**Attachment F**

**Aquatic Invertebrate 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., Keller, A.E and S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. Environ. Toxicol. Chem. 10(4): 539-546.)

**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:**

|  |  |
| --- | --- |
|  | 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 (> 10% for acute and > 20% for chronic) |
|  | Bioaccumulation: steady state not reached |
|  | Dermal or Injection Exposure Pathway |
|  | Review paper or previously published without modification |
|  | 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 (lifestage)** | **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, Diet=dietary, MT=maternal transfer

b Verification following completion of Part C of the DER

Chronic:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species (lifestage)** | **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 or µg/g)** | **Verified Chronic Valueb(mg/L or µg/g)** | **Chronic ValueEndpoint** | **Classification** |
|  |  |  |  |  |  |  |  |  |  |  |  | Quantitative / Qualitative  |

a S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved, Diet=dietary, MT=maternal transfer

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 and include data not provided in* Table A.II.1*.*

**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** | **Sample Size** | **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., muscle, liver, blood, etc.).*

**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 number of immobilized] 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:** *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 [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 Growth****[Length/Weight]****(units)** | **Sample Size** | **[Standard Deviation or Standard Error]** | **Mean Percent Change in [Length/ 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 Spawns]** | **Sample Size** | **[Standard Deviation or Standard Error]** | **[Mean Number of Eggs]** | **Sample Size** | **[Standard Deviation or Standard Error]** | **[Mean Number of Offspring]** | **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 behavioral abnormalities 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., behavioral abnormalities, etc.)]* 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 Name: Scientific Name:Order Name:Family Name: |

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

 |
| **Strain/Source:*** If wild caught, organisms should be from unpolluted areas from the same source/population [9]
	+ Quarantine for at least 7 days or until they are disease free, before acclimation [9]
	+ Wild caught Gammarids should be quarantined at least 14 days [2]
	+ Wild caught shrimp should be quarantined at least 10 days [5]
* Daphnids should be cultured at the test facility [1,7]
	+ Should originate from same culture population [1,7]
* Should not be used:
	+ If appeared stressed, such as discoloration or unusual behavior [9]
	+ If more than 5% die during the 48 hours before test initiation [1,2,4,7,9]
	+ If they were used in previous test treatments or controls [1,2,4,10]
	+ If culture contains ephippa, adults do not produce at least 3 offspring/day in 7 days before test, or animals are first brood progeny (Daphnids) [1,7]
	+ If adult brood stock (bivalves) were injured during handling, exhibit abnormal shell development, or underwent unplanned spawning [6]
	+ If >5% culture or brood stock (*C. virginica*) dies or shows signs of stress [3]
* No treatments of diseases may be administered:
	+ Within 16 hours of field collection [9]
	+ Within 10 days of testing or during testing [9]
 |  |  |
| **Age at Study Initiation:****Acute:*** Larval stages preferred [9]
* Mayflies and Stoneflies
	+ Early instar [9]
* Daphnids/cladocerans:
	+ < 24-hr old [1,7,9]
* Midges:
	+ 2nd or 3rd instar larva [9]
* Gammarid amphipods
	+ <24-hr post release [2]
* *Hyalella azteca* (chronic exposure)
	+ Generally, 7 - 8 days old [11]
* Freshwater mussels (chronic exposure)
	+ Generally, 2 month old juveniles [12]
* Oysters (C. virginica)
	+ 30-50mm valve height and as similar in size as possible (<20% coefficient of variation) [3]
* Mysids
	+ < 24-hr post release [4,9]
* Penaeid shrimp
	+ Post-larval juveniles [5]
 |  |  |
| **Was body weight or length recorded at test initiation and/or at regular intervals?** |

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

 |  |
| **Was body weight or length 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:*** At least 2 replicates/treatment recommended for most tests [1-6,9,14]
* 4 replicates/treatment recommended for *C. virginica* acute limit test [3]
* 4 replicates/treatment (flow-through) or 10 replicates/treatment (renewal) recommended for Daphnia chronic tests [7]
 | Control(s): |  |
| Treatment(s): |  |
| **Number of Organisms per Replicate/ Treatment Group:*** Unless otherwise specified, at least 10 organisms/replicate recommended for most tests [1-6,9]
* 8 organisms/replicate recommended for *C. virginica* limit test [3]
* 15-30 embryos/replicate for Bivalves [6]
* 5 organisms/replicate (flow-through) or 1 organism/replicate (renewal) recommended for Daphnia chronic tests [7]
 | Control(s): |  |
| Treatment(s): |  |
| **Exposure Pathway:***(i.e., water, sediment, or diet). Note: all other pathways (e.g., dermal, injection) are unacceptable.* |  |  |
| **Exposure Duration:****Acute*** Cladocerans and midges should be 48 hours [1.10]
	+ Longer durations acceptable if test species not fed and had acceptable controls [10]
* Freshwater mussel glochidia should be a maximum of 24 hours [12]
	+ Shorter durations (6, 12, 18 hours) acceptable so long as 90% survival of control animals achieved (see below) [12]
* Embryo/larva (bivalve mollusks, sea urchins, lobsters, crabs, shrimp and abalones) should be 96 hours, but at least 48 hours [6,10]
* Other invertebrate species should be 96 hours [2-5,2]

**Chronic*** Daphnids/cladocerans should be 21 days (3-brood test) [7,10]
	+ Exception 7 days acceptable for *Ceriodaphnia dubia* [10]
* Freshwater juvenile mussels should be at least 28 days [12]
* *Hyalella azteca* should be at least 42 days
	+ Beginning with 7 - 8 day old animals [11]
* Mysids should continue until 7 days past the median time of first brood release in the controls [12]
 |

|  |  |
| --- | --- |
|  | Acute |
|  | Chronic |
|  | Other *(please remark):*  |

 |  |

|  |  |  |  |
| --- | --- | --- | --- |
| *For Both Controlled Experiments and Field Observations* | **Observation Intervals:** Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [9] |  |  |
| **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. Certain specific test protocols require more (e.g., [1-6] require five concentration plus a control)* | 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/Holding:*** Should be placed in a tank along with the water in which they were transported [9]
	+ Water should be changed gradually to 100% dilution water (usually 2 or more days) [5,9]
	+ For wild-caught animals, test water temperature should be within 5°C of collection water temperature [9]
	+ Temperature change rate should not exceed 3°C within 72 hours [9]
* Test specific recommendations:
	+ Mysids: should be maintained in dilution water 48 hours before test, with < 1ºC/day variation in water temperature [4]
	+ Gammarids: should be acclimated to dilution water conditions over 48 hours, then held an additional 7 days [2]
	+ Bivalves: Brood adults should be held for at least 14 days in test dilution water prior to spawning [6]
	+ *C. virginia*: on arrival at test facility, brush shells clean of fouling organisms [3]
		- Acclimate to dilution water and hold for 10-12 days, until demonstrated they are not stressed or diseased [3]
		- Maintain in dilution water at test temperature for 48 hours before test [3]
* To avoid unnecessary stress and promote good health:
	+ Organisms should not be crowded [9]
		- See “Biomass/Loading Rate” for guidance on holding densities
	+ Water temperature variation should be limited
	+ Dissolved oxygen:
		- Maintain between 60 - 100% saturation [9]
		- Continuous gentle aeration if needed [9]
	+ Unionized ammonia concentration in holding and acclimation waters should be < 35 µg/L [9]
	+ Mortality during the week preceding the test (following a 48 hour adjustment period) must be < 10%, or the batch should be rejected [2,3,5,6]
	+ If between 5-10%, holding should be extended an additional 7 days [2,3,5,6]
 | Duration: | *Identify number of individuals excluded from testing and/or analysis (if any):*  |
| Feeding: |
| Water: |
| Temperature (°C):  |
| Dissolved Oxygen (mg/L): |
| Health (*any mortality observed?*):  |
| **Acclimation followed published guidance?***Describe, if any* |

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

*If yes, indicate which guidance:* |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Test Vessel:*** Test chambers should be loosely covered [9]
* Test chamber material:
	+ Should minimize sorption of test chemical from water [9]
	+ Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [9]
	+ Glass, No. 316 stainless steel, nylon screen and perfluorocarbon (e.g. Teflon) are acceptable [8,9]
		- Other materials recommended for specific chemicals should be used when appropriate (e.g., polyethylene for PFAS chemicals [13]
	+ Rubber, copper, brass, galvanized metal, epoxy glues, lead and flexible tubing should not come into contact with test solution, dilution water or stock [8,9]
		- Stainless steel should not be used for saltwater tests [8]
* Size/volume should maintain acceptable biomass loading rates (see below) [9]
* Substrate:
	+ Required for some species (e.g., *Hyalella azteca*) [11]
	+ Common types: stainless steel screen, nylon screen, quartz sand, cotton gauze and maple leaves [11]
	+ More inert substances preferred over plant material, since plants may break down during testing and promote bacterial growth [11]
	+ Consideration should be given between substrate and toxicant [11]
		- Hydrophobic organic compounds in particular can bind strongly to Nitex® screen, reducing exposure concentrations, especially for studies using static or intermittent renewal exposure methods [11]
	+ Examples:
		- Acid-washed sand, free of excess organic matter, 2-3 cm depth (Penaeid shrimp) [5]
		- Bent piece of stainless steel screen (Gammarid amphipods) [2]
 | Material:  | *Briefly describe the test vessel here* |
| Size:  |
| Fill Volume:  |
| Substrate Used *(if applicable)*: |
| **Test Solution Delivery System/Method:*** Flow-through preferred for some highly volatile, hydrolyzable or degradable materials [10]
	+ Concentrations should be measured often enough using acceptable analytical methods [10]
* Acute *C. virginica* shell deposition test must be flow through [3]
* Chronic exposures:
	+ Flow-through, measured tests required [10]
	+ Exception: renewal is acceptable for Cladocerans [7,10,14]
 | Test Concentrations Measured

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

Test Solution Delivery System:

|  |  |
| --- | --- |
|  | Static |
|  | Renewal |
|  |  *Indicate Interval:*  |
|  |  |
|  | Flow-through |
|  |  *Indicate Type of Diluter:*  |
|  |  |

 |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Dilution Water Source & Characteristics:*** Dilution water must be characterized (natural surface water, well water, etc.) [10]
	+ Clean surface water, ground water, reconstituted water, or natural or artificial seawater (for saltwater species) are acceptable [1-7]
	+ Dechlorinated tap water should not be used as some forms of chlorination difficult to adequately remove [1,2,7,8]
	+ Distilled/deionized water without the addition of appropriate salts should not be used [10]
* Freshwater hardness range should be < 5 mg/L or < 10% of the average (whichever is greater) [9]
	+ Recommended hardness <250 mg/L (preferably <180 mg/L); or 40-50 mg/L for metals [1,2,7]
	+ Unless study is examining effects of hardness on toxicity.
* Saltwater salinity range should be < 2 g/kg or < 20% of the average (whichever is greater) [9]
	+ Recommended salinity 20±2 ‰ [3-6]
		- For *C. virginica* test with unfiltered seawater, recommended salinity >12±5‰ [3]
	+ Unless study is examining effects of salinity on toxicity.
* Dilution water in which total organic carbon (TOC) > 2 mg/L [OCSPP Guidance – 1-7] should not be used (U.S. EPA Guidelines recommends limit of >5 mg/L – 2)
	+ Unless data show that TOC or particulate matter do not affect toxicity [10], or the study is examining effects of TOC on toxicity.
* Dissolved oxygen in dilution water at start of test recommended to be 90-100% of saturation [1-7]
* pH should be between 6-8.5 for freshwater species and 7.5-8.5 for saltwater species and should vary by less than 1 pH unit during the test and between concentrations [1-7]
* Dilution water for tests with *Hyalella azteca*
	+ Reconstituted waters should have at least 0.02 mg bromide/L; natural ground or surface water presumed to have sufficient bromide [11]
	+ Recommended that control/dilution waters have chloride concentrations at or above 15 mg/L [11]
 |  |  |
| **Dilution Series** (*e.g., 0.5x, 0.6x, etc.*): |  |  |
| **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)* | Dissolved Oxygen (mg/L): |  |
| pH:  |
| Temperature (°C):  |
| Hardness (mg/L as CaCO3): |
| Salinity (ppt): |
| Total Organic Carbon (mg/L):  |
| Dissolved Organic Carbon (mg/L): |
| **Aeration:*** Strongly discouraged unless dissolved oxygen (D.O.) in danger of falling below 60% [1-7,9]
	+ Preferably performed prior to addition of test substance [1-7]
* Avoid aeration when testing highly oxidizable, reducible and volatile materials
* Assurances should be made to prevent stress on test organisms [1-7,9]
* Aeration should be the same in all test chambers at all times [1-7,9]
 |

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

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|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Describe Preparation of Test Concentrations (e.g., water exposure, diet):** |  |  |
| **Test Chemical Solubility in Water:*** List units and conditions (e.g., 0.01% at 20ºC)
 |  |  |
| **Were concentrations in water or diet 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 (e.g., blood, liver, muscle) 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/diet determined?** |

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

 |  |
| **Was test material regurgitated/avoided?** |

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

 |  |
| **Solvent/Vehicle Type**:* When used, a carrier solvent should be kept to a minimum concentration [1]
* Should be restricted to situations where no other acceptable method of media preparation is available [8]
* Should not affect either survival or growth of test organisms [9]
* Should be reagent grade or better [9]
* 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 [14]
* Should not exceed 0.1 mL/L [1-8]
	+ Solvent concentration as low as 0.02 mL/L recommended [1-8]
* Examples of preferred solvents include dimethylformamide, triethylene glycol, methanol, acetone, and ethanol [8].
 |  |  |
| **Test Temperature*** Start between 18-22 ºC. Maintain ±1ºC of starting temperature throughout test [1]
* 18ºC recommended [2]
* 20 ºC recommended [3]
* 25 ºC recommended [4]
* 23 ºC recommended [5]
* Between 16-25ºC depending on test species [see ref. 6]
* Should be 20±2ºC [7]
 |  |  |
| **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* | **Biomass Loading Rate:*** Loading should be limited so as not to affect test results. Loading will vary depending on temperature, type of test (static vs. flow-through), species, food/feeding regime, chamber size, test solution volume, etc. [9]
* 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.
* Loading should be sufficiently low to ensure:
	+ Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [9,15]
	+ Unionized ammonia does not exceed 35 µg/L [9]
	+ Uptake by test organisms does not lower test material concentration by > 20% [9]
	+ Growth of organisms is not reduced by crowding
* Generally, at the end of the test, the loading (grams of organisms; wet weight; blotted dry) in each test chamber should not exceed the following:
	+ Static or renewal tests: > 0.8 g/L (lower temperatures); > 0.5 g/L (higher temperatures) [9]
	+ >1 Daphnid / 20 mL [1]
	+ >1 Daphnid / 40 mL for chronic renewal tests [7]
	+ >30 Mysids / L [4]
	+ >30 bivalve embryos/mL [6]
	+ >0.8 g/L [2,5]
	+ Flow through tests: > 1 g/L/day or > 10 g/L at any time (lower temperatures); > 0.5 g/L/day or > 5 g/L at any time (higher temperatures) [9]
	+ > 0.5 g/L/day or > 5 g/L [1,2,4,5,7]
	+ C. virginica loading based on flow rate adequate to promote shell growth [3]
		- Flow rate of 1 L/hr/individual using unfiltered natural seawater [3]
	+ When loading based on temperature, lower temperatures are defined as the lower of 17˚C or the optimal test temperature for that species. [9]
 |  |  |
| **Feeding:*** Unacceptable for acute tests [1,2,5,6,10]
	+ Exceptions:
		- Data indicate that the food did not affect the toxicity of the test material [10]
		- Test organisms will be severely stressed if they are unfed for 96 hours [10]
			* Mysids should be fed daily during testing (same diet as during culturing and acclimation) [4]
			* C. virginica should be fed during testing [3]
		- Test material is very soluble and does not sorb or complex readily (e.g., ammonia) [10]
 |

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

 |  |
| **Lighting:*** Generally, ambient laboratory levels (540-1080 lux or 50 - 100 foot candles) or natural lighting should be acceptable, with a constant photoperiod between 12 light:12 dark and 16 light: 8 dark [1-7]
* Artificial light cycles should have a 15 - 30 minute transition period to avoid stress due to rapid increases in light intensity [1-7,9]
 |  |  |

**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:****Acute*** Daphnids/cladocerans:
	+ EC50 based on percentage of organisms immobilized plus percentage of organisms killed [10]
* Embryo/larva (bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones):
	+ EC50 based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed [10]
		- If available, the 96 hour EC50 based on the percentage of organisms with incompletely developed shells and the 96-hr LC50 should also be reported separately [2]
* Freshwater mussel (glochidia and juveniles):
	+ Glochidia: EC50 based on 100 x number closed glochidia after adding NaCl solution - number closed glochidia before adding NaCl solution) / Total number open and closed glochidia after adding NaCl solution [12]
	+ Juvenile: EC50 based on percentage exhibiting foot movement within a 5-min observation period [12]
* All other species and older life stages:
	+ EC50 based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed [10]
		- If not available, the 96 hour LC50 should be used [10]

**Chronic*** Daphnid:
	+ Survival and young per female [10]
* Mysids:
	+ Survival, growth and young per female [10]
 | *List parameters:* |  |
| **Were controls acceptable?****Acute*** > 90% control survival at test termination [10]
	+ Glochidia 90% after 24 hours, or, the next longest duration less than 24 hours that had at least 90% survival [12]
	+ >70% normal oyster embryos, or >60% normal hard clam or mussel larvae at the end of the test [6]
	+ >90% surviving and free of disease or stress with an overall mean new shell growth of 2mm for *C. virginica* [3]

**Chronic*** > 80% control survival at test termination [10]
	+ >80% in 42 day test with *Hyalella azteca*, slightly lower in tests substantially longer than 42 days [11]
	+ > 80% surviving and free of disease or stress that did not produce ephippia and that produced at least 60 offspring in 21 days in Daphnid tests [7]
 |

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

Control survival (%): |  |

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Details** | **Remarks** |
| **Were individuals excluded from the analysis?** |

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

*If yes, describe justification provided:* |  |
| **Was water quality in test chambers acceptable?*** If appropriate, describe any water quality issues

(e.g., dissolved oxygen level below 60% of saturation) |

|  |  |  |  |
| --- | --- | --- | --- |
|  | 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 |

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.*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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):**

**II. Toxicity Values:** *Include confidence intervals if applicable*

**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 is 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

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

1. U.S. EPA. 2016a. OCSPP 850.1010: Aquatic invertebrate acute toxicity test, freshwater Daphnids. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-013. October 2016.
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14. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
15. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.