**Attachment G**

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

**March 2024**

***Part A: Overview***

**I. Test Information**

**Chemical name:**

CAS name: CAS Number:

Purity: Storage conditions:

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., Levy, J.L., J.L. Stauber, and D.F. Jolley. 2007. Sensitivity of marine microalgae to copper: The effect of biotic factors on copper adsorption and toxicity. Sci. Total Environ. 387(1–3):141-154)

**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 | | |
|  | Bioaccumulation: steady state not reached | | |
|  | Review paper or previously published without modification | | |
|  | Excessive EDTA or similar complexing agent | | |
|  | Other: *(if any, list here, e.g. use of distilled water)* | | |

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

***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)**

DESCRIPTION OF UNMEASURED TEST CONCENTRATIONS: *Describe concerns with unmeasured test concentrations and the influence of the study classification.*

DESCRIPTION OF CONCERNS WITH DILUTION WATER: *Describe concerns with characterization of and/or deficiencies with dilution water (e.g., uncharacterized stream or lake water, potential presence of unknown containments, high organic content, extreme hardness, pH, etc).*

***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 for the most sensitive endpoint (apical and/or non-apical) and modify as needed. If study is classified as “Not Acceptable for Use” DO NOT complete summary tables.*

Acute:

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species**  **(age of inoculum)** | **Methoda** | **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 Concentrationb (mg/L)** | **Classification** |
|  |  |  |  |  |  |  |  |  |  |  | Quantitative / Qualitative |

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

b Verification following completion of Part C of the DER

Chronic:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species**  **(age of inoculum)** | **Methoda** | **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 Valueb (mg/L)** | **Chronic Value Endpoint** | **Classification** |
|  |  |  |  |  |  |  |  |  |  |  |  | Quantitative / Qualitative |

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

b Verification following completion of Part C of the DER

**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/Tissues**

[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. Superscript(s) used to identify the values reported to be significantly different from control as p value of [0.05/ or any other provided by authors].

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

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

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

**General Notes:** *Compare response on growth (such as cell density, biomass in dry weight, and growth rate) with control treatment and/or the reference chemical. Also indicate if exponential growth in the control was observed.*

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

Mean growth [e.g., cell density, chlorophyll*a* concentration, length and/or weight] of [test organism] exposed to [test substance] for [test duration] under [static/renewal/flow-through] conditions. Superscript(s) used to identify the values reported to be significantly different from control as p value of [0.05/ or any other provided by authors].

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **[Mean Cell Density]** | **Sample Size** | **[Standard Deviation or Standard Error]** | **[Mean Percent Change in Biomass]** | **Sample Size** | **[Standard Deviation or Standard Error]** |
| *Control* |  |  |  |  |  |  |
| [1] |  |  |  |  |  |  |
| [2] |  |  |  |  |  |  |
| [3] |  |  |  |  |  |  |
| [4] |  |  |  |  |  |  |
| [5] |  |  |  |  |  |  |
| [6] |  |  |  |  |  |  |
| *j* |  |  |  |  |  |  |
| [ECx] |  | | |  | | |
| NOEC |  | | |  | | |
| LOEC |  | | |  | | |

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

**Reproductive**: *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. Superscript(s) used to identify the values reported to be significantly different from control as p value of [0.05/ or any other provided by authors].

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment**  **(units)** | **[Mean Number of Spores]** | **Sample Size** | **[Standard Deviation or Standard Error]** | **[Mean Number of Cystocarps]** | **Sample Size** | **[Standard Deviation or Standard Error]** |
| *Control* |  |  |  |  |  |  |
| [1] |  |  |  |  |  |  |
| [2] |  |  |  |  |  |  |
| [3] |  |  |  |  |  |  |
| [4] |  |  |  |  |  |  |
| [5] |  |  |  |  |  |  |
| [6] |  |  |  |  |  |  |
| *j* |  |  |  |  |  |  |
| [ECx] |  | | |  | | |
| NOEC |  | | |  | | |
| LOEC |  | | |  | | |

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

**Sublethal Toxicity Endpoints**: *Include other sublethal effect(s), including unusual cell shape, color differences, flocculation, adherence of algae to test vessels, aggregation of algal cells, precipitation in the test solution, 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., developmental abnormalities, loss of color, morphological changes, necrosis, and/or floccing.)]* in *[test organism]* during [test duration (*acute/chronic*)] exposure to *[test substance]* under *[static/renewal/flow-through]* conditions. Superscript(s) used to identify the values reported to be significantly different from control as p value of [0.05/ or any other provided by authors].

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

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

**Reported Statistics**:*Copy and paste statistical section from publication.*

***Part B: Detailed Review***

**I. Materials and Methods**

**Protocol/Guidance Followed:** *Indicate if provided by authors.*

**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 under 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 Grouping Name:  Scientific Name:  Order Name:  Family 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 or culture collection   + Specify clone if identified [1] * Obtained from unpolluted areas in the wild   + Quarantine for at least 14 days or until they are disease free, before acclimation [4] * Must originate from same source and population [4] * Should not be used:   + If mortality observed [1]   + If unusual shapes [1]   + If color differences [1]   + If there are differences in chloroplast morphology [1]   + If clumping or flocculation [1]   + If used in a previous test, including as a control [1] |  |  |
| **Age of stock culture at Study Initiation (microalgae):**   * Generally 3-7 days old recommended (when logarithmic growth is occurring) [1] * 1985 Algal acute toxicity test (5-10 days) [5] |  |  |
| **Was growth (**e.g., cell density, chlorophyll concentration, length and/or weight) **recorded at test initiation?** | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| **Was growth (**e.g., cell density, chlorophyll concentration, length and/or weight) **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:** | Control(s): |  |
| Treatment(s): |  |
| **Cell Density or Biomass per Replicate/ Treatment Group:** | Control(s): |  |
| Treatment(s): |  |
| **Exposure Pathway:**  *(i.e., water, sediment, or mix).* |  |  |
| **Exposure Duration:**   * Static algal tests are generally 72-120 hours * OECD 201: recommends 72 hours for algal growth inhibition test [6] * 48 hour exposure (followed by 5-7 day development period for reproduction tests (e.g., *C. parvula* test) [7] * Algistatic tests are generally 13-14 days (4-5 day exposure plus up to 9 day recovery period) [1] |  |  |
| **Observation Intervals:**   * Generally microalgal enumeration recommended daily if possible using indirect methods [1]   Water quality (e.g., temperature, pH, light) should be monitored throughout the test. Additional vessels can be used for some measurements to avoid disturbing test vessels [1] |  |  |
| **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:**   * Should be incubated under test conditions [1] * Should be used when still growing exponentially [1]   + Water should be changed gradually to 100% dilution water (usually 2 or more days) [4]   + 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 [1]   + Lighting should be maintained on a light:dark cycle and at an intensity optimal for the test species [1]     - Light intensity should be measured for each culture vessel at the level of the culture solution [1]   + Stock algal cultures should be shaken to prevent clumping (at least daily) [1]   + pH of nutrient medium in which algae is cultured should be maintained at optimal conditions for the test species [1]     - Should not be adjusted after adding test organism [1]   + Growth medium chelators:     - Not to be used if suspected to interact with test chemical [1]     - Recommended growth media:       * OECD TG 201: 0.0027 mM EDTA [6]       * EPA AAP: 0.00081 mM EDTA [6]     - Acceptable provided concentrations are not excessive for test chemicals subject to interferences by the chelator (e.g., > 200 µg/L EDTA for metals) [8] | 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 algae, 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 | |  | Spore Germination | |  | Other *(please remark):* | |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Test Vessel:**   * Test chambers should be covered [1]   + Acceptable covers include foam plugs, stainless steel, glass, or plastic screw caps [1]   + Covers that contact test solution should 1) minimize sorption of test chemical from water; 2) not contain substances that will leach or interact with test solution or affect results [1] * Test chamber material:   + Should minimize sorption of test chemical from water [1,9]   + Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical   + May not contain substances that inhibit the growth of test organisms [1]   + Erlenmeyer flasks or culturing apparatus are recommended for growth / growth inhibition tests [1]     - Sizes between 125-500 mL are suggested. All vessels should be the same size [1]     - Test solution volume should not exceed 50% of test chamber volume [1]   + Erlenmeyer flasks or polystyrene cups are acceptable for algal reproduction tests [7]     - Test chamber size and solution volume should be appropriate for the species tested [7] * Size/volume should maintain acceptable cell density | Material: | *Briefly describe the test vessel here* |
| Size: |
| Fill Volume: |
| **Test Solution Delivery System/Method:** | Test Concentrations Measured   |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No |   Test Solution Delivery System:   |  |  | | --- | --- | |  | Static | |  | Renewal | |  | *Indicate Interval:* | |  |  | |  | Flow-through | |  | *Indicate Type of Diluter:* | |  |  | |  |
| **Dilution Water Source & Characteristics:**   * 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.) [10]   + Distilled/deionized water without the addition of appropriate salts should not be used [8] * Dilution water in which total organic carbon or particulate matter exceed 5 mg/L should not be used [8]   + Unless data show that organic carbon or particulate matter do not affect toxicity [8] |  |  |
| **Dilution Series** (*e.g., 0.5x, 0.6x, etc.*):   * 0.667x or 0.5x recommended [2] * < 0.25x not recommended [2] |  |  |
| **Water Pretreatment** | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Intervals of water quality measurement:** |  |  |
| **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   + ~ 7.5 for most freshwater algal species [1]   + ~8 for *Skeletonema* spp. [1]   + Recommend measuring at start and end of test [1] * Temperature   + 24-25 ºC for most species [1]   + 20ºCfor *Skeletonema* spp. [1]   + 22-24 ºC for *Champia parvula* reproduction test [7] * Salinity   + 28-32 ‰ for *C. parvula* reproduction test [7] | Dissolved Oxygen (mg/L): |  |
| Temperature (°C): |
| Light:dark cycle: |
| pH (test initiation): |
| pH (test termination): |
| Hardness (mg/L as CaCO3): |
| Salinity (for marine algae, ppt): |
| Total Organic Carbon (mg/L): |
| Dissolved Organic Carbon (mg/L): |
| Chelator Used: |
| Carbon source: |
| **Aeration or Agitation:**   * Aeration not recommended unless appropriate for the test substance * *Raphidocelis* (formerly *Selanastrum*) and *Skeletonema* should be shaken during test [2] * *C. parvula* should be shaken or swirled 2x/day [7] | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| **Describe Preparation of Test Concentrations**: |  |  |
| **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 | |  |
| **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 [4] * Should be reagent grade or better [4] * Should not exceed 0.5 ml/L (static) or 0.1 ml/L (flow through) unless it was shown that higher concentrations do not affect toxicity [USEPA Guidelines Addendum - 10] * Should not exceed 0.1 mL/L [OCSPP - 1,3] * Solvent concentration as low as 0.02 mL/L recommended [3] * Examples of preferred solvents include dimethylformamide, triethylene glycol, methanol, acetone, and ethanol [3]. |  |  |
| **Negative Control:** | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| **Reference Toxicant Testing:** | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No |   *If Yes, identify substance:* |  |
| **Other Control:** *If any (e.g. solvent control)* |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Initial Cell Density:**   * Recommended: ~1 x 104 cells/mL for *R. subcapita* (formerly *S. capricornatum*), *N. pelliculosa*, *A. flos-aquae*; and 7.7 x 104 cells/mL for *S. costatum* [2] * Recommended ~5 x 103 - 104 cells/mL for *R. subcapita* , 2-5 x 103 *S. subspicatus*, 5 x 104 – 105 *S. leopoldensis*, and comparable biomass for other species [6] * Biomass density should not exceed 0.5 mg/L dry weight [6] * This maximum number would have to be determined for the species, test duration, temperature, flow rate, test solution volume, chamber size, food, feeding regime, etc. |  |  |
| **Feeding:**   * + - Nutrient medium added during renewal tests? | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| **Lighting:**   * Continuous lighting for *Raphidocelis, Navicula, Anabaena* [2] * 14:10 light:dark for *Skeletonema* [2] * Fluorescent lights recommended [2] * ~4.3 K lx (4,306 lm/m2) for *Raphidocelis, Navicula, and Skeletonema* [2] * ~2.2 K lx for *Anabaena* [2] * Light should have PAR of ~60-70 µE/m2/s [2] * Lighting conditions should be consistent with conditions during culturing/acclimation [2] |  |  |

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

***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.*

**Clarifying Questions for Study Authors and the Other Pertinent Information/Notes from Discussion:** *Provide clarifying questions for study authors.*

**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:**  **Common Apical Parameters Include:**  **Growth**   * ECx, ICx based on growth inhibition relative to control [1] * Algicidal or algistatic [1]   **Other Endpoints**   * Chlorophyll *a*, etc. [1] | *List parameters:* |  |
| **Was control cell density or biomass acceptable?**   * Did controls reach logarithmic growth by 96 hr? * How was logarithmic growth determined? | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| **Were individuals excluded from the analysis?** | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No |   *If yes, describe justification provided:* |  |
| **Was test chemical algicidal or algistatic?**   * What method was used to make this determination?   + Evans stain, reincubation of subculture, etc. | Algicidal  Algistatic |  |
| **Additional observations**   * Changes in cell sizes or shapes (deformations) [1] * Unusual colors [1] * Differences in chloroplast morphology [1] * Flocculation, clumping, adhering to test containers [1] |  |  |
| **Was water quality in test chambers acceptable?**   * If appropriate, describe any water quality issues | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| **Availability of concentration-response data:** |  |  |
| * Were treatment level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)?   *specify endpoints in remarks* | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| * Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)?   *specify endpoints in remarks* | |  |  |  |  | | --- | --- | --- | --- | |  | Yes |  | No | |  |
| * If treatment and/or replicate level concentration-response data were included, how was 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 |  | No |   *If yes, requested by:*  *Request date:*  *Date additional data received::* |  |
| *If concentration-response data are available, complete* ***Verification of Statistical Results (Part C)*** *for sensitive species*. |  |  |

***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.*

|  |  |  |  |  |  |  |  |  |  |
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| **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*

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|  | 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*

**III.** **Attachments:** *Include all attachments listed above after the table below.*

**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 850.4500: Algal toxicity. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-006. January 2012.
2. U.S. EPA. 1996. OPPTS 850.5400 algal toxicity, tiers I and II. Ecological effects test guidelines. Prevention, Pesticides and Toxic Substances. EPA 712-C-96-164. April 1996.
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. U.S. EPA. 2002. 40 CFR 797.1050 - Algal acute toxicity test. Source: 50 FR 39321, Sept. 27, 1985, as amended at 52 FR 19058, May 20, 1987. July 1, 2002 Edition. pp. 101:105.
6. OECD. 2011. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, <https://doi.org/10.1787/9789264069923-en>.
7. ASTM Standard E 1498, 1992. 2012. Standard guide for conducting sexual reproduction tests with seaweeds. ASTM International, West Conshohocken, PA.
8. 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.
9. Boudreau, T.M., Sibley, P.K., Mabury, S.A., Muir, D.G.C., and Solomon, K.R. 2003. Laboratory Evaluation of the Toxicity of Perfluorooctane Sulfonate (PFOS) on *Selenastrum capricornutum*, *Chlorella vulgaris*, *Lemna gibba*, *Daphnia magna*, and *Daphnia pulicaria*. Archives of Environmental Contamination and Toxicology. 44: 307-313.
10. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.