**Attachment H**

**Aquatic Vascular Plant Data Evaluation Record (DER) Template**

**March 2024**

***Part A: Overview***

**I. Test Information**

**Chemical name:**

 CAS name: not provided CAS Number: not provided

 Purity: not provided Storage conditions: not provided

 Solubility in Water (units):

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Controlled Experiment** |  | **Field Study/Observation** | (*Place X by One*) |
|  | (*manipulated*) |  | (*not manipulated*) |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Primary Reviewer:** |  | **Date:** |  |  |  | **EPA** |  | **Contractor** | (*Place X by One*) |
| **Secondary Reviewer:** |  | **Date:** |  |  |  | **EPA** |  | **Contractor** | (*Place X by One*) |
| (*At least one reviewer should be from EPA for sensitive taxa*) |

**CITATION**: *Indicate: author(s), year, study title, journal, volume, and pages*.

(e.g., Antunes, P.M.C., M.L. Scornaienchi, H.D. Roshon. 2012. Copper toxicity to Lemna minor modelled using humic acid as a surrogate for the plant root. Chemosphere. 88 (4):389-394.)

**Companion Papers:** *Identify any companion papers associated with this paper using the citation format above.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Were other DERs completed for Companion Papers?** |  |  | **Yes** |  |  | **No** | (*If yes, list file names of DERs below*) |

**Study Classification for Aquatic Life Criteria Development:** *Place X by One Based on Highest Use*

|  |  |
| --- | --- |
|  | Acceptable for Quantitative Use |
|  | Acceptable for Qualitative Use |
|  | Not Acceptable for Use/Unused |

**General Notes:** *Provide any necessary details regarding the study’s use classification for all pertinent endpoints, including non-apical endpoints within the study (e.g., note all study classifications for each endpoint if the use varies)*

**Major Deficiencies (note any stated exclusions)**: *Check all that apply. Checking any of* t*hese items make the study “****Not Acceptable for Use****”*

|  |  |  |  |
| --- | --- | --- | --- |
|  | Mixture (for controlled experiments only) |  | No Controls (for controlled experiments only) |
|  | Excessive Control Mortality |
|  | Dilution water not adequately characterized |  | Bioaccumulation: steady state not reached |
|  | Review paper or previously published without modification |
|  | Excessive EDTA or similar complexing agent |
|  | Other: [Add Text if Applicable] |

POTENTIAL CHEMICAL MIXTURES:*Describe any potential chemicals mixtures as characterized by study authors (including any confirmation of chemical mixtures).*

DESCRIPTION OF DILUTION WATER: *Describe concerns with characterization of and/or major deficiencies with dilution water.*

***General Notes:***

**Minor Deficiencies:** *List and describe any minor deficiencies or other concerns with test. These items may make the study “****Acceptable for Qualitative Use****”* **(exceptions may apply as noted)**

***For Field Studies/Observations****: A field study/observation may be considered “****Acceptable for Quantitative Use****” if it consisted of a range of exposure concentrations and the observed effects are justifiably contributed to a single chemical exposure*

|  |  |
| --- | --- |
|  | Mixture (observed effects not justifiably contributed to single chemical exposure) |
|  | Uncharacterized Reference Sites/Conditions |

POTENTIAL CHEMICAL MIXTURES PRESENT AT SITE: *Describe any potential chemicals mixtures present at the site as characterized by study authors (including any confirmation of chemicals present at study site).*

EXPOSURE VARIABILITY ACROSS STUDY SITE(S): *Describe any exposure variability across study site(s) as characterized by study authors (i.e., description of study design with reference and contaminated sites).*

***General Notes:***

**Reviewer’s Comments:** *Provide additional comments that do not appear under other sections of the template*.

**ABSTRACT**: *Copy and paste abstract from publication*.

**SUMMARY***: Fill out and modify as needed.*

Acute:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** **(life stagea)** | **Methodb** | **Categoryc** | **Test duration** | **Chemical / Purity** | **pH** | **Temp.(°C)** | **Hardness(mg/L as CaCO3) or Salinity (ppt)** | **DOC(mg/L)** | **Effect** | **Reported Effect Concentration****(mg/L)** | **Verified Effect Concentration(mg/L)** | **Classification** |
|  |  |  |  |  |  |  |  |  |  |  |  | Quantitative / Qualitative / Unused |

a e.g., seed, seedling, adult

b S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved

c B=benthic, E=emergent, Sub=submerged

Chronic:

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** **(life stagea)** | **Methodb** | **Categoryc** | **Test duration** | **Chemical / Purity** | **pH** | **Temp.(°C)** | **Hardness(mg/L as CaCO3)****or Salinity (ppt)** | **DOC(mg/L)** | **Chronic Limits** | **Reported Chronic Value****(mg/L)** | **Verified Chronic Value(mg/L)** | **Chronic ValueEndpoint** | **Classification** |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Quantitative / Qualitative / Unused |

a e.g., seed, seedling, adult

b S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved

c B=benthic, E=emergent, Sub=submerged

**II. Results** *Provide results as reported in the publication (including supplemental materials). Include screen shots of tables and/or figures reporting results from the article following tabulated data table in each associated results section for all studies*. *Complete tabulated data tables for all studies for studies marked “****Acceptable for Quantitative Use”*** *and* ***“Acceptable for Qualitative Use****”*.

**Water Quality Parameters**: *If only general summary data of water quality parameters is provided by study authors (i.e., no specific details of water quality parameters on a treatment level is provided), summarize any information regarding water quality parameters under General Notes below.*

**General Notes:** *For aquatic life criteria development, measured water quality parameters in the treatments nearest the toxicity test endpoint(s), e.g., LC50, EC20, etc., are most relevant.*

**Table A.II.1. Measured Water Quality Parameters in Test Solutions.**

Dissolved oxygen, temperature, pH and [other parameters (hardness, salinity, DOC)] in test solutions during the *[X]*-day exposure of *[test organism]* to *[concentration of treatment(s)]* of *[test substance]* under *[static renewal/flow-through]* conditions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Treatment** | **Mean** | **Range** |
| **Dissolved oxygen****(% saturation or mg/L)** | *[1]* |  |  |
| *[2]* |  |  |
| *j* |  |  |
| *j* |  |  |
| **Temperature (̊C)** | *[1]* |  |  |
| *[2]* |  |  |
| *j* |  |  |
| *j* |  |  |
| **pH** | *[1]* |  |  |
| *[2]* |  |  |
| *j* |  |  |
| *j* |  |  |
| **Other (e.g., hardness, salinity, DOC)** | *[1]* |  |  |
| *[2]* |  |  |
| *j* |  |  |
| *j* |  |  |

**Chemical Concentrations**: *Summarize the concentration verification data from test solutions/media. Expand table to include each measured concentration data for each media type (i.e., water, tissue, cells).*

**General Notes:** *Provide any necessary detail regarding the measured concentrations, including any identified cause for substantial differences between nominal and measured concentrations, if samples were collected on separate days (and if so provide details), and any potential cross contamination.*

**Table A.II.2. Measured (and Nominal) Chemical Concentrations in Test Solutions/Media.**

[Analytical Method] verification of test and control concentrations during an [X]-day exposure of [test organism] to [test substance] under [static renewal/flow-through] conditions.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Nominal Concentration (units)** | **[Mean] Measured Concentration (units)** | **Number of Samples** | **Non-Detecta** | **Number of Samples Below Non-Detect** | **[Standard Deviation or Standard Error]** | **Range** |
| *Control* |  |  |  |  |  |  |  |
| [1] |  |  |  |  |  |  |  |
| [2] |  |  |  |  |  |  |  |
| [3] |  |  |  |  |  |  |  |
| [4] |  |  |  |  |  |  |  |
| [5] |  |  |  |  |  |  |  |
| [6] |  |  |  |  |  |  |  |
| *j* |  |  |  |  |  |  |  |

aNon-Detect: 0 = measured and detected; 1=measured and not detected; if not measured or reported enter as such

**Mortality**: *Briefly summarize mortality results (if any).*

**General Notes:** *Comment on concentrations response relations and slope of response if provided. Compare mortality with control treatment and/or the reference chemical.*

**Table A.II.3. Mean Percent [Mortality or Survival].**

Mean percent mortality or survival of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

|  |  |  |
| --- | --- | --- |
| **Treatment** | **[Mean % Mortality]** | **[Standard Deviation or Standard Error]** |
| *Control* |  |  |
| [1] |  |  |
| [2] |  |  |
| [3] |  |  |
| [4] |  |  |
| [5] |  |  |
| [6] |  |  |
| [LCx] |  |
| NOEC |  |
| LOEC |  |

 a Use superscript to identify the values reported to be significantly different from control.

**Growth**: *Briefly summarize growth results (if any).*

**General Notes:** *Comment on concentrations response relations and slope of response if provided. Compare growth endpoints with control treatment and/or the reference chemical.*

**Table A.II.4. Mean [Growth].**

Mean growth [e.g., length and/or weight, chlorophyll*a* concentration] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Mean Growth** **[Length/Weight]****(units)** | **[Standard Deviation or Standard Error]** | **Mean Percent Change in [Length/ Biomass]** | **[Standard Deviation or Standard Error]** |
| *Control* |  |  |  |  |
| [1] |  |  |  |  |
| [2] |  |  |  |  |
| [3] |  |  |  |  |
| [4] |  |  |  |  |
| [5] |  |  |  |  |
| [6] |  |  |  |  |
| *j* |  |  |  |  |
| [ECx] |  |  |
| NOEC |  |  |
| LOEC |  |  |

a Use superscript to identify the values reported to be significantly different from control.

**Reproduction**: *Briefly summarize reproduction endpoint results (if any). For multi-generational studies, copy and paste* Table A.II.5 *below for each generation with reproductive effects data.*

**General Notes:** *Comment on concentrations response relations and slope of response if provided. Compare reproduction endpoints with control treatment and/or the reference chemical.*

**Table A.II.5. Mean [Reproductive] Effect.**

Mean [reproductive] effects for [generation] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment****(units)** | **[Mean Reproductive Effect]** | **[Standard Deviation or Standard Error]** | **[Mean Reproductive Effect]** | **[Standard Deviation or Standard Error]** |
| *Control* |  |  |  |  |
| [1] |  |  |  |  |
| [2] |  |  |  |  |
| [3] |  |  |  |  |
| [4] |  |  |  |  |
| [5] |  |  |  |  |
| [6] |  |  |  |  |
| *j* |  |  |  |  |
| [ECx] |  |  |
| NOEC |  |  |
| LOEC |  |  |

a Use superscript to identify the values reported to be significantly different from control.

**Sublethal Toxicity Endpoints**: *Include other sublethal effect(s), including unusual colors or shapes, or other signs of toxicity, if any. Copy Table A.II.6 as needed to provide details for each sublethal effect observed.*

**General Notes:** *Briefly summarize observed sublethal effects otherwise not captured in the results table(s) below.*

**Table A.II.6. Mean [Sublethal] Effect.**

*Mean [*Sublethal effect*, (e.g., morphological changes, etc.)]* in *[test organism]* during [test duration (*acute/chronic*)] exposure to *[test substance]* under *[static/renewal/flow-through]* conditions.

|  |  |  |
| --- | --- | --- |
| **Treatment** | **[Mean Sublethal Response]****(units)** | **[Standard Deviation or Standard Error]** |
| *Control* |  |  |
| [1] |  |  |
| [2] |  |  |
| [3] |  |  |
| [4] |  |  |
| [5] |  |  |
| [6] |  |  |
| *j* |  |  |
| [ECx] |  |
| NOEC |  |
| LOEC |  |

a Use superscript to identify the values reported to be significantly different from control

**Reported Statistics**: *List and briefly summarize the statistical tests that were performed for each of the response parameters that were analyzed (or, copy and paste statistical section from publication).*

***Part B: Detailed Review***

**I. Materials and Methods**

PROTOCOL/GUIDANCE FOLLOWED: *If indicated by authors, provide protocol that was followed (e.g., U.S. EPA, ASTM, OECD, Environment Canada, European Union, etc.).*

DEVIATIONS FROM PROTOCOL: *If authors report any deviations from the protocol noted above indicate here.*

**Study Design and Methods:** *Copy and paste methods section from publication.*

TEST ORGANISM: *Provide information in details and any relevant or related information or clarifications in remarks.*

| **Parameter** | **Details** | **Remarks** |
| --- | --- | --- |
| **Species:**Useful sites include:* <https://www.itis.gov/>
* <https://www.fws.gov/endangered/>
* <https://www.fisheries.noaa.gov/find-species>
 | Common or Group Name: Scientific Name: |

|  |  |
| --- | --- |
| North American species?  |  |
| Surrogate for North American Taxon? |  |
| FIFRA 5 Species?Is this species Threatened or Endangered? | \_\_\_\_ |
| *(Place X if applicable)* |  |

 |
| **Strain/Source:*** Obtained from laboratory culture or commercial source. [1]
	+ Identification of clone is desirable [1]
* Obtained from unpolluted areas in the wild
	+ If collected from the field, plants should be maintained in culture in the same medium as used for testing for a minimum of eight weeks prior to use. [2]
* *Oryza sativa* (rice) are obtained as seeds and can be kept in a cool area for one year [5]
* Must originate from same source and population [4, 5]
* Should not be used:
	+ If contaminated by other organisms such as algae and protozoa [2]
	+ If visible lesions or discoloration (chlorosis) [2]
	+ If large number of plants with single fronds [2]
 |  |  |
| **Age of inoculum at Study Initiation:*** EPA recommends 7-12 day old cultures for *Lemma spp.* [1]
* *Oryza sativa* (rice) approximately 8-10 cm tall [5]
 |  |  |
| **Was growth recorded at regular intervals?** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Yes |  |  | No |

*If yes, describe regular intervals:* |  |

**STUDY PARAMETERS:** *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for both Controlled Experiments and Field Studies/Observations.*

| *For Both Controlled Experiments and Field Observations* | **Parameter** | **Details** | **Remarks** |
| --- | --- | --- | --- |
| **Number of Replicates per Treatment Group:*** EPA recommends 4 replicates for *Lemna* test [1]
* ASTM: 5 replicates for rice and other macrophytes [5]
 | Control(s): |  |
| Treatment(s): |  |
| **Plants per Replicate/ Treatment Group:*** EPA recommends 3-5 plants/replicate for *Lemna* test [1]
 | Control(s): |  |
| Treatment(s): |  |
| Fronds per Plant (e.g., *Lemna spp.*)* EPA recommends 3-4 fronds/plant for *Lemna* test [1]
 | Control(s): |  |
| Treatment(s): |  |
| **Exposure Pathway:***(i.e., water, sediment, or mixed). Note: all other pathways are unacceptable.* |  |  |
| **Exposure Duration:*** EPA recommends 7 days for *Lemna spp.* Test [1, 2]
* ASTM: 2 weeks for rice and other macrophytes [5]
 |  |  |
| **Observation Intervals:** * For *Lemna* test, EPA recommends every three days during the test and at test termination [1]
* Should be an appropriate number of observations over the exposure duration to establish the shape of the toxicity curve
* Should allow for mathematical/statistical determination of point estimates
 |  |  |
| **Test Concentrations (remember units):***Recommended test concentrations include at least three concentrations other than the control; four or more will provide a better statistical analysis.*  | Nominal:  |  |
| Measured:  |
| Media measured in: |
| **What analytic methods were used to measure test concentrations?** |  |  |
| **What was the recovery of the test material?** |  |  |
| **What was the reporting limit of the analytical method used to measure the test concentrations?** |  |  |
| **Were standards used as part of the analytical method?** |  |  |

**CONTROLLED EXPERIMENT STUDY PARAMETERS:** *Provide information under Details and any relevant information of deficiencies in Remarks. Complete for Controlled Experiments only.*

| *For Controlled Experiments Only* | **Parameter** | **Details** | **Remarks** |
| --- | --- | --- | --- |
| **Acclimation/Culturing:*** Test plants should be from cultures maintained at test conditions for an appropriate amount of time
	+ EPA recommends 8 weeks for *Lemna*. [1]
	+ Temperature change rate should not exceed 2°C during acclimation or testing [1]
* To avoid unnecessary stress and promote good health:
	+ Organisms should not be crowded [1]
	+ Temperature should be maintained at optimal test conditions for the test species, and temperature variation should not exceed 2°C during acclimation or testing (25 ±2°C for *Lemna*, 20-30°C for other macrophytes) [1, 5]
	+ Lighting should be maintained on a light:dark cycle and intensity at test conditions optimal for the test species.
		- Continuous light recommended for *Lemna* test (4,200-6,700 lux) [1]
		- Minimum of 16 hours light for other macrophytes (30-40 W/m2) [5]
		- Light intensity should be measured at test initiation for each culture vessel at the level of the culture solution. [1]
	+ pH of nutrient medium in which plant is cultured should be maintained at optimal conditions for the test species.
		- EPA recommends pH of 6.5 for *Lemna minor* tests, pH 7.5 for *Lemna gibba* tests. [1]
	+ Growth medium (growth chelators):
		- EPA recommends 20x-AAP growth medium for *L. gibba*, and modified Swedish Standard (SIS) growth medium for *L. minor.* [1]
		- OECD recommends pH buffer addition for test substances where pH stability is important. [2]
		- OECD recommends less than 0.001 mmol/L chelator (if used). [2]
		- EPA Guidelines note acceptable provided concentrations not excessive for test chemicals subject to interferences by the chelator (e.g., > 200 µg/L EDTA for metals) [7]
		- Were details provided if non-standard growth medium used (yes/no)?
 | Duration: | *Identify number of individuals excluded from testing and/or analysis (if any):* |
| Standard Nutrient Medium Used:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*If no, provide details of composition of the nutrient medium under the remarks section* |
| Water type: |
| Temperature (°C):  |
| Light:dark cycle: |
| Salinity (for marine plants, ppt): |
| Chelator used: |
| Carbon source: |
| Dissolved Oxygen (mg/L): |
| Health (*any mortality, abnormalities observed?*): |
| **Acclimation followed published guidance?***Describe, if any* |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*If yes, indicate which guidance:* |  |
| **Test Type:** |

|  |  |
| --- | --- |
|  | Acute |
|  | Partial Life Cycle |
|  | Chronic |
|  | Germination |
|  | Other *(please remark):*  |

 |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Test Vessel:*** Test chambers should be loosely covered [1]
	+ Test vessels/covers should not create shadows or otherwise affect light levels. [1]
* Test chamber material:
	+ Should minimize sorption of test chemical from water [1]
	+ Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [1]
	+ May not contain substances that inhibit the growth of test organisms. [1]
* Test chamber type:
	+ Erlenmeyer flasks, crystallizing dishes, glass petri plates, or other container can be suitable.
		- Sizes between 250-1,000 mL are suggested. All vessels should be the same size. [1]
		- Vessels for *Lemna* tests should be > 20 mm depth and > 100 mL volume. [1]
		- Should be wide enough so that fronds in control vessel do not overlap (*Lemna* tests). [1]
	+ Plastic pots with drainage holes in the bottom are used for culturing and exposing other macrophytes. [5]
* Size/volume should maintain acceptable biomass loading rates (see below)
 | Material:  | *Briefly describe the test vessel here* |
| Size:  |
| Fill Volume:  |
| **Test Solution Delivery System/Method:** |  Static Renewal; Interval: Flow-through: Delivered to water or sediment? Test concentrations measured? |  |
| **Sediment Used (For Rooted Plants):*** Origin (e.g., natural, artificial, field collected, reference)
* Textural Classification (% sand, silt, clay)
* Organic Carbon (%)
* Geographic Location
* Chemical quality confirmed?
 |  |  |
| **Source of Dilution Water:*** Freshwater hardness range should be <5 mg/L or <10% of the average (whichever is greater) [4]
* Saltwater salinity range should be <2 g/kg or <20% of the average (whichever is greater) [4]
* Dilution water must be characterized (natural surface water, well water, etc.) [8]
	+ Distilled/deionized water without the addition of appropriate salts should not be used. [7]
* Dilution water in which total organic carbon or particulate matter exceed 5 mg/L should not be used [7]
	+ Unless data show that organic carbon or particulate matter do not affect toxicity. [7]
 |  |  |
| **Dilution Series** (*e.g., 0.5x, 0.6x, etc.*):* 0.667x or 0.5x is recommended. [1]
* <0.25x not recommended. [1]
 |  |  |
| **Water Pretreatment** |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| **Intervals of water quality measurement:** |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Dilution Water Parameters:***Measured at the beginning of the experiment or averaged over the duration of the experiment (details of water quality parameters measured in test solutions should be included under the results section)*Recommendations:* pH
	+ 6.5 for *L. minor* tests and 7.5 for *L. gibba* tests. [1]
	+ Recommend measuring at beginning and end of test. [1]
* Temperature
	+ EPA and OECD recommend 24-25 ºC (±2 ºC) for *Lemna spp.* tests
* Can be measured in growth medium of extra chambers during test.
* ASTM recommends 20-30°C for other macrophytes [5]
 | Dissolved Oxygen (mg/L): |  |
| Temperature (°C): |
| Light:dark cycle: |
| pH (test initiation): |
| pH (test termination): |
| Hardness (mg/L as CaCO3): |
| Salinity (for marine plants, ppt): |
| Total Organic Carbon (mg/L):  |
| Dissolved Organic Carbon (mg/L): |
| Chelator used: |
| Carbon source: |
| **Aeration or Agitation:** (Describe if yes)* Aeration not recommended unless appropriate for the test substance [7]
 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| **Describe Preparation of Test Concentrations**:(Indicate how test material was added to the growth medium (e.g., added directly or used stock solution) |  |  |
| **Test Chemical Solubility in Water:**List units and conditions (e.g., 0.01% at 20ºC) |  |  |
| **Were concentrations in water or nutrient medium verified by chemical analysis?***Measured test concentrations should be reported in* Table A.II.2 *above.* |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*Indicate media:* |  |
| **Were test concentrations verified by chemical analysis in tissue?***Measured test concentrations can be verified in test organism tissue alone if a dose-response relationship is observed.**Measured test concentrations should be reported in* Table A.II.2 *above.* |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*Indicate tissue type:* | *If test concentrations were verified in test organism tissue, was a dose-response relationship observed?* |
| **Were stability and homogeneity of test material in water/nutrient medium determined?** |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Solvent/Vehicle Type**:* When used, a carrier solvent should be kept to a minimum concentration. [4]
* Should not affect either survival or growth of test organisms (e.g., N, N-dimethyl-formamide recommended for *Lemma* spp. instead of acetone). [1]
* Should be reagent grade or better [4]
* Should not exceed 0.1 ml/L, unless it was shown that higher concentrations do not affect toxicity [1]
 |  |  |
| **Negative Control:** |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| **Reference Toxicant Testing:** |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*If Yes, identify substance:* |  |
| **Other Control:** *If any (e.g. solvent control)* |  |  |
| **Biomass Loading Rate:** |  |  |
| **Feeding:*** Nutrient medium added during renewal tests?
 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| **Lighting:*** Lighting conditions should be consistent with conditions during culturing/acclimation [1, 2]
* Should be measured in the growth chamber at the same distance from light source as test plants [1]
* Should be measured before, after, and at least once during test [1]
* Day:night period should be appropriate to the test species (e.g., continuous lighting recommended for *Lemna spp.*) [1]
* Fluorescent lighting recommended [1, 2]
* OECD recommends 6,500-10,000 lux (85-125 µE/m2/s) for *Lemna spp.* test. [2]
* EPA recommends 4,200-6,700 lux (57-90 µE/m2/s) for *Lemna spp.* test. [1]
* Should not change by more than 15% during test [2]
	+ - Measure daily if suspected
 |  |  |

**Study Design/Methods Classification:** *(Place X by One Based on Overall Use)*

***Provide details of Major or Minor Deficiencies/Concerns with Study Design in Associated Sections of Part A: Overview***

*This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A.*

|  |  |
| --- | --- |
|  | Study Design Acceptable for Quantitative Use |
|  | Study Design Acceptable for Qualitative Use |
|  | Study Design Not Acceptable for Use |

**Additional Notes:** *Provide additional considerations for the classification of study use based on the study design.*

**OBSERVATIONS:** *Provide information under Details and any relevant information in Remarks. This information should be consistent with the Results Section in Part A.*

| **Parameter** | **Details** | **Remarks** |
| --- | --- | --- |
| **Parameters measured including sublethal effects/toxicity symptoms:****Growth*** ECx, ICx based on growth inhibition (e.g., biomass, frond reduction, etc.) relative to control. [1, 5]

Reproduction* Seed germination, seedling production, etc. [5]

**Other Endpoints*** + Chlorophyll a, pigment content, etc. [5]
 | *List parameters:* |  |
| **Was control growth acceptable?*** Change in frond number, area, size (e.g., *Lemna*)?
* How was acceptable control growth determined?
 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| **Were controls acceptable?*** Relative increase in frond number or size (e.g., *Lemna*) [1]
* Growth rate, etc.
 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*If yes, describe justification provided:* |  |
| **Were individuals excluded from the analysis?** |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*If yes, describe justification provided:* |  |
| **Additional observations*** Unusual colors [1]
* Differences in chloroplast morphology [1]
* Changes in test solution appearance (e.g., clarity, films, precipitates, etc.) [1]
* Flocculation, clumping, adhering to test containers [1]
* Was crowding observed? [1]
 |  |  |
| **Was water quality in test chambers acceptable?*** If appropriate, describe any water quality issues

 (e.g., EPA and OECD recommend temperature of  24-25 ºC (±2 ºC) for *Lemna spp.* tests) [1, 2] |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| **Availability of concentration-response data:** |  |  |
| * Were treatment level concentration-response data included? (specify endpoints in remarks)
 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| * Were replicate level concentration-response data included? (specify endpoints in remarks)
 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

 |  |
| * How was concentration-response data presented? (check all that apply)
 |

|  |  |
| --- | --- |
|  | Tables |
|  | Graphs |
|  | Supplemental Files |

 |  |
| * Were concentration-response data estimated from graphs study publication or supplemental materials?
 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Yes |  | No |

*If yes, indicate software used:* |  |
| * Should additional concentration-response data be requested from study authors?
 |  Yes NoRequested by:Request date:Date additional data received: |  |
| *If concentration-response data are available, complete* ***Verification of Statistical Results (Part C)***. |  |  |

***Part C: Statistical Verification of Results***

**I. Statistical Verification Information:** *Report the statistical methods (e.g., R, EPA TRAP, BMDS, other) used to verify the reported study or test results for the five (5) most sensitive genera and sensitive apical endpoints (including for tests where such estimates were not provided). If values for the LC50, LT50 and NOEC are greater than the highest test concentration, use the “>” symbol.*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Primary Reviewer:** |  | **Date:** |  |  |  | **EPA** |  | **Contractor** | (*Place X by One*) |
| **Secondary Reviewer:** |  | **Date:** |  |  |  | **EPA** |  | **Contractor** | (*Place X by One*) |
| (*At least one reviewer should be from EPA for sensitive taxa*) |

**Endpoint(s) Verified:**

**Additional Calculated Endpoint(s):**

**Statistical Method (e.g., TRAP, BMDS, R, other):**

**Fitted Model:**

**II. Toxicity Values:** *Include confidence intervals (CI) if applicable. 95% CI unless otherwise noted.*

|  |  |
| --- | --- |
| **NOEC:**  |  |
| **LOEC:**  |  |
| **MATC:**  |  |
|  |  |
| **EC5:**  |  |
| **EC10:**  |  |
| **EC20:**  |  |
| **EC50 or LC50:**  |  |

**Dose-Response Curve Classification:** *(Place X by One)*

*This classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A*

|  |  |
| --- | --- |
|  | Dose-Response Curve Acceptable for Quantitative Use |
|  | Dose-Response Curve Acceptable for Qualitative Use |
|  | Dose-Response Curve Not Acceptable for Use |

**Summary of Statistical Verification:** *Provide summary of methods used in statistical verification.*

**Additional Notes:**

**Attachments:**

1. *Provide attachments to ensure all data used in Part C are captured, whether from study results reported in the publication and/or from additional data requested from study authors*
	* *Data from study results of the publication should be reported in Results section of Part A*
	* *Additional data provided upon request from study authors should be reported in Table C.II.1 below and original correspondence with study authors should be included as attachments*
2. *Model assessment output (including all model figures, tables, and fit metrics)*
3. *Statistical code used for curve fitting*

**Additional Data Used in Response-Curve**: *Provide all data used to fit dose-response curve not captured in Results section of DER above in Part A, rows as needed. First row in italicized text is an example.*

**Table C.II.1 Additional Da****ta Used in Dose-Response Curve.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Curve ID** | **Species** | **Endpoint** | **Treatment** | **Replicate** | **[Standard Deviation or Standard Error]** | **# of Survivors** | **Na** | **ka** | **na** | **Response** | **Response Unit** | **Conc** | **Conc units** |
| *Alchronic1* | *Ceriodaphnia dubia* | *# of young/female* | *0* | *6* |  |  | *10* | *10* | *1* | *18* | *count* | *0.03* | *mg/L* |
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a N = number of individuals per treatment; k = number of replicates per treatment level; n = number of individuals per replicate

**III. Attachments:** *Include model assessment output (including all model figures, tables, and fit metrics) here*

***Part D: References to Test Guidance***

1. U.S. EPA. 2012. OCSPP 850.4400: Aquatic plant toxicity test using *Lemna spp*. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-008. January 2012.
2. OECD. 2002. Test No. 221. *Lemna sp*. Growth Inhibition Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. 22 pp.
3. U.S. EPA. 2016. OCSPP 850.1000: Background and special consideration-tests with aquatic and sediment-dwelling fauna and aquatic microcosms. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-014. October 2016.
4. ASTM Standard E 739, 1980. 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA.
5. ASTM Standard E 1841-04. 2012. Standard Guide for Conducting Renewal Phytotoxicity Tests with Freshwater Emergent Macrophytes. ASTM International, West Conshohocken, PA. 10 pp.
6. U.S. EPA. 2012. OCSPP 850.4600: Rhizobium-legume toxicity. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-004. January 2012.
7. Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses. PB85-227049. National Technical Information Service, Springfield, VA.
8. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.