**Attachment I**

**Amphibian 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., Fort, D.J., E.L. Stover, J.A. Bantle, J.N. Dumont and R.A. Finch. 2001. Evaluation of a reproductive toxicity assay using *Xenopus laevis*: boric acid, cadmium and ethylene glycol monomethyl ether. J. Appl. Toxicol. 2: 41-52.)

**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 (> 10% for acute and > 20% for chronic) |
|  | Diet not adequately characterized |  | 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)** | **Exposure****Methoda** | **Test duration** | **Chemical / Purity** | **pH** | **Temp.(°C)** | **Hardness(mg/L as CaCO3) or Salinity (ppt)** | **DOC(mg/L)** | **Relative Humidity** | **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, FETAX=Frog Embryo Teratogenesis Assay-Xenopus

b Verification following completion of Part C of the DER

Chronic:

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species****(lifestage)** | **Exposure****Methoda** | **Test duration** | **Chemical / Purity** | **pH** | **Temp.(°C)** | **Hardness(mg/L as CaCO3)****or Salinity (ppt)** | **DOC(mg/L)** | **Relative Humidity** | **Chronic Limits** | **Reported Chronic Value****(mg/Lor 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, FETAX=Frog Embryo Teratogenesis Assay-Xenopus

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., 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 [Biomass]** | **Sample Size** | **[Standard Deviation or Standard Error]** | **Mean Time to [Developmental Stageb]** | **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.

**b** Developmental staging can be general (e.g., larval, metamorphosis, etc.) or it can be specific. Xenopus are staged using the Nieuwkoop and Faber (1994) system, anurans are staged using the Gosner (1960) system, and salamanders are staged using the Harrison (1969) system.

Nieuwkoop, P.D. and J. Faber. 1994. Normal table of *Xenopus laevis* (Daudin). Garland Publishing Inc, New York.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16(3): 183-190.

Harrison R. 1969. Harrison stages and description of the normal development of the spotted salamanders, *Ambystoma punctatum* (Limm.). Pages 44-66 in Harrison R, ed. Organization and Development of the Embryo. New Haven, CT: Yale University Press.

**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 Eggs]** | **Sample Size** | **[Standard Deviation or Standard Error]** | **[Mean Percent Hatch]** | **Sample Size** | **[Standard Deviation or Standard Error]** | **[Mean Number of Larva/Metamorphosed]** | **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 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 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:*** Wild caught from unpolluted areas [2]
	+ Quarantine for at least 14 days or until they are disease free, before acclimation [2]
* Must originate from same source and population [2]
* Should not be used:
	+ If appeared stressed, such as discoloration or unusual behavior [2]
		- Should avoid crowding or rapid changes in temperature or water quality to avoid stress [3]
	+ If more than 5% die during the 48 hours before test initiation [2]
	+ If they were used in previous test treatments or controls [4]
* No treatments of diseases may be administered:
	+ Within 16-hr of field collection [2]
	+ Within 10 days or testing or during testing [2]
 |  |  |
| **Age at Study Initiation:****Acute:*** Young larvae should be used whenever possible [2]

**FETAX:*** (*Xenopus* *laevis*)- embryos (cysteine-treated to remove jelly coat) [5]

**Chronic:*** Partial life-cycle test:
	+ Immature juveniles at least 2 months prior to active gonad development [4]
* *Xenopus* LAGDA test: newly spawned embryos (Nieuwkoop and Faber (NF) stage 8-10), also cysteine-treated to remove jelly coat [6]
* *Xenopus* AMA test: NF stage 51 [3]
 |

|  |  |
| --- | --- |
|  | Embryonic |
|  | Larval |
|  | Juvenile |
|  | Adult |

*Specify stage if provided:* |  |
| **Was body weight or length recorded at test initiation?***For field observations, was body weight measured in a consistent manner (e.g., during blood sample collection) detailed in methods?* |

|  |  |  |  |
| --- | --- | --- | --- |
|  | 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:*** FETAX: recommends 2 replicates per test concentration and 4 replicates for controls [5]
* LAGDA: recommends 4 replicates per test concentration and 8 replicated for controls [6]
* AMA: recommends at least 4 replicates per treatment/control [3]
* At least 2 replicates/treatment recommended for chronic tests [2]
* At least 2 replicates/treatment recommended for chronic tests [7]
 | Control(s): |  |
| Treatment(s): |  |
| **Number of Organisms per Replicate/ Treatment Group:*** Unless otherwise specified, at least 10 organisms/treatment recommended [7]
* FETAX: 20 or 25 (*X. laevis* embryos) per replicate [5]
* LAGDA: recommends 20 animals (*X. laevis* embryos)/tank (replicate) at exposure initiation and 10 animals (juveniles)/tank (replicate) after NF stage 66 to exposure termination [6]
* AMA: 20 (*X. laevis* embryos) per replicate at test initiation. 5 indiv/replicate randomly removed after 7d for growth & development measurements [3]
 | Control(s):

|  |  |
| --- | --- |
| Male: |  |
| Female: |  |

 |  |
| Treatment(s):

|  |  |
| --- | --- |
| Male: |  |
| Female: |  |

 |  |
| **Exposure Pathway:***(i.e., water, sediment, or diet). Note: all other pathways (e.g., dermal, injection) are unacceptable.* |  |  |
| **Exposure Duration:****Acute*** Should be 96 hours [4]

**FETAX*** Must be 96 hours [5]

**Chronic*** Partial life-cycle tests:
	+ Begin with embryos or newly hatched tadpoles, continue through completed metamorphosis
* Larval growth and development assay (LAGDA): from NF stage 8-10 to ten weeks after the median time to NF stage 62 in water and/or solvent control group (maximum 17 weeks) [6]
* Amphibian metamorphosis assay (AMA): 21-day exposure beginning with NF stage 51 tadpoles. Final NF stage is one of the measured endpoints [3]
 |

|  |  |
| --- | --- |
|  | Acute |
|  | Partial Life Cycle |
|  | Larval Growth and Development Assay (LAGDA) |
|  | Amphibian Metamorphosis Assay (AMA) |
|  | Other *(please remark):*  |

 |  |
| **Observation Intervals:** * No specific guidance on number of observation intervals for changes in survival, deformities, behavior, etc. of study organisms during a test.

Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [2] |  |  |
| **Observations:**Parental:(*e.g., mortality, body weight, mean feed consumption*)Offspring:*(e.g., mortality, time to metamorphosis, snout-vent lent, external abnormalities)* |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| *For Both Controlled Experiments and Field Observations* | **Parameter** | **Details** | **Remarks** |
| **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/Holding:*** If aquatic phase, should be placed in a tank along with the water in which they were transported [2]

Water should be changed gradually to 100% dilution water (usually 2 or more days) [2]* + For wild-caught animals, test water temperature should be within 5˚C of collection water temperature [2]
	+ Temperature change rate should not exceed 3°C within 72 hours [2]
* To avoid unnecessary stress and promote good health:
	+ Organisms should not be crowded [2]
	+ Water temperature variation should be limited (e.g., <3°C in any 12 hour period) [2]
	+ Water dissolved oxygen:
		- Maintain between 60-100% saturation [2]
		- Continuous gentle aeration if needed [2]
	+ Un-ionized ammonia concentration in holding and acclimation waters should be <35 µg/L [2]
 | Duration: |  |
| Feeding: |
| Water type: |
| Temperature (°C):  |
| Dissolved Oxygen (mg/L): |
| Health (*any mortality observed?*):  |
| Number of individuals excluded from analysis:  |
| **Acclimation followed published guidance?***Describe, if any* |

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

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

|  |  |
| --- | --- |
|  | Acute |
|  | Partial Life Cycle |
|  | Larval Growth and Development Assay (LAGDA) |
|  | Amphibian Metamorphosis Assay (AMA) |
|  | Other *(please remark):*  |

 |  |
| **Test Vessel/Enclosure Size:*** Test chambers should be loosely covered [2]
* Test chamber material:
	+ Should minimize sorption of test chemical from water [2]
	+ Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [2]
	+ Glass, No. 316 stainless steel, nylon screen and perfluorocarbon (e.g. Teflon) are acceptable [1,2]
		- Other materials recommended for specific chemicals should be used when appropriate (e.g., polyethylene for PFAS chemicals [8]
	+ Rubber, copper, brass, galvanized metal, epoxy glues, lead and flexible tubing should not come into contact with test solution, dilution water or stock [1,2]
* Size/volume should maintain acceptable biomass loading rates (see below) [2]
 | Material:  | *Briefly describe the test vessel here* |
| Size:  |
| Fill Volume:  |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *For Controlled Experiments Only* | **Test Solution Delivery System/Method:*** Flow-through preferred for some highly volatile, hydrolysable or degradable materials [4]
	+ Concentrations should be measured often enough using acceptable analytical methods [4]
* Chronic exposures:
	+ Flow-through, measured tests required [4]
* LAGDA: designed using a flow through system [6]
 | 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) [2]
* Saltwater salinity range should be <2 g/kg or <20% of the average (whichever is greater) [2]
* Dilution water must be characterized (natural surface water, well water, etc.) [4]
	+ Distilled/deionized water without the addition of appropriate salts should not be used [4]
* Dilution water in which total organic carbon or particulate matter exceed 5 mg/L should not be used [4]
	+ Unless data show that organic carbon or particulate matter do not affect toxicity [4]
* FETAX: FETAX solution preferred [5]
* LAGDA: any water that permists normal growth and development of *X. laevis* (e.g., spring water or charcoal filtered tap water) [6]
 |  |  |
| **Dilution Series** (*e.g., 0.5x, 0.6x, etc.*): |  |  |
| **Test Conditions/****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)** FETAX: 24 ± 2°C recommended [5]
* LAGDA: 21 ± 1°C recommended [6]
* FETAX: pH should be between 6.5 and 9.0 [5]
* LAGDA: pH should be between 6.5 and 8.5 [6]
* LAGDA: D.O. should be ≥40% of air saturation [6]
 | Dissolved Oxygen (mg/L): |  |
| pH:  |
| Temperature (°C):  |
| Relative Humidity: |
| Hardness (mg/L as CaCO3): |
| Salinity (ppt): |
| Total Organic Carbon (mg/L):  |
| Dissolved Organic Carbon (mg/L): |
| **Aeration:*** Acceptable to maintain dissolved oxygen at 60-100% saturation at all times [2]
* Avoid aeration when testing highly oxidizable, reducible and volatile materials
* Turbulence should be minimized to prevent stress on test organisms and/or re-suspend fecal matter [2]
* Aeration should be the same in all test chambers at all times [2]
 |

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

 |  |
| **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)* |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **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 |

 |  |
| **Test Chemical Solubility in Water:*** List units and conditions (e.g., 0.01% at 20ºC)
 |  |  |
| **Solvent/Vehicle Type**:* When used, a carrier solvent should be kept to a minimum concentration [2]
	+ Should be restricted to situations where no other acceptable method of media preparation is available [1]
* Should not affect either survival or growth of test organisms [2]
* Should be reagent grade or better [2]
* 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 - 7]
* Should not exceed 0.1 mL/L [OCSPP - 1]
	+ Solvent concentration as low as 0.02 mL/L recommended [1]
* Examples of preferred solvents include dimethylformamide, triethylene glycol, methanol, acetone, and ethanol [1]
 |  |  |
| **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 [2]
* Loading will vary depending on temperature, type of test (static vs. flow-through), species, food/feeding regime, chamber size, test solution volume, etc.
* This maximum number would have to be determined for the species, temperature, flow rate or test solution volume, chamber size, food, feeding regime, etc.
* For all species, loading should be sufficiently low to ensure:
	+ Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [2,9]
	+ Unionized ammonia does not exceed 35 µg/L
	+ Uptake by test organisms does not lower test material concentration by >20% [2]
	+ 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 tests: >0.8 g/L (lower temperatures); >0.5 g/L (higher temperatures) [2]
	+ 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) [2]
	+ Lower temperatures are defined as the lower of 17˚C or the optimal test temperature for that species [2]
 |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Parameter** | **Details** | **Remarks** |
| *For Controlled Experiments Only* | **Feeding:*** Unacceptable for acute tests [4]
	+ Exceptions:
		- Data indicate that the food did not affect the toxicity of the test material [4]
		- Test organisms will be severely stressed if they are unfed for 96 hours [4]
		- Test material is very soluble and does not sorb or complex readily (e.g., ammonia) [4]
 |

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

*Describe diet as provided:* |  |
| **Lighting:*** No specific requirements for lighting
	+ Embryos should be incubated under dim incandescent lighting (≤20 fc) or total darkness during early life-stage toxicity testing
	+ Embryos must not be subjected to prolonged exposure to direct sunlight, fluorescent lighting, or high intensity incandescent lighting
* Generally, ambient laboratory levels (540-1080 lux or 50-100 foot candles) or natural lighting should be acceptable, as well as a diurnal cycle consisting of 50% daylight or other natural seasonal diurnal cycle;
* Artificial light cycles should have a 15-30 minute transition period to avoid stress due to rapid increases in light intensity [2]
* Depends on the type of test (acute or chronic) and endpoint (e.g., reproduction) of interest.
* LAGDA: recommends fluorescent bulbs (wide spectrum), 600-2000 lux (lumens/m2) at the water surface and photoperiod of 12 h light:12 h dark [6]
 |  |  |

**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*** EC50 based on percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus percentage of organisms killed
	+ If not available, the 96-hr LC50 should be used [4]

**FETAX** * Mortality, malformation, and growth inhibition [5]

**Chronic*** Partial Life-cycle test:
	+ Survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability, and hatchability [4]
* LAGDA: Mortality (and abnormal appearances), time to NF stage 62, growth (weight and length) [6]
* AMA: mortality, hind limb length, snout to vent length, developmental stage, wet weight, thyroid histology [3]
 | *List parameters:* |  |
| **Egg Collection Interval:** |  |  |
| **Egg Storage Conditions:** | Temperature: |  |
| Relative Humidity: |
| **Was control survival acceptable?****Acute*** >90% control survival at test termination [4]

**Chronic*** >80% control survival at test termination [4]
 |

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

Control survival (%): |  |
| **Were individuals excluded from the analysis?** |

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

*If yes, describe justification provided:* |  |
| **Were exposure conditions or water quality in test chambers acceptable?*** If appropriate, describe any water quality issues

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

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

 |  |

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Details** | **Remarks** |
| **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):**

**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*

**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. 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.
2. ASTM Standard E 729, 1980. 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA.
3. OECD 407. 2008. T*est No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, <https://doi.org/10.1787/9789264070684-en>.
4. 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.
5. National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). 2000. Frog embryo teratogenesis assay – *Xenopus* (FETAX). Background Review Document. National Institute of Environmental Health Sciences (NIEHS). Research Triangle Park, NC, 273 pp.
6. OECD 241.2015. The larval amphibian growth and development assay (LAGDA). OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, <https://doi.org/10.1787/9789264242340-en>.
7. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
8. 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.
9. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.