | **Dry Weight on Day**  ***[xn]***  **(g)** | **% Inhibition on Day**  ***[xn]*** |
| Negative control |  |  |
| *Treatment 1* |  |  |
| *Treatment 2* |  |  |
| *Treatment n* |  |  |
| *Positive control/Reference chemical (if used)* |  |  |
| EC50 | *[insert [***>***] if greater than] [biological or bioactivity units/L or ha]* | |
| NOEC  *(if applicable)* |  | |

* 1. **Growth Rate:** *[Report only for algae testing]*

**TABLE *[#]*.** Effect of *[test substance]* on the growth of the *[test organism]*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment (mg a.i/L)** | **Initial Cell Number (cells/mL)** | **Cell Number/mL** | | | | | **%**  **Growth Inhibiti on** |
| ***Time 1*** | ***Time 2*** | ***Time 3*** | ***Time 4*** | ***Time n*** |
| Negative control |  |  |  |  |  |  |  |
| *treatment 1* |  |  |  |  |  |  |  |
| *treatment 2* |  |  |  |  |  |  |  |
| *treatment n* |  |  |  |  |  |  |  |
| EC50 | *[insert [***>***] if greater than]* | | | | | | |

# *(The following section is applicable to both terrestrial and aquatic plant testing-* Resume input of data)

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

***U.S. EPA OCSPP 885.4300*** *No specific statistical recommendations. From* ***U.S. EPA OCSPP 885.0001-*** *Appropriate statistical methods are to* be used to summarize experimental data, to express trends, and to evaluate the significance of differences in data obtained from different test group and methods used shall reflect the current state-of-the art. All data averages or means must be accompanied by standard deviations and the standard errors of the means should also be calculated; however, notations of statistically significant differences accompanied by the confidence level or probability should also be used in place of standard error determinations. Other methods of expressing data dispersion may also be used when appropriate.

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

***Environment Canada EPS 1/RM/44*** *Single concentration test: percent emergence on Day 7 and at test end, percent of surviving (emerged)* plants showing atypical appearance at test end; mean (± SD) length of roots and longest roots at test end, mean (±SD) dry weight of shoots and roots at test end, comparing MHC to controls. Multi-concentration test: percent emergence on Day 7 and at test end, percent of surviving (emerged) plants showing atypical appearance at test end; mean (± SD) length of roots and longest roots at test end, mean (±SD) dry weight of shoots and roots at test end, comparing each test chamber and treatment. Data permitting, 7- and 21-day EC50 for emergence, 21-day EC50 for atypical appearance, 21-day IC25 for length and dry weight of shoots and roots or surviving plants.

***OECD 208*** *The LC50 should be calculated from the effect of the test substance on emergence, and the EC50 should be calculated from the effect* of the test substance on growth.

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *Lemna minor: In single concentration test: mean (±SD) number of fronds; mean (±SD) dry weight of* fronds, comparing MHC to controls. Multi-concentration test: mean (±SD) number of fronds; mean (±SD) dry weight of fronds, comparing each test chamber and treatment. Data permitting, 7-day IC25 for attained number of fronds, 7-day IC25 for frond dry weight, NOEC/LOEC for attained number of fronds and frond dry weight. Champia parvula: In single concentration test: percent survival of female branch tips; mean (±SD) number of cystocarps per plant; percent surviving plants showing atypical appearance including lesions or abnormally developed cystocarps, comparing MHC to controls. Multi-concentration test: percent survival of female branch tips; mean (±SD) number of cystocarps per plant; percent surviving plants showing atypical appearance including lesions or abnormally developed cystocarps, comparing each test chamber and treatment. Data permitting, 9-day LC50 for female plants; 9-day EC50 for female plants showing atypical appearance; 9-day IC25 for attained number of cystocarps per female, NOEC/LOEC for attained number of cystocarps per female.

***OECD 201*** *The mean value of the cell concentration for each test substance concentration and for the controls is plotted against time to* produce growth curves. The concentration-effect relationship is determined by comparison of areas under the growth curves, or by comparison of growth rates (see guideline for specific calculations).

1. **VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:** *[If applicable- Report the statistical methods used by the reviewer to verify the applicant’s results (such as when using for multi- concentration testing); If values for LC50, EC50, NOEC are greater than the MHC level, use* ***<*** *symbol.]*

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

##### *conclusions e.g., values for LC50, EC50, 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 toxicity and pathogenicity study for nontarget plant testing (OCSPP 885.4300, PMRA: M9.8.1, M9.8.2 and OECD: IIM 8.6, IIM 8.4, IIM 8.5, 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 885.4300*** *No specific validity criteria.*

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

***Environment Canada EPS 1/RM/44*** *The test is invalid if, for plants in negative control soil, any of the following occurs at test end: mean* emergence rate: <60% in carrot, <70% in alfalfa, cucumber, blue grama grass, lettuce, red fescue, or tomato, <80% in barley, northern wheatgrass, Durum wheat, or red clover, <90% in radish; mean root length: <40 mm in carrot or tomato, <70 mm in blue grama grass or red fescue, <100 mm in lettuce or red clover, <120 mm in radish, northern wheatgrass, alfalfa, cucumber, <200 mm in barley or Durum wheat; mean shoot length: <20 mm in lettuce or red clover, <40 mm in carrot, <50 mm in radish, alfalfa, cucumber, blue grama grass, carrot, tomato,

*<80 mm in northern wheatgrass or red fescue, <130 mm in barley or Durum wheat.*

***OECD 208*** *The seedling emergence in controls must be at least 70%; control seedlings must not exhibit visible phytotoxic effects (e.g. chlorosis,* necrosis, wilting, leaf and stem deformations) and control plants must exhibit only normal variation in growth and morphology for that particular species; the mean survival of emerged control seedlings must be at least 90% for the duration of the study; environmental conditions for a particular species must all be identical and growing media must contain the same amount of soil matrix, support media, or substrate from the same source.

###### *For Aquatic Plants and Algae:*

***Environment Canada EPS 1/RM/44*** *Lemna minor: the test is invalid if the increase in the number of fronds in the negative control during the 7-* day test period is less than 8-fold (i.e., the mean number of fronds for the negative control must be ≥48 at the end of the test, for the test to be valid). Champia parvula: the test is invalid if <80% survival in the negative controls, or if the mean number of cystocarps in negative controls is

*<10/plant.*

***OECD 201*** *The cell concentration in the control cultures should have increased by a factor of at least 16 within three days. The mean* coefficient of variation for section-by-section specific growth rates (e.g., days 0–1, 1–2 and 2–3) in the control cultures must not exceed 35%. The coefficient of variation of average specific growth rates during the whole study period in replicate control cultures must not exceed 7% in tests with Pseudokirchneriella subcapitata and Desmodesmus subspicatus (for other less frequently tested species, the coefficient of variation should not exceed 10%).

1. **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 nontarget plant studies (OCSPP 885.4300). The waiver request is based on the rationale that *[name of active ingredient]* is a naturally-occurring *[soil/water/plant-surface. etc.]* colonizer, whose level in the environment will not significantly increase with the use of *[product name]* and that an extensive literature search yielded no *[or no significant]* reports of adverse effects in terrestrial and aquatic nontarget plants in greenhouse and/or field trials.

The proposed uses of *[product name]* on *[identify use sites/crops]* is not expected to result in increased exposure or adverse effects in plants. Therefore, additional testing is not considered necessary to assess the risks of the *[product name]* to nontarget plants. The *[applicant]* requests a waiver of terrestrial and aquatic nontarget plant studies.

***(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. ***[If applicable]* No adverse effects on plants observed in greenhouse and field trials.** *[Number of]* research trials on *[name of crops]* were conducted to determine the efficacy and/or plant host spectrum of *[product name]*. Observations for phytotoxicity/phytopathogenicity were included in each trial. No adverse effects on nontarget plants were observed. *[If comparison of the quality of plants treated with the product to those treated with chemical (positive control) products and to untreated (negative) control plants was conducted, then report the findings and conclusions.]*
  2. **No evidence of adverse effects.** A literature search of the *[e.g., AGRICOLA, TOXLINE, BIOLOGICAL ABSTRACTS, CHEMTOX, PUBMED, etc.]* 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 terrestrial and aquatic plants. *[Name the metabolites found to be produced - does the MPCA strain also produce these or other metabolites? Have*

##### *natural populations of the MPCA or its metabolites been associated with adverse effects in terrestrial or* aquatic plant 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 survive or persist in aquatic ecosystems, as relates to the potential exposure to aquatic plants. Does the MPCA appear on any authoritative list of plant pathogens? Name the lists examined.]*

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

##### Use of *[product name]* will be limited to *[soil, seed, foliar, greenhouse, etc.]* applications *[by spray, dip,* soil incorporation, aerial, etc.] on *[name crops/use sites]*, thus minimizing direct exposure to non-target plants. *[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. Include any other factors that would limit exposure to non-target plants.

***[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 PLANT 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 Mendel (1890), the effects of this MPCA on plants have been studied in three plant families.*

|  |  |  |  |
| --- | --- | --- | --- |
| *e.g.,* | ***A.*** | ***LILIACEAE:*** |  |
|  | ***1.*** | ***Article 1:*** | *(summarize and report findings)* |
|  | ***2.*** | ***Article 2:*** | *(summarize and report findings)* |
|  | ***B.*** | ***MALVACEAE:*** |  |
|  | ***1.*** | ***Article 1:*** | *(summarize and report findings)* |
|  | ***2.*** | ***Article 2:*** | *(summarize and report findings)* |
| ***C. POACEAE (GRAMINEAE):*** | | | |
| ***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]**
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