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Standard Operating Procedures for Systematic Review of Ecological Toxicity Data in Support of Ambient Water Quality Criteria, Benchmark and Screening Value Derivation for Aquatic Life and Aquatic Dependent Wildlife

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United States Environmental Protection Agency
Office of Water
Office of Science and Technology
Health and Ecological Criteria Division
Ecological Risk Assessment Branch

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1 Introduction

1.1 Purpose

The purpose of this Standard Operating Procedures (SOP) document is to provide information on the long-standing process used to assist the EPA Office of Water (OW) scientists and the EPA contractors in systematic review of the quality of ecological effects studies in the development of numeric Ambient Water Quality Criteria (AWQC) for the protection of aquatic life and aquatic-dependent wildlife. In addition, EPA is making these materials publicly available to offer information to criteria developers outside of the EPA, including states and authorized Tribes, that could consider conducting systematic review of ecological effects data following OW's process described in this SOP. This document is only intended to be informative and descriptive and does not provide or make recommendations about what other entities should do to conduct systematic review in the development of AWQC. This document supports the screening, review, documentation, and use of data from various sources and can assist criteria developers in determining if and in what manner (e.g., quantitatively or qualitatively) a study could be used in an effects assessment to support development of aquatic life criteria and other protective values (e.g., aquatic life benchmarks and screening values). The SOP is generally consistent with evaluation guidelines developed by the EPA Office of Chemical Safety and Pollution Prevention (OCSPP) (e.g., U.S. EPA 2011) to support a common approach to data evaluation for chemicals.

1.2 Background

One of the objectives of the Clean Water Act (CWA) (33 U.S.C. Sections 1251-1387is to protect and restore the biological, chemical, and physical integrity of waters in the United States. Pursuant to CWA Section 304(a), the EPA is required to publish, and from time to time thereafter revise, criteria for water quality to accurately reflect the latest scientific knowledge. AWQC are levels of individual pollutants, water quality characteristics, or descriptions of conditions of a water body that, if met, are expected to protect the aquatic community. AWQC for the protection of aquatic life and aquatic-dependent wildlife, developed under Section 304(a), reflect current scientific knowledge and are based on data and scientific determinations of the relationship between concentrations of a pollutant and its effects on aquatic life. The EPA's national recommended AWQC are not rules, nor do they automatically become part of a state's water quality standards. The EPA develops recommended AWQC based on the best available science, a scientific literature review, established procedures for risk assessment, EPA policy, and external scientific peer review and public input.

States and authorized Tribes may adopt criteria that the EPA publishes under CWA Section 304(a), modify the CWA 304(a) criteria to reflect site-specific conditions, or adopt criteria based on other scientifically defensible methods (40 C.F.R. § 131.11(b)(1)). Under CWA Section 303 and the EPA's implementing regulations at 40 C.F.R. Part 131, the EPA must review and approve new or revised state water quality standards, including criteria. A state must adopt criteria that protect the designated use and are based on a sound scientific rationale (40 C.F.R. §

131.11(a)(1)).

Since 1985, the EPA's AWQC for the protection of aquatic life have been derived based on methods outlined in the EPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (hereafter referred to as "*Guidelines;*" US EPA 1985). The *Guidelines* have provided consistency and transparency in the derivation methodology of AWQC for pollutants. The *Guidelines* include detailed direction on acceptability of toxicity test results and minimum data requirements (see **Attachment A**) to ensure the quality and sufficiency of data used in the derivation of nationally recommended AWQC. This SOP was developed by synthesizing the principles in the 1985 *Guidelines* and other EPA data quality guidance to transparently and consistently document the systematic review of data used in the development of AWQC.

AWQC are based on an evaluation of available toxicological effects data for a pollutant for aquatic life and/or aquatic-dependent wildlife [taxa that depend on aquatic prey (e.g., fish and emergent aquatic insects) as their major food source]. Most ecological effects data for pollutants are obtained from the U.S. EPA ECOTOXicology Knowledgebase [1] (ECOTOX, Olker et al. 2022), maintained by the EPA's Office of Research and Development. Another important source of information used in the development of AWQC for pesticides is toxicity data acquired through U.S. EPA's Office of Pesticide Programs (OPP) (under the Federal Insecticide, Fungicide, and Rodenticide Act, 40 CFR Part 158). Applicable toxicity data from EPA researchers and sources external to the EPA, such as other federal agencies, academic researchers, and other international environmental regulatory authorities are also considered during the development of AWQC.

1.3 Organization of the Document

This document is divided into the following three sections that describe a systematic review approach:

- **Screening the Open Literature**: Discusses the general screening of papers and categorization of acceptability (*i.e.*, applicable, non-applicable, and "others").

- **Reviewing the Open Literature**: Provides technical support for reviewing studies that pass the screening process to assess the quality of the study and determine its usability in AWQC development.
- **Documenting the Open Literature**: Provides technical support for the efficient and consistent process for documenting reviews of the open literature and avoiding duplicative and possibly conflicting efforts associated with study reviews across reviewers.

[1] ECOTOX is a publicly available database that curates the ecological effects of single chemicals to aquatic and terrestrial plants and animals. For more information on ECOTOX literature review and data curation processes, database documentation, controlled vocabularies, and guides for users, see the overview in Olker et al. 2022 and visit EPA's ECOTOX website at https://cfpub.epa.gov/ecotox/.

The flowchart depicted in **Figure 1** summarizes the Systematic Review Process for open literature studies.

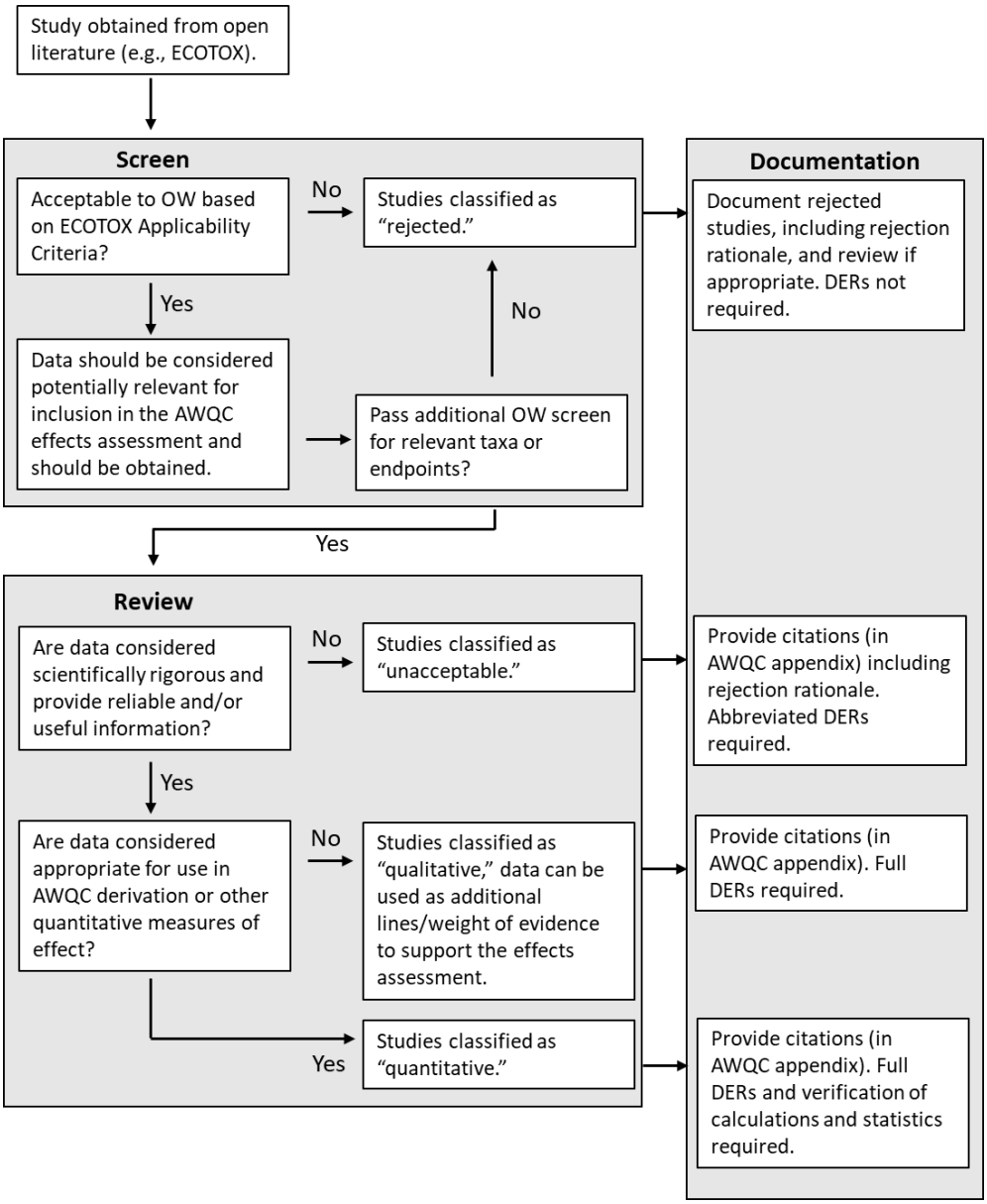

Figure 1. The EPA Office of Water's screening, review, and documentation steps in overarching systematic review process of open literature studies.
ECOTOX = US EPA Ecotoxicology Database; AWQC = Ambient Water Quality Criteria; DER = Data Evaluation Record.

2 Screening the Open Literature

The purpose of this section is to discuss the screening process applied to identify potentially useful open literature studies. OW uses ECOTOX as its primary search engine to obtain relevant acute and chronic toxicity information for AWQC development.

ECOTOX is a source for locating single chemical toxicity data for aquatic life, terrestrial plants, and wildlife. ECOTOX was created and is maintained by the U.S. EPA's Office of Research and Development/Center for Computational Toxicology and Exposure's Great Lakes Toxicology and Ecology Division, and has well-established systematic review and data curation processes used for both Program offices and database chemical searches procedures. The screening process begins with a comprehensive chemical-specific literature search of the open literature conducted according to ECOTOX Standard Operating Procedures. The search terms in ECOTOX are comprised of chemical terms, synonyms, degradates, and verified Chemical Abstracts Service (CAS) numbers. For example, the literature search terms for Phosphoric acid, triphenyl ester are as follows: *"Antioxidant TTP" OR "BRN 1888236" OR "Celluflex TPP" OR "DHPF 005" OR "Disflamoll TP" OR "NSC 57868" OR "O,O,O-Triphenyl phosphate" OR "Phenyl phosphate" OR "Phoscon FR 903N" OR "Phosflex TPP" OR "Phosphoric acid, triphenyl ester" OR "Phosphoric acid, triphenyl ester radical ion(1+)" OR "Reofos TPP" OR "Sumilizer TPP" OR "Triphenol phosphate" OR "Triphenoxyphosphine oxide" OR "Triphenyl phosphate" OR "Triphenylphosphat" OR "Triphenylphosphate" OR "UN 3077" OR "UNII-YZE19Z66EA" OR "Wako TPP" OR "WSFR-TPP"*. Once the references are generated, they are tagged with exclusion keywords (see **Figure 2** and **Attachment C**).

After developing the literature search strategy, the ECOTOX literature review process includes conducting the searches, identifying potentially applicable studies based on title and abstract, acquiring potentially applicable studies, applying the ECOTOX applicability criteria (see **Section 2.1**), and abstracting the data, with respect to the acceptability of the study report for inclusion in the knowledgebase, into ECOTOX (see **Figure 2**). Studies that meet the applicability criteria are coded to reflect information on the chemical, species, habitat, test location, exposure route, control type, endpoint and effect. At each step, search terms and results, screening decisions, and respective tags are recorded and stored electronically in the ECOTOX Knowledgebase for both applicable and non-applicable studies. This process is documented in U.S. EPA (2023) and described in Olker et al. (2022).

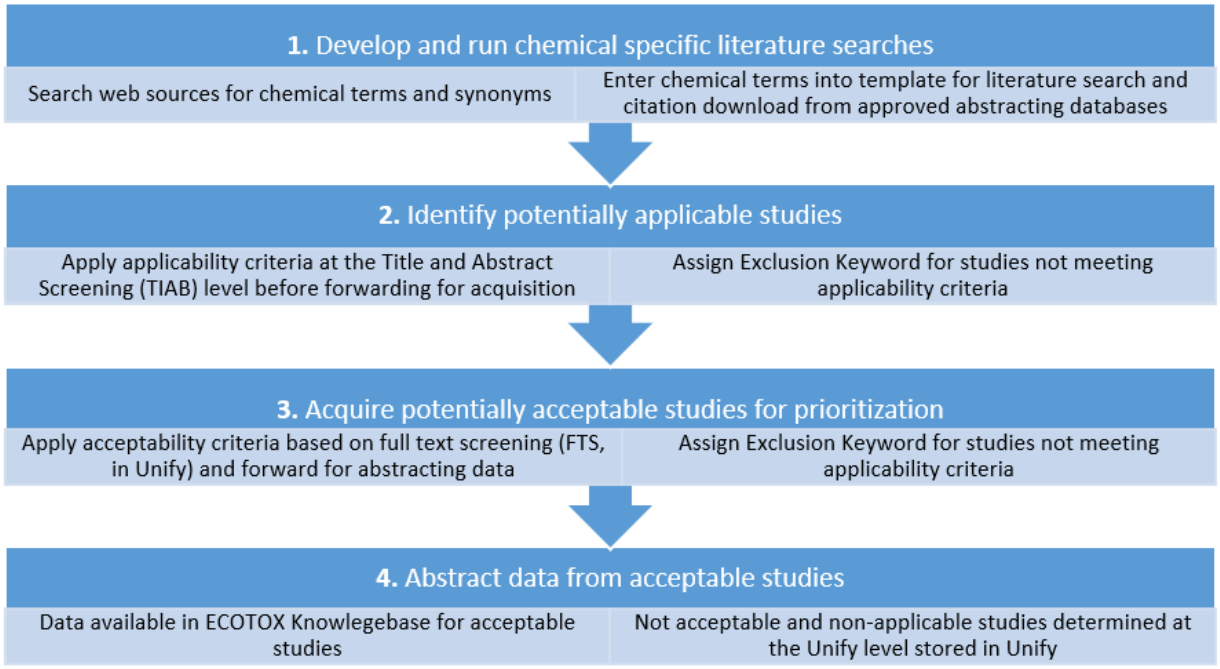

Figure 2. Overview of the literature searches, citation identification, and applicability criteria used by the ECOTOX Knowledgebase as described in U.S. EPA (2023).

Although this section of this SOP focuses on the evaluation of toxicological studies obtained through ECOTOX, many of the approaches applied to the evaluation of these studies are also applicable to the evaluation of studies from other sources. All papers identified in the ECOTOX database search as having data that are potentially relevant to AWQC derivation are obtained and screened for data quality. When requesting the data pull from ECOTOX, OW obtains all applicable studies (with pdfs), as well as the non-applicable studies (with reason for exclusion), and the studies in the "other" category. To ensure data quality and verifiability, these studies should meet certain applicability criteria described to be considered for use in the development of AWQC. Studies are assigned to one of three categories based on the applicability evaluation:

(1) Studies accepted by ECOTOX and OW (**Section 2.1**);
(2) Studies accepted by ECOTOX but not by OW (**Section 2.2**);
(3) Studies rejected by either ECOTOX or OW (**Section 2.3**); and
(4) "Other" studies (**Section 2.4**).

2.1 Studies Accepted by ECOTOX and OW

Traditionally, toxic effects of a chemical should be relevant to a whole organism (including aquatic animal, aquatic-dependent wildlife or aquatic plant species) to be considered for use in the development of AWQC. However, increasingly *in vitro* methods are used to assess toxicity for priority setting and screening assessments, and as quantitative linkages between these *in vitro* endpoints and apical endpoints of regulatory concern (survival, growth, and reproduction) are established, they may be considered for quantitative risk assessment. To be accepted by OW, regardless of whether the study is identified through the ECOTOX database or outside literature sources, papers should meet the following minimum criteria based on the ECOTOX applicability criteria:

(1) The toxic effects are related to single chemical exposure (unless the study is being considered as part of a mixture effects assessment);
(2) There is a biological effect on live, whole organisms or *in vitro* preparation including gene chips or omics data on adverse outcome pathways potentially of interest;
(3) Chemical test concentrations are reported;
(4) There is an explicit duration of exposure;
(5) Toxicology information that is relevant to OW is reported for the chemical of concern;
(6) The paper is published in the English language;
(7) The paper is available as a full article (not an abstract);
(8) The paper is publicly available;
(9) The paper is the primary source of the data;
(10) A calculated endpoint is reported or can be calculated using reported or available information;
(11) Treatment(s) are compared to an acceptable control;
(12) The location of the study (*e.g.*, laboratory vs. field) is reported; and
(13) The tested species is reported (with recognized nomenclature).

Attachment B discusses the ECOTOX and OW applicability criteria in detail, as well as the criteria for studies that are rejected from the ECOTOX database and thus tagged as non-applicable.

"Acceptable" studies from the open literature papers that pass the ECOTOX and OW screens of applicability should be considered potentially relevant for inclusion in the AWQC effects assessment and should be obtained for further review.

2.2 Studies Accepted by ECOTOX but not by OW

After studies from the open literature that pass the ECOTOX applicability criteria are obtained, an additional [title and abstract] scan of the "acceptable" studies should be conducted to identify

studies with taxa that are not relevant to AWQC (*e.g.*, strictly terrestrial species). If non-relevant studies are identified, the studies will follow the process outlined in **Section 2.3**. All remaining acceptable studies will be further reviewed. Additional discussion of the review of studies and documentation for use in AWQC development is provided in **Sections 3** and **4**, respectively.

2.3 Studies Rejected by either ECOTOX or OW

Open literature studies found not acceptable for ECOTOX or OW, based on the factors summarized in **Section 2.1** and **2.2**, are not typically used to derive AWQC; however, they may provide some useful information for AWQC development. Studies that do not meet the criteria for ECOTOX are designated as "non-applicable," and are given rejection keywords (see **Attachment C**), which can be cited to explain the reason for excluding a study from consideration. The EPA, may review these rejected studies, if appropriate, to determine whether the study includes information useful to the effects assessment or effects characterization. For example, studies may be rejected by ECOTOX because they include data on mixtures of chemicals. Studies that are coded with the rejection code "MIXTURE" during the initial evaluation may still be considered as a "line of evidence" during AWQC development. Other studies that are likely to be rejected by ECOTOX, but that may be useful to the EPA, as a "line of evidence" include those with data related to modeling, monitoring, incident reports, and review articles.

2.4 Consideration of Papers in the "Other" Category

In addition to providing citations for "acceptable" (*i.e.*, acceptable for ECOTOX and OW) and "rejected" (*i.e.*, not acceptable for ECOTOX or OW) studies, a file of citations called "Other" is provided to the EPA as part of the ECOTOX search. The "Other" category documents citations for studies into one of the following four categories:

(1) Target (for pesticides): toxicity of chemical on intended pest including efficacy studies;
(2) On Order: potentially acceptable but publication has not been received;
(3) To Abstract: applicable but not coded; and
(4) To Apply Criteria: potentially acceptable but not evaluated.

Papers included in the "Other" category are not coded into ECOTOX and do not appear as "applicable" data. Depending on the chemical, citations for "target" data are routinely included, whereas citations from the other three categories of "on order," "to abstract" and "to apply criteria" are encountered less frequently. In an effort to address the omission of information included in the "Other" category, all citations listed in the ECOTOX file name "Other" should be reviewed for potential inclusion in acceptable studies category.

3 Reviewing the Open Literature

All studies identified through ECOTOX or other relevant sources that are identified as potentially useful based on the screening process discussed in **Section 2** should be reviewed and classified as described in this section.

When reviewing ecotoxicity data, it is important to have a systematic method for reviewing and evaluating the quality of a study. This applies to all types of ecotoxicity studies that have the potential to be included in AWQC development (*e.g.*, single-species toxicity data, multispecies laboratory studies, mesocosm studies, and field tests). Information that should be considered when evaluating the test data quality includes:

- species and test properties (*e.g*., age, weight, lifestage)
- test compound and dosing (nominal and measured doses)
- dosing methodology (static, static renewal, or flow-through)
- exposure duration (acute or chronic, or subchronic)
- water quality data during the test (dissolved oxygen levels, pH, temperature, etc.)
- ecotoxicological endpoint data for both control and treatment groups
- statistical methods used to analyze the test results
- any unexpected observations or unusual circumstances that would be important in the interpretation of the test (*e.g.*, tank overflow, malfunction of aeration system)

High quality data should be both relevant and reliable for AWQC development. The Information Quality Act of 2001 (Data Quality Act) charged the Office of Management and Budget (OMB) with issuing government-wide guidelines that "provide policy and procedural guidance to Federal agencies for ensuring and maximizing the quality, objectivity, utility, and integrity of information (including statistical information) disseminated by Federal agencies." In 2002, the OMB issued Information Quality Guidelines and the EPA then developed the policy and procedural guidance, *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency* (EPA/206R-02-008, October 2002).

Data quality, as defined by OMB and in the Informational Quality Guidelines, is the overarching data condition that must be determined by evaluating each of the following:

1. **Utility** refers to the usefulness of the literature to its intended purpose and use. Studies, data, information and methods must only be relied upon to the extent their use is scientifically justified.
2. **Objectivity** refers to whether the information in the study is presented in an accurate, clear, complete, appropriate, and unbiased manner.

3. **Transparency** refers to the clarity of the process. For example, uncertainties and error sources, assumptions, statistical methods, and justifications must be identified, as this high degree of transparency facilitates reproducibility by third parties.
4. **Integrity** refers to ensuring the information is not compromised through alteration or improper interpretation.

In the derivation of AWQC, ecotoxicity data need to be particularly evaluated based on three major points: 1) relevance to criteria derivation; 2) level of documentation; and 3) acceptability. The level of documentation and acceptability together define the reliability of a study. The ECOTOX system for documentation of aquatic and terrestrial toxicity data from laboratory and field studies is widely accepted. International jurisdictions use similar processes (*e.g.*, Dutch National Institute for Public Health and the Environment (RIVM) 2001) and acceptance criteria (*e.g.*, Klimisch et al. 1997) in the data evaluation process.

To minimize uncertainty in AWQC, only data that meet stated data quality guidelines should be used for criteria derivation (**Section 3.2.2**). Toxicity and physical-chemical data should be from studies conducted according to accepted protocols that are appropriate for the chemical and organism being tested. As *in vitro* methods develop, technical support information on accepted protocols will be incorporated. Some international jurisdictions simply state that tests must have been conducted according to accepted, standardized protocols or according to principles of good laboratory practices (GLP) while the U.S. EPA (U.S. EPA 1985, U.S. EPA OSCPP Section 850 Guidelines for aquatic species) and European Commission (2011) list very specific data requirements.

3.1 Study Classifications

Studies identified in ECOTOX and other sources that may provide data relevant to criteria derivation should be reviewed and classified. Data should be classified into one of three general categories:

- Quantitative: Appropriate for use in AWQC derivation or other quantitative measures of effect (*e.g.*, Sensitivity Distribution development, acute-to-chronic ratio calculation);

- Qualitative: Not appropriate for quantitative use, but of sufficient quality to be used descriptively; for example, as a line of evidence in the effects assessment; and

- Unacceptable: Inappropriate for quantitative and qualitative use in AWQC development due to insufficient quality and/or lack of scientific defensibility.

General guidelines for reviewing open literature studies and identifying data usability from a study as “quantitative,” “qualitative,” or “unacceptable” are provided in the following section (**Section 3.2)**.

3.2 Guidance for Open Literature Data Review

The 1985 *Guidelines* provides guidance to enable classification of data from open literature studies into one of three categories, as discussed in the preceding section. OW has developed this SOP document as an enhancement to the 1985 *Guidelines,* to support transparency and consistency in evaluations. The approach outlined in this SOP document is based on the 1985 *Guidelines* and additionally reflects information available in the EPA OCSPP Series 850 Ecological Effects Test Guidelines for aquatic and aquatic-dependent species.

Prior to development of this SOP, OW a conducted a review comparing OW's data quality and test acceptability guidelines for the review of open literature studies on aquatic and aquatic-dependent species, as presented in the 1985 *Guidelines,* with OPP's 40 CFR Part 158 requirements and guidance on reviewing open literature studies (U.S. EPA 2011) for the evaluation of data quality and test acceptability under the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. § 136 *et seq*., 1996). The results of this review indicate that test acceptability and data quality requirements do not differ substantively between the two programs, and that data would generally be excluded or included based on similar data quality requirements. The approach outlined in this SOP document is also generally consistent with data quality and test acceptability criteria applied in data quality review for aquatic and aquatic-dependent species' data by the EPA's Office of Pollution Prevention and Toxics under the Toxics Substances Control Act (15 U.S.C. §§ 2601 et seq., 1976).

EPA reviewers also apply their best professional judgment to determine the appropriate classification for aquatic toxicity studies. The templates for taxa-specific Data Evaluation Records (DERs) found in **Attachment D** of this document include detailed information for the study reviewer on specific attributes necessary to consider a study acceptable for use in criteria development. The information in the DER templates on specific attributes of a study reflect the EPA (1985 *Guidelines,* 1995 *Addendum*, and EPA OCSPP's *Series 850 Ecological Effects Test Guidelines*), ASTM (American Society for Testing and Materials), and OECD (Organisation for Economic Co-operation and Development) standardized test guidelines. The sources for each specific attribute of the study are cited on the DER.

While a single factor may result in a study being considered unacceptable (*e.g.*, excessive control mortality), more typically, several factors in combination render a study unacceptable. The following should be considered when evaluating open literature studies:

- **Data used in derivation of AWQC**. The data should be from a primary source published in English and available either as a publication or in the form of a dated and signed document (*e.g.*, manuscript, memo, letter; U.S. EPA 1985). Reports should include enough supporting information to evaluate the acceptability of test procedures and reliability of study results. Unpublished studies deemed useful for criteria derivation should undergo focused the EPA review, and in some cases external peer review, prior to usage in criteria derivation. The 1985 *Guidelines* (U.S. EPA 1985) and associated Technical Support Document (U.S. EPA

1987) provide specific data quality guidance and information on quality criteria for acceptance/rejection of toxicity tests. This SOP document summarizes and updates previous data quality information found in the 1985 *Guidelines* (U.S. EPA 1985) and the Technical Support Document (U.S. EPA 1987).

- **Nature of the test substance (percent active ingredient; source/manufacturer).** The study should indicate the exact nature and source of the chemical toxicant being tested, including the grade and purity. Data for test substances less than 80% pure and chemical mixtures are typically deemed unacceptable (*e.g.*, drilling muds, effluents, sludges), but may be considered on a case-by-case basis. For most metals, acceptable data for only a few salts (chloride, nitrate, and sulfate) are used quantitatively. Data for other salts are typically classified as either qualitative or unacceptable but may be used on a case-by-case basis. If the chemical is a pesticide, the percent active ingredient and/or the purity of the test compound should be reported. If a vehicle is used for solubilization of the chemical in the test dilution water, the vehicle should be identified and should be known not interfere with the absorption, distribution, metabolism, or the elimination (ADME) of the test substance, nor alter the behavior/response of the test organisms. Toxicity studies which rely on solvents should include solvent controls to document that the solvent did not affect the organism response.

- **Species, age, sex, size, and life stage and source of the test species.** The test organism should be identified by scientific name and the health of the test organism should be reported. The test organisms, to the extent possible, should be of uniform weight, size and age, and life stage, and have no history of pre-exposure to other chemicals. Any acclimation of test organisms prior to testing should be reported and adhere to established protocols (*e.g.*, U.S. EPA or ASTM). Observed diseases and treatment should be reported and may disqualify test animals or a specific study from quantitative use. Data obtained with species non-resident in North American were previously generally categorized as unacceptable and rejected without further review based on discussions in the 1985 *Guidelines*. Current scientific approaches reflect the interest in considering all quality toxicity tests on aquatic species as potential surrogate data for the thousands of untested species in the environment and thus may be included on a case-by-case basis in the systematic review process, so that data needed to fill information gaps is not unnecessarily excluded without appropriate consideration of their utility. Species native to temperate regions are considered more representative surrogates for potential untested species in the U.S. than tropical species.

- **Method of chemical addition and exposure.** The test material is typically dosed to the dilution water by either static, static-renewal or flow-through procedures. Acute tests can use any of these three methods, if the chemical is known to be sufficiently stable in water over the test duration, but chronic tests should use either static-renewal or flow-through procedures. Data from tests in which the test organisms were exposed to the test material by injection or gavage are typically classified as unacceptable, but may be considered as part of

the weight of the evidence. Data from tests with dietary exposure will be considered for bioaccumulative chemicals.

- **Number of organisms tested per concentration/dosage level and number of concentrations/dosage levels evaluated, as well as the number of replicates for each concentration/dosage level**. This type of information should be reported and be sufficient to yield statistically-sound results. An inadequate number of test organisms per test level or not randomly assigning test organisms to the different treatments, can produce unreliable results. U.S. EPA (*e.g.*, Office of Chemical Safety and Pollution Prevention 850 Draft or Final Test Guidelines[2]) or ASTM standardized test guidelines should be consulted for further information on the adequate number of test organisms per test level and on assigning test organisms. Data from tests in which enzymes, excised or homogenized tissue or cell cultures can be considered in examining adverse outcome pathways or used if a quantitative relationship between the effect and an apical endpoint (*e.g.*, survival, growth, and reproduction) has been determined.

- **Quantification of exposure.** The concentration (and total volume of test material administered, if available) in water or sediment should be reported, as well as the type of test (static, renewal, flowthrough) and duration of exposure. For all studies, the exposure conditions should be clearly described and documented. Measured concentrations are strongly preferred over nominal concentrations for acute tests, while measured concentrations are required for chronic tests and field studies. Measured and nominal concentrations should be reported. Measured concentrations should be analyzed according to procedures set forth in standardized analytical chemistry test guidelines, such as USEPA, ASTM standard test guidelines or other analytical methods determined to be of high performance (*e.g.*, documented research method). Measured concentrations should not vary excessively during the study period. Studies should not be used quantitatively where measured concentrations deviate more than identified as acceptable in the published analytical method. Studies should also not be used quantitatively if variability in concentration between the different treatment groups is sufficiently high (*e.g.*, results deviate by more than 20%) to render mean exposure concentrations between the different treatment groups statistically indistinguishable and/or overlapping concentrations occur between different treatments. The following factors should be considered when reviewing a study with high variability between treatment group measured concentrations: a) the treatment group with high variability is not an endpoint of concern (*e.g.*, different than the no observed effect concentration [NOEC] or lowest observed effect concentration [LOEC] value); b) the dose-response is strong despite the +/- 20% variance; c) the frequency and duration of occurrence (*i.e.*, variance at a low frequency vs. all the time); d) the study authors have provided justification for variability in measured concentrations and identified all measures

[2] https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines

taken to mitigate the problem; and e) the duration of the study (*i.e.*, <20% variability is rarely achieved in fish full lifecycle studies). Where only nominal concentrations are reported, the reviewer should consider whether the test compound is subject to degradation, volatilization, partitioning and/or a combination of these properties such that exposure levels may be considerably different than any reported nominal values (discussed under "Test chemical properties"). Additionally, the reviewer should consider whether test conditions could allow exposure to other chemicals that could potentially confound the study outcomes.

- **Control conditions and performance.** A suitable number of controls should be run concurrently with treatments, and control performance should be used as an indicator of whether study conditions and animal health are acceptable. Negative controls should be run concurrent with the study and failure to do so would typically invalidate the study; exceptions can be made under special circumstances (*e.g.*, lab with a strong history of known, non-variable control responses and outcomes with toxicants consistent with other studies). Positive controls should also be run with a reference toxicant if organisms are field collected or if sublethal biochemical endpoints are assessed to ensure responsiveness of the organisms tested. Studies which rely on co-solvents should also report concurrent solvent control performance. As an indicator of study conditions, control performance in terms of mortality and disease should be carefully evaluated and reported to determine the adequacy of the study. For most species, mortality of greater than 10% in controls for acute tests and greater than 20% in controls for chronic studies is sufficient to invalidate studies. Ideally, studies should also report the measured concentrations of test chemical in the controls. Studies reporting test chemical residues in the controls greater than the limit of detection (LOD) should be invalidated, as the ability of the study to discriminate a treatment effect may be compromised. Exceptions may occur for naturally occurring test materials (*e.g.*, copper, other metals, various salts) and in situations where the presence of the test material in controls can be discounted given acceptable performance in the controls and where effect concentrations in the treatment groups are orders of magnitude higher than the control. Normal growth/development times (where available) for the tested species should be compared to those reported for the study controls. Where the growth/development of the control organisms differs substantially from normal reported values, the reviewer must determine whether study conditions have impaired the organisms' ability to thrive. In cases where growth or development in the controls is substantially different than typically observed for the test organisms, the study results should not be used because the ability to distinguish treatment effects is uncertain.

- **Wild-caught animals.** Tests using wild-caught animals with unknown previous exposure histories are acceptable provided their source is not an area where prior exposure to pollutants is likely. Test organisms that are reported as having been previously exposed to the test material or other contaminants are invalid. Likewise, wild caught test organisms collected from water with high natural concentrations of potential environmentally-derived toxicants (*e.g.*, metals) should not be used. Also, wild caught animals used in tests that are not quarantined and sufficiently acclimated should be invalidated. Documentation of

quarantine and acclimation should be available for all wild caught test organisms used in toxicity tests. As an exception, wild caught organisms collected from high and low exposure sites may be used to generate an exposure gradient for maternal transfer studies for certain chemicals (*e.g.*, selenium maternal transfer studies where effects were observed in offspring).

- **Macroscopic observations of the test animals.** During a study, a detailed description of the nature, incidence, time of occurrence, severity, and duration of all observed effects, including death and any other abnormal or unusual signs and symptoms (*i.e.*, sublethal effects) should be reported for controls and treatment groups.

- **Husbandry and test conditions.** Standardized conditions (U.S. EPA OSCPP 850 Guidelines, ASTM studies) have been established in part to minimize the potential for husbandry conditions to confound the study outcome. Reviewers should be cognizant of husbandry conditions and verify that the study conditions are adequately described and acceptable. This description should include:

    - number of animals per cage or test container (*i.e.*, biological loading rate)
    - test organism health (treatment or observation of disease/stress should be reported)
    - ambient temperature (use of water temperature outside the range of values that are acceptable for the test species is invalid, unless temperature-related responses are an experimental factor)
    - humidity (as applicable)
    - photoperiod
    - dietary composition and feeding rate
    - source of food
    - dimensions of the test container
    - source of the dilution water and description of its chemical characteristics (use of distilled or deionized water without reconstitution is invalid)
    - description of the toxicant delivery system and flow rate (expressed as the average water volume of test solution passing through each test chamber per unit time).

  Reviewers should consider whether the water exchange (static, static renewal, or flow-through conditions) is adequate to support the number of test animals in the selected test chambers and is appropriate given the test chemical's stability. Control performance and variability should be used as an indicator of the test environment suitability. Results from tests using nonstandard protocols are acceptable for use if the conditions described above are adequately documented and no unexplained irregularities are observed.

- **Feeding during acute tests.** Results of acute tests during which test organisms were fed should not be used (except for tests using certain species such as saltwater annelids and mysids) unless data indicate that food did not affect the toxicity of the test material and/or the test material has a low K_{ow} value (< 2). For compounds with a log K_{ow} of less than two, the presence of food is assumed to be not likely to significantly alter the dissolved concentration

or bioavailability of the test material. For pesticides with log K_{ow} values between 5 and 7, laboratory toxicity data should be carefully reviewed to ensure that feeding regimes are eliminated to minimize any effects from interaction of the pesticide with food particles (*e.g.*, reduction of test solution concentration as a result of partitioning into the food particles, or introduction of a dietary exposure route if animals ingest food that has sorbed to the pesticide, if the test is intended to capture only water column-based effects).

- **Test chamber material.** Tests conducted with organic materials in plastic test chambers (test vessels constructed from materials other than glass) without measurement of test material should be considered invalid unless it can be confirmed that the test material is stable and/or has a low K_{ow} value. Tests conducted in plastic test chambers should be documented in the study evaluation because there is the potential for plasticizers to leach into the dilution water, which could compromise the test results.

- **Test chemical properties (e.g., solubility, K_d and K_{oc}, vapor pressure/Henry's law constant).** This information is important in determining if actual exposure concentrations could differ substantially from nominal concentrations and where it might be critical to have measured values throughout the study. The U.S. EPA OCSPP Guideline 850.1000 (Draft; U.S. EPA 1996) provides useful guidance on the design and conduct of aquatic studies with difficult to test substances and should be considered when determining the acceptability of a toxicity study. The solubility and stability of the test material should be known for the conditions under which the test is being conducted to provide scientifically defensible information. This representative analysis of the material should be conducted under the same conditions as those used for the test. The limit of detection (LOD) and limit of quantification (LOQ) for the chemical being analyzed should be identified. Incomplete dissolution of materials with low water solubility, such as evidenced by the presence of precipitates or films in or on the water could affect actual exposure levels, and the solubility of the chemical in relation to the reported test concentrations should be considered when evaluating such a test outcome. The use of aerated treatment units when testing a chemical that is volatile is likely to overestimate exposure concentrations, and should be considered invalid, unless exposure concentrations are regularly measured. For any chemicals where stability, solubility, volatility, and/or sorption may be issues, chemical measurements at the study initiation and termination are considered important and should be considered when determining the validity of the test.

- **Water quality**. All relevant water quality parameters (*e.g.*, dissolved oxygen, temperature, pH) should be reported. Hardness, Dissolved Organic Carbon (DOC), and pH are crucial for the evaluation of metals toxicity, particularly for those where bioavailability is affected by these parameters. However, results of acute tests conducted in dilution water with total organic carbon or particulate matter exceeding 5 mg/L should not be used, unless a relationship between acute toxicity and organic carbon or particulate matter has been established and/or data show that organic carbon, particulate matter, or similar substances do not affect exposure/toxicity. In addition, biological loading rates should be suitable for the

test container and not compromise water quality during the study. For water column tests, mean dissolved oxygen concentrations should not drop below 60% saturation for prolonged periods, unless justification is provided indicating the dissolved oxygen suppression did not interfere with the test outcomes.

- **Negative and solvent control performance.** The concentration of organic solvent in test solution should not exceed 0.1 mL/L for acute and/or chronic invertebrate studies or acute fish flow-through studies; the solvent should not exceed 0.5 mL/L for acute fish static or static-renewal studies (see the appropriate comparable U.S. EPA or ASTM study guideline). OPP has developed guidance (U.S. EPA 2008) for aquatic studies with pesticides for determining whether negative and solvent control performance is adequate. This guidance should be considered when determining whether an aquatic toxicity study conducted with high K_{ow} organic compounds is valid.

- **Endpoint selection.** Measured toxicity outcomes should be representative and applicable to the assessment endpoint being evaluated (*e.g.*, risks to species, populations, communities). For acute tests, these endpoints would typically include acute median effective concentration (EC_{50}) or median lethal concentration (LC_{50}) values. For chronic studies, endpoints may include NOEC and LOEC, and/or preferably ECx (*e.g.*, EC_{10} or EC_{20}) values. Where toxicity data are available for multiple life stages of the same species (*e.g.*, eggs, juveniles, adults), OW uses the data from the most sensitive life stage to develop AWQC. This helps to ensure that a given species can survive an exposure during the most sensitive stages of its life cycle, and thus maintain a viable population. Studies reporting sublethal endpoints other than those traditionally used in AWQC development may be used qualitatively as additional lines of evidence to support tests that report direct effects on survival, growth, and reproduction. Sublethal effects can include hormonal, biochemical, cellular, osmoregulatory, and behavioral measurement endpoints, among others. Some of these endpoints, such as adaptational behaviors (*e.g.*, predator avoidance, feeding behaviors), reproductive behaviors (*e.g.*, mating behavior, nest guarding behavior), and morphological effects are important to the overall fitness of both the individual and the population. Consideration should be given to whether there is a quantitative relationship between the observed sublethal effect and an effect that is relevant to organism and/or population viability and is of regulatory concern (*e.g.*, survival, growth, reproduction). These types of endpoints (Kramer et al. 2011) may also be linked via Adverse Outcome Pathways (AOP) for survival and reproduction to population-level effects in aquatic animals (Ankley et al. 2010).

- **Statistical methods used to analyze the test outcome.** Verification of the statistical analysis is an integral part of the data evaluation process. As such, studies should report the specific measures of central tendency (*e.g.*, means, medians) and dispersion (*e.g.*, standard deviations, standard errors) that were used, along with associated sample sizes (N values). The report should state which methods of statistical comparison (*e.g.*, t-test, ANOVA, chi square) were used and the assumed data distribution (parametric versus nonparametric). Tests using parametric statistics should indicate whether the conditions for such tests (*i.e.*, normal

distribution and homogeneity of variance) have been met. Specific statistical software used should be identified.

- **Information necessary to provide a complete and accurate evaluation of test outcomes**. Each report should include a summary of the data, a description of the statistical analysis of the data, and a statement of conclusions drawn from the analysis that allows the reader to independently evaluate the conclusions of the author. The availability of raw data is particularly important when needed to recalculate an endpoint for a study that could substantively affect the criteria value (*e.g.*, studies indicating effects near the 5th percentile), and efforts should be made to obtain the raw data from the study author if these data are not reported in the study.

- **Important information to determine study reliability.** Inconsistencies or deviations with recommended methodologies should be reported, as discussed in the applicable guidelines (U.S. EPA guidelines, ASTM test methods, and/or OSCPP guidelines Standard Evaluation Procedure [SEP]), for each of the respective studies. The U.S. EPA specific test guidelines can provide additional measures to gauge the reliability of study conditions.

3.2.1 Studies Classified as Unacceptable

Open literature studies classified as unacceptable are those that are not considered sufficiently scientifically rigorous for use in criteria derivation and do not provide useful and/or reliable information. Studies classified as unacceptable can include those performed under conditions that deviated so significantly from the recommended protocols that they bring into question the validity of the toxicity test results.

Aquatic studies commonly placed in this category include those with improper test conditions (*e.g.*, static exposures with volatile chemicals in aerated test chambers), test vessels constructed of materials other than glass coupled with a test substance that is expected to sorb to the test chamber walls, excessive mortality of control animals, substantial amounts of missing test information, a study where the test material was not properly identified, and/or environmental conditions or results that cannot be readily interpreted from the information provided. A detailed list of factors that could result in invalidation of open literature data is provided above in **Section 3.2**.

3.2.2 Studies for Quantitative and Qualitative Use

If a study is determined to be acceptable based on the guidelines described above, a determination is made regarding whether the information provided in the study is adequate for ″quantitative″ or ″ qualitative″ use in AWQC derivation. For OW's purposes, 'quantitative' means the data from the study can be used to derive a numeric AWQC. ″ Qualitative″ refers to data that are not adequate for the derivation of numeric aquatic life criteria, but that can be used as additional lines/weight of evidence to support the effects assessment as described in the

effects characterization.

As previously discussed, to be used quantitatively, the endpoint(s) reported in the open literature should meet all the following general guidelines:

- The endpoint is reported in (or can be converted to) acceptable units (*e.g.*, µg/L)
- The endpoint reported can be used to derive AWQC (*e.g.*, LC_{50} for acute exposure in fish) for apical endpoints of concern: survival, growth and reproduction; and
- Sufficient information is provided in the study to substantiate or independently evaluate whether the reported study conclusions (*i.e.*, dose-response) and endpoints are accurate.

Depending on the measured endpoint, study evaluation criteria similar to those in OCSPP 850 Test Guidelines or ASTM methods, should be used to gauge the utility of the study. If a study does not contain sufficient information to meet the key acceptance criteria including the general guidelines summarized above in this SOP, the data from the study should be classified as "qualitative."

OW recognizes that the third criterion of "sufficient information" listed above requires best professional judgment. The most reliable means of determining whether study conclusions can be verified is through accessing the raw data for a study; however, it is recognized that many open literature papers, particularly older ones, may not provide this type of information. Therefore, the quantitative use of open literature requires that the study provide a relatively comprehensive description of the conditions under which the study was conducted, and the data generated by the study. The study should report detailed measures of the variability associated with the data and the methods used to analyze the data. Reviewers should note whether the statistical tests used in the study are appropriate for the study design, the nature of the measured endpoints, and the data generated by the study.

Where raw data are not available to verify the study endpoints, the reviewer should discuss the uncertainties associated with quantitative use of the data. Consideration should be given to the extent to which the measured test endpoints align with other lines of evidence.

4 Documentation of Open Literature Used in AWQC Development

This section discusses the process of tracking and documenting studies obtained from the open literature through the screening (**Section 4.1**) and reviewing process (**Section 4.2**), and how this information is presented in AWQC documents (**Section 4.3**).

4.1 Documenting Study Screening Outcomes

All studies identified through an ECOTOX search or other relevant searches should be documented throughout the screening process. The files provided with the results of the ECOTOX database search and screen can include the studies accepted by ECOTOX as well as studies considered non-applicable with defined rejection reasons and "other" studies with identification terms. These files should be retained to document which studies require further review and which studies do not. The files should also be updated if studies are found from sources other than the ECOTOX database and in cases when a study is accepted by ECOTOX but not by OW.

4.2 Documenting Study Review Outcomes

Studies identified in the open literature (ECOTOX and other sources) that may provide data relevant to criteria derivation should be reviewed and classified (*i.e.*, quantitative, qualitative, unacceptable), and the evaluation should be documented as described in this section.

Data Evaluation Record (DER) templates have been developed by OW to describe specific recommendations for toxicity test aspects and to document review outcomes for studies considered for use in AWQC development. The purpose of completing the DER for each study as outlined below is to ensure a transparent, efficient, and consistent process for completing and documenting reviews of studies and avoiding duplicative and possibly conflicting conclusions associated with study reviews by different reviewers. The EPA is using an electronic format of the DERs housed under the EPA's ECOTOX database. The information captured in the DER is identical to those included below. In the future, the information captured in the DER could be captured in another electronic format. DER templates for fish, aquatic and terrestrial invertebrates, plants, amphibians, and avian species are included in **Attachment E** through **Attachment J**.

Data Evaluation Records (or an equivalent documentation approach) should be completed:

- For open literature studies that pass the initial screening phase as described in **Section 2**.
- For all studies with acceptable endpoints that are classified as 'quantitative,' 'qualitative,' or 'unacceptable' as described in **Section 3**.
    - 'Unacceptable' studies receive an abbreviated DER review, as noted below.
- By a primary and secondary reviewer.

The DERs are separated into three parts:

- Part A includes a general overview (citation, summary of any deficiencies, study classification [quantitative, qualitative, unacceptable]), abstract, summary of relevant endpoints, and results.
- Part B includes information on materials and methods.

- Part C includes verification of calculations and statistical results (if raw data are provided or obtained from the study author, and statistical re-analyses are deemed useful or important).

The DERs should be saved with a file name that includes:

- Chemical (*e.g.*, "Hg" for mercury)
- Taxonomic group (*e.g.*, "invert" for invertebrate)
- Species name or common name (*e.g.*, "C. dubia" or "Cladoceran"). If data for more than one species are provided in the paper, then use term that best conveys the information (*e.g.*, "multiple inverts")
- Abbreviated citation beginning with year published followed by first author (*e.g.*, year and first author [et al.])
- Reviewer's initials and date

For example, **BaCl_Fish_ D. rerio _2016_Kwon et al._ CB_05-09-19** would be the file name of the study review completed by "Catherine Brown" of:

> Kwon, B., N. Ha, J. Jung, P.G. Kim, Y. Kho, K. Choi and K. Ji. 2016. Effects of barium chloride exposure on hormones and genes of the hypothalamic-pituitary-gonad axis, and reproduction of zebrafish (*Danio rerio*). Bull. Environ. Contam. Toxicol. 96(3): 341-346.

The procedures for completion of the DERs for endpoints that are classified as "quantitative", "qualitative", or "unacceptable" are described below.

4.2.1 Completion of DERs for Endpoints Used Quantitatively

DERs of open literature data and other studies that are used quantitatively to derive numeric criteria should be completed in their entirety (*i.e.*, Parts A, B, and C). The DER should include: the basic study requirements, such as test organism source/acclimation, use of solvent and negative controls, control mortality rates (or other issues with controls that could affect the study validity), number of test concentrations, number of treatments (*e.g.*, 3 applications, 7-day application interval), water quality parameters, and verification of suitable replication. The review should also document all statistically or biologically significant effects. In addition, the duration of exposure, the magnitude of the effect, and the test concentration (nominal, measured, and time-weighted average, if it can be determined) at which the effect was observed should be documented. Each documented endpoint should specify the affected taxa and/or individual species. In addition, the reviewer should include relevant figures and tables from the study that include key findings (*e.g.*, a screenshot from the publication); table and figure captions should properly cite the relevant publication if the figure and/or table is copied from the publication. Statistical software and methods (*e.g.*, R, TRAP, BMDS, with associated version number/date identified) used to verify the reported study or test results and calculate point estimates should be completed when possible and deemed necessary, and reported in Part C. This step may occur separately in time, after Parts A and B have been completed. All open literature studies that are

classified as ‘quantitative’ and used to derive criteria should undergo two levels of internal review, including a primary review of the study, typically by the EPA contractor, and a secondary review by staff typically within OW’s Ecological Risk Assessment Branch (ERAB). In the future the information captured in a DER could be captured in another electronic format.

4.2.2 Completion of DERs for Endpoints Used Qualitatively

DERs should be completed for open literature studies that include endpoints to be used qualitatively in criteria development. To the extent possible, DERs for qualitative endpoints should include the same type of information and level of detail as reviews that are completed for quantitative endpoints, but only through Parts A and B. In addition, DERs for qualitative endpoints should include a description of the study limitations which preclude its quantitative use.

4.2.3 Completion of DERs for Unacceptable Open Literature Studies

For those open literature studies that are classified as “unacceptable,” DERs should be completed, however, the length and level of detail relative to “quantitative” and “qualitative” reviews should be significantly reduced. The abbreviated DER for unacceptable studies should consist of Part A only and focus on the limitations of the study which preclude its use in criteria development. Detailed description of the experimental design is not required for studies that are classified as “unacceptable,” however, screenshots of figures and tables of relevant results should be included.

4.3 Open Literature Review Documentation in AWQC Documents

Studies identified that may provide data relevant to criteria derivation and are reviewed and classified as quantitative, qualitative, or unacceptable are listed as appendices in AWQC documents as described below.

4.3.1 Documentation for Endpoints Used Quantitatively

Numeric AWQC development by OW is detailed in the Effects Analysis section of aquatic life AWQC documents, along with the final acute and chronic criteria (with allowed durations and frequencies). Numerous quantitative data are provided in this section, as is an overview of key drivers of the criteria magnitude. The final main section of the aquatic life AWQC document, the Effects Characterization, describes confidence and uncertainties in those studies and includes relevant qualitative studies as other lines of evidence.

A table listing the studies used quantitatively can also be found in appendices labeled “quantitative toxicity data” (previously referred to as “acceptable toxicity data” in aquatic life AWQC developed prior to 2017). This table includes relevant study information including, but

not limited to, the species tested, notes on the test method (*e.g.*, static versus flow-through), test material, water quality, acute or chronic effect value, and the study reference.

4.3.2 Documentation for Endpoints Used Qualitatively

Although endpoints from studies that are classified as 'qualitative' are not appropriate for quantitative use (*i.e.*, numeric AWQC derivation), they should be discussed in the Effects Characterization section of the AWQC document as additional lines of evidence to support conclusions. A clear rationale should be provided in an appendix. The Effects Characterization section of the AWQC document should focus on how these studies provide supporting lines of evidence and briefly reference why the endpoints were not used quantitatively. These reasons might include test duration, limitations in the study design, lack of sufficient information to substantiate the test results/conclusions, or other uncertainties that confound the ability to discriminate a quantitative treatment-related effect. As previously stated, best professional judgment should be used to determine the appropriate use of studies in criteria development.

A table listing the studies categorized as qualitative can also be found in appendices labeled "qualitative toxicity data" (previously referred to as "other toxicity data"). This table includes relevant study information including, but not limited to, the species tested, test material, test duration, water quality, acute or chronic effect value, species mean acute or chronic value (SMAV or SMCV), the study reference, and notes on the reason it is categorized for qualitative use.

4.3.3 Documentation for Unacceptable Endpoints

To create and maintain a record of all studies reviewed and considered in criteria development, a table listing the studies determined as unacceptable for use is also included in appendices labeled "unacceptable toxicity data" (previously referred to as "unused toxicity data") in each AWQC document. This table includes the citation and rationale for considering the study unacceptable for criteria development.

5 References

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U.S. EPA. 2008. Memorandum on Guidance for Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicity Studies. From Mary Frankenberry, Carolyn Hammer, and Keith Sappington to Donald Brady (EFED Division Director). September 25, 2008.

U.S. EPA. 2011. Evaluation Guidelines for Ecological Toxicity Data in the Open Literature: Procedures for Screening, Reviewing, and Using Published Open Literature Toxicity Data in Ecological Risk Assessments. Office of Pesticide Programs. May 9, 2011.

U.S. EPA. 2013. Aquatic Life Ambient Water Quality Criteria for Ammonia - Freshwater. Office of Water. Washington, DC. EPA-822-R-13-001.

U.S. EPA. 2022. ECOTOX ECOTOXicology Knowledgebase System User Guide – Version 5.5. August 2022.

U.S. EPA. 2023. ECOTOX ECOTOXicology Knowledgebase System SOP: ECOTOX Literature Searches, Citation Identification and Applicability Criteria. September 2023.

Attachment A 1985 Guidelines Minimum Data Requirements for Acute and Chronic AWQC

- The acute freshwater toxicity testing requirement is fulfilled with the following eight minimum data requirements:
    - the family Salmonidae in the class Osteichthyes;
    - a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (*e.g.*, bluegill, channel catfish);
    - a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.);
    - a planktonic crustacean (*e.g.*, cladoceran, copepod);
    - a benthic crustacean (*e.g.*, ostracod, isopod, amphipod, crayfish);
    - an insect (*e.g.*, mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge);
    - a family in a phylum other than Arthropoda or Chordata (*e.g.*, Rotifera, Annelida, Mollusca); and
    - a family in any order of insect or any phylum not already represented.

- The acute estuarine/marine requirement is fulfilled with the following eight minimum data requirements:
    - two families in the phylum Chordata;
    - a family in a phylum other than Arthropoda or Chordata;
    - either the Mysidae or Penaeidae family;
    - three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above); and
    - any other family.

- Chronic toxicity test data (longer-term survival, growth, or reproduction) are required for a minimum of three taxa, with at least one chronic test being from an acutely-sensitive species. Acute-chronic ratios can be calculated with data from species of aquatic animals from at least three different families if the following data requirements are met:
    - at least one is a fish;
    - at least one is an invertebrate; and
    - for freshwater chronic criterion: at least one is an acutely sensitive freshwater species (the other two may be estuarine/marine species) or for estuarine/marine chronic criterion: at least one is an acutely sensitive estuarine/marine species (the other two may be freshwater species).

- At least one acceptable test with a freshwater alga or vascular plant is required. If plants are among the aquatic organisms most sensitive to the material, results of a plant in another phylum should also be available.

- At least one acceptable bioconcentration factor determined with an appropriate freshwater species is required.

Attachment B Acceptability Criteria for Aquatic Effects Data

ECOTOX Reference No.: _____

General instructions: If more than one experimental design is used in the study, multiple Literature Acceptance Criteria Checklist forms may be required, but the acceptability of the paper is based on at least one experimental design meeting all the Acceptability Criteria.

No.	Criteria / *Instructions*	Yes / No
1	The paper reports effects associated with a single chemical exposure.	
	However, OW will consider studies examining additivity, synergism, or antagonism of two or more chemicals where pertinent to the derivation of an aquatic life criterion. Effluents, leachates, drilling muds, fly ashes, natural sediments, and sludges are not considered single chemicals. In addition, the single chemical cannot be introduced as a component of an effluent, etc. *Formulated products, such as emulsifiable concentrates and wettable powders while considered single chemicals, may not be used for quantitative criteria derivation unless this is the only material that has valid data. The data can be used qualitatively in the effects characterization section.*	
2	The paper reports a biological effect on live, whole organisms or *in vitro* preparation.	
	The authors clearly identify an observed effect response related to the exposure of a live organism to the chemical of concern. In vitro studies may be considered in the criteria derivation process. "*Positive" effects (e.g., increased reproduction) will be recorded and considered in the effects characterization section.*	
3	The paper reports a concurrent environmental chemical concentration/dose or application rate.	
	Authors clearly report a concentration/dose or application rate associated with the observed effect response. If the study results are only available in a graphical format, add a comment to the remarks field.	
4	The paper reports an explicit duration of exposure.	
	Authors must explicitly report the duration of the exposure related to the observed effect. *Durations can include qualitative terms (e.g., at hatch, at harvest).*	
5	The paper reports toxicology information for the pollutant of concern to OST/OW.	
6	The article is published in the English language.	
	The full article is published in the English language. Translations will not be conducted.	
7	The study is presented as a full article.	

No.	Criteria / *Instructions*	Yes / No
	Abstracts from journal publications where the full article is published in non-English, the abstract is published in English and conference proceedings published as brief abstracts will not be considered.	
8	The paper is a publicly available document.	
	Publications that are not publicly available (e.g., internal memoranda, government reports not readily available from NTIS) may be considered. However, the documents must be made available to the public via the Federal docket when the assessment goes out in the Federal Register. In addition, certain key studies that may not be in press may be used if the reviewer is provided the opportunity to conduct an internal/external review and the author's permission to publicly disclose the information. If a registrant-sponsored study for a pesticide, all information needed for criteria derivation would need to be made available to OW through a data evaluation record (DER) or similar vehicle.	
9	The paper is the primary source of the data.	
	A document is considered a primary source if at least one of the investigators who conducted the toxicity test is an author, and the authors do not cite another publication as the original source of the data.	
10	The paper reports a calculated endpoint.	
	For the purposes of this evaluation, an endpoint is defined as the quantification of an observed effect obtained through statistics or other means of calculation for the expressed purpose of comparing equivalent effects (e.g., LC_{50}, BCF, NOEC). If within a single experiment, the authors report the same endpoint at multiple durations, the duration most relevant to the OW's standard acceptable test durations for acute and chronic studies (e.g., 96-hr LC/EC_{50s} will be used and note the other endpoints in the remarks field). If NOEC and LOEC endpoints are not explicitly reported by the authors, reviewers should interpret endpoints based on levels of significance reported in the paper. If more than one measurement (e.g., juveniles per litter, juveniles per females) is observed for a particular effect (e.g., reproduction), then only the most sensitive measurement will be used, and the remaining measurements are noted in the remarks field.	
11	The paper reports that treatment(s) were compared to an acceptable control.	

No.	Criteria / *Instructions*	Yes / No
	The control treatment must be comparable to the other treatments and must be free of the chemical stressor. Appropriate controls include: baseline or background control - parameters of actual or representative test species measured before and after administration of test chemical, though not as part of the same test scenario; negative control - organisms maintained under conditions identical to exposed organisms except for the absence of the test substance; positive controls - organisms maintained under conditions identical to the exposed organisms except the test substance is replaced with a substance known to elicit a consistent toxic response; and solvent controls - organisms exposed to carrier or solvent that is used as a vehicle for administrating the test substance to exposed organisms. *The number of treatments (other than controls) should be reported in the data summary table.*	
12	The paper reports the location of the study (*e.g.*, laboratory vs. field).	
	Authors clearly state the locations of the study, either in a controlled laboratory setting or in the field. Field studies are not typically used in a quantitative manner in the criteria derivation process, but may be used qualitatively to support quantitative laboratory studies. Field studies can include natural or artificial settings (e.g., microcosms, mesocosms).	
13	The paper reports the species that was tested; and this species can be verified	
	The authors clearly identify the test species and the organism's scientific name can be verified in a reliable reference. The preferred scientific name should be reported. If a specific genus/species is not reported, reviewers report the species information at the lowest taxonomic level.	

Attachment C ECOTOX Exclusion Reasons

General Instructions: The following is a list of ECOTOX exclusion keywords and definitions utilized under the ECOTOX database efforts.

Exclusion keywords	Description
ABSTRACT	Study published as an abstract only.
ARCHIVED CHEMICAL	When all chemical name(s) in a publication are unable to be verified. Also used for individual chemicals on the second page of the screening module.
BACTERIA	Bacteria; includes microbes and Microtox tests. Only use when COC is affecting/effecting bacteria. **If bacteria is creating the effect, use NO TOXICANT.*
BENEFICIAL EFFECT	Study reports only a positive effect (improving the health of the organism). Also used for individual chemicals on the second page of the screening module.
BIOLOGICAL TOXICANT	Biological toxicants including venoms, fungal toxins, and plant, animal or microbial extracts or toxins not purified. This is used only when the toxicant (COC) is in a biological toxicant form. For example, if acetylsalicylic acid is derived from birch bark rather than prepared in lab. **This is rarely used in TIAB.*
CHEM METHODS	The description of chemical analysis procedures and measurements in a laboratory setting. No organism effects are reported in the paper.
EFFICACY	A secondary positive benefit to one organism; for example insect species are reduced and agricultural yield is improved.
FATE	Chemical distribution in natural media (water, soil, air, and tissue if no biological effect).
HUMAN HEALTH	Studies with human subjects or with surrogate animal subjects. Also includes human or human surrogate species DNA injected into non-human cells and studies on food.
INCIDENT	Reports of accidental or intended animal deaths by exposure to a toxicant or poison; not a controlled experiment.
METHODS	Publication only provides documentation for toxicology test methods, experimental design, statistical methods, standard terminology, recently developed test methods. **must include COC*
MIXTURE	No single chemical effects reported. This includes ambient toxicants in lakes, rivers, soil, air and co-exposure with other chemicals, including microplastics. Howeverm 'Chemical 1' mixed with 'Chemical 2' based on abstract alone is not sufficient enough to exclude as there may be single exposures as well within the full test. **Not commonly used in TIABing, except for 'effluent,' indication an unknown mixture.*
MODELING	Modeling or QSAR papers. **must include COC*
NO CONC	Not including search chemical, no usable dose or concentration reported after examination of the entire paper; includes lead shot studies lacking dose information (i.e., report only the number of pellets) as well as endpoint concentrations reported in log units only.
NO DURATION	No duration reported (entire publication examined).

Exclusion keywords	Description
NO TOXICANT	No chemical toxicant added as stressor; ambient air components not included in ECOTOX. - ambient air component chemicals (ozone, CO2, SO2) and pollution - ambient conditions, including radioactivity, ultraviolet light (UV), temperature, pH, salinity, dissolved oxygen (DO), or other water, air or soil parameters
NUTRIENT	In situ chemical of interest used as nutrient.
PEST MANUAL	More than one insect species is tested; and some are in the PEST group and some are not; Unify does not make this distinction. Used with a second inclusion/exclusion keyword, e.g., OK.
REVIEW	All toxicity tests reported in other primary publications; REVIEW bibliography may be Full Text Screened to identify relevant citations
SEDIMENT CONC	Chemical concentration reported in sediment only (if pore or overlying water concentrations reported, then applicable).
SURVEY	Effects observed in field collected organisms and/or brought to laboratory for residue measurement.
TARGET MANUAL	Used when more than one species group is Full Text Screened and different categories are needed, e.g., and one is a target species and the other has beneficial effect associated with the chemical application. For example, a plant is tested (with positive/efficacy effects) and the insect is a target species. Often accompanied by the exclusion keyword EFFICACY.
VIRUS	Virus used as a test organism. **If virus is causing the effect, use NO TOXICANT*
YEAST	Yeast used as test organism.
WEEDS MANUAL	Used when more than one plant species is attached to the paper and there is only endpoint data for the weed species. For example, a pine tree has no endpoint data, but knapweed has endpoint data.
ADDENDUM [Bibliographic]	Publication is a supplement to another publication. The Addendum citation is cross-referenced to the original publication and the PDFs are merged (erratum or addendum). Erratums and Addendums are ordered and attached to the back of the corresponding publication.
DATASET* [Bibliographic]	Author linked repository dataset.*
DUPLICATE [Bibliographic]	Publication duplicated in different journal or source.
ECOCHEM VERIFICATION SOURCE [Bibliographic]	Publication used to verify chemical CAS or physical/chemical properties.
NO SOURCE [Bibliographic]	Source of publication undetermined, publication unavailable; order status ARCHIVE (includes internal chemical company document and personal communication citations).
NON-ENGLISH [Bibliographic]	Paper's full text language other than English
PUBL AS [Bibliographic]	Entire study was published in another source; only data from one source is abstracted. The second source is linked by exclusion keyword.
REFS CHECKED [Bibliographic]	References in a REVIEW have been checked.
RETRACTED [Bibliographic]	Retracted article from publication by journal.
SCREENED [Bibliographic]	Identifies a book or journal where applicability criteria have been applied to all chapters or publications.

Exclusion keywords	Description
SPECIES VERIFICATION SOURCE [Bibliographic]	Publication used to verify species.

Attachment D Instructions for Completing Data Evaluation Records (DERs) for Toxicity Studies in the Open Literature (September 2024)

Attachment D
Instructions for Completing Data Evaluation Records (DERs) for Toxicity Studies in the Open Literature

The purpose of Attachment D is to provide instructions for completing DERs while reviewing toxicity studies from the open literature and to ensure that DERs are completed in a consistent manner across reviewers.

All DERs should be:

- Completed in their entirety (except for noted exemptions) following the instructions provided below
    - Do not leave sections/tables blank unless noted otherwise. Use *not applicable (NA), not calculable, not provided, not verified,* or *not measured* as appropriate
- Completed for each chemical and species combination (*i.e.*, if a study focused on 2 separate chemicals and 2 separate species, 4 individual DERs should be completed for the one study)
- Saved with a file name that follows:
    - DER_ Chemical_Taxa_ Abbreviated Citation (First author [et al.])_ Year of Publication_ Species Name _ Reviewers Initials - *Example:* DER_Barium_Fish_Kwon et al._2016_D. rerio_CB

Part A: Overview *Complete an abbreviated DER of* ***Part A only*** *for studies marked "****Not Acceptable for Use****"*

I. Test Information

Chemical Information: Complete information as provided.

Test Type: Place *X* by one classification. These test type classifications should be applied when completing Part B: Detailed Review

- **Controlled Experiments** are defined here as studies where the chemical exposure and test community is manipulated (*e.g.*, laboratory, microcosm, and mesocosm tests regardless if conducted indoors or outdoors)
- **Field Study/Observations** are defined here as studies where the either the chemical exposure **and/or** test community is not manipulated and the exposure observations occur in a natural waterbody (*e.g.*, observed effects of a chemical at a contaminated stream, lake, pond, and in-situ stream or a whole lake experiments)

Reviewer Information: Complete reviewer information. Primary and secondary reviewer should be designated by the EPA project lead and will typically consist of a contractor as the primary reviewer and EPA staff as the secondary reviewer.

Citation: Complete citation as noted in SOP and on DER, being sure to indicate author(s), year, study title, journal, volume and pages.

Companion Papers: Provide a list of any companion papers, including other publications, reports, or theses associated with the current publication being reviewed using the same citation format noted in the SOP and on the DER. If companion papers are listed, identify if separate DERs were completed for companion papers and list file names for each of the DERs.

- Companion papers include separate publications reporting other aspects of the experimental design and/or results or other papers used in the development of the experimental design of the publication being reviewed

Attachment D
Instructions for Completing Data Evaluation Records (DERs) for Toxicity Studies in the Open Literature

Study Classification for Aquatic Life Criteria Development: Place *X* by one classification based on the study's highest use (*e.g.*, mark "*Acceptable for Quantitative Use"* only if a study has both Quantitative and Qualitative Use endpoints).

- This overall Study Classification for Aquatic Life Criteria Development should take the Study Design/Methods Classification (in Materials and Methods section of Part B) and Response-Curve Classification (in Statistical Verification of Results section of Part C) into consideration.
- Provide any necessary details related to the study's use classification for all pertinent endpoints, including non-apical endpoints (*i.e.*, differences in the study's use classification for certain endpoints).

Major Deficiencies: Check all that apply, paying attention to any noted exceptions. Checking any of these items may make the study "*Not Acceptable for Use*". If occurrence of mixtures is identified as a major deficiency, describe potential chemical mixtures in areas provided. Identify any other notable concerns that may classify the study as "*Not Acceptable for Use*" under *General Notes*.

Minor Deficiencies: List and describe any minor deficiencies or other concerns with test. Listing any items in this section may make the study "*Acceptable for Qualitative Use*" (exceptions apply to field studies as noted on the DER) and listing one (particularly chemical solubility issues, anomalous or inconsistent results, previous or variable exposure, dosing via gavage) or several items may make the study "*Not Acceptable for Use*".

The EPA project lead will typically make this distinction in use classification. A study may be considered "*Acceptable for Quantitative Use"* even if one or more minor deficiencies are identified and the study results are consistent with similar studies focused on a related taxon (*i.e.*, up to a family level) and measured the similar endpoints. For field studies/observations check mixtures if observed effects are not justifiably contributed to single chemical exposure and uncharacterized reference sites/conditions if appropriate. If either of these are checked, that may make the study "*Not Acceptable for Use*". Describe potential chemical mixtures present at the site and exposure variability across the study site(s). Identify any other notable concerns that may classify the study as "*Acceptable for Qualitative Use*" or "*Not Acceptable for Use*" under *General Notes*.

- **Minor Deficiencies may include**: analytical or chemical solubility issues, problems encountered with the test organisms or treatments (minor variability in concentrations, loss of replicate(s), small sample sizes, only one test concentrations), description of dilution water not provided (*e.g.*, uncharacterized stream water or potential presence of unknown containments, high organic content, extreme hardness, pH), too few exposure concentrations, anomalous or inconsistent results, unmeasured test concentrations, previous or variable exposure, dosing via gavage, insufficient details regarding methods or analyses.
- **For field studies/observations**: Only publications with a range of exposure concentrations (*i.e.*, study design incorporates both low and high exposure concentrations) and those where observed effects are justifiably contributed to a single chemical exposure (within a mixture of concentrations) should be considered "*Acceptable for Quantitative Use.*"

Reviewer's Comments: Add comments not captured elsewhere on DER, including pertinent information for drafting study summaries for the Effects Analysis and/or Effects Characterization sections of the AWQC.

Abstract: Copy and paste abstract from publication.

Summary Tables: Complete acute and chronic tables for *"Acceptable for Quantitative Use*" and "*Acceptable for Qualitative Use*" studies with information on the most sensitive apical and/or non-

apical endpoint measured (*i.e.*, those usually captured in the data appendices/tables of the criteria document). Modify tables as needed under the direction of the EPA project lead. DO NOT complete tables for studies classified as "*Not Acceptable for Use."*

- To fill in Reported Effect Concentration see Part A Section II: Results below.
- To fill in Verified Effect Concentration see Part C: Statistical Verification below. If Statistical verification not preformed (*i.e.*, paper marked "*Not Acceptable for Use*"), indicate *not verified.*

II. Results: Provide results as reported in the publication (including supplemental materials). Add pertinent information for drafting study summaries that is otherwise not captured in the result section of the DER under *General Notes* for the relevant section. Complete each results section as follows:

- **For all studies**, paste screen shots of tables and/or figures reporting results from the article, including those tables and/or figures found in the supporting materials, in the respective subsections and after the associated pre-tabulated results table. If a particular result (*i.e.*, mortality, growth, reproduction, and/or sublethal effects) was not part of the study design note this under the *General Notes* area of the associated results section.

- **For studies marked "*Acceptable for Quantitative Use*" or "*Acceptable for Qualitative Use*"** complete all pre-tabulated tables (including any needed modifications to supplied tables) as follows:

  - The table headers, particularly the supplied entries in brackets (*Example: Mean percent mortality [or number of immobilized] of [test organism] exposed to [test substance] for [test duration])*
  - The number and titles of treatments, including controls (including negative and solvent controls if any) by adding rows as needed and changing the treatment name in brackets to a consistent nomenclature in publication (*e.g.*, categorical names used by study authors (low, medium, high), nominal, or measured concentrations). Ensure all treatment group labels are consistent throughout the results section.
  - The observed effect(s) in Tables A.II.3 through Table A.II.6 to be consistent with the observed effects reported in the study (*e.g.*, mean percent mortality, growth, or reproductive effects). Common observed effects are supplied in each pre-tabulated table. Include units when appropriate. Add columns for additional effects (and standard deviation or standard error), if needed.
  - Edit Standard Deviation or Standard Error Column headers based on which is reported.
  - Copy and paste additional reproductive effects tables for each generation of a multi-generational study (remembering to label each generation) and the sublethal effect table for each observed sublethal effect.
  - Identify the values that are reported to be significantly different from control with a superscript.
  - Provide the toxicity values (*e.g.*, LC_x, EC_x, NOEC and LOEC) identified in the study, whether stated by the study authors or not. Edit toxicity values (*e.g.*, LC_x and EC_x) provided in brackets as needed. If values for LC_{50}, LT_{50}, NOEC are greater than the highest treatment concentration, use > symbol.

Water Quality Parameters: Summarize water quality parameters measurements made in test solutions. If only general summary data of water quality parameters are provided by study authors (*i.e.*, specific details of water quality parameters on a treatment level is not provided), summarize any information regarding water quality parameters under *General Notes*.

Chemical Concentrations: Summarize the concentration verification data from the test solutions/media and discuss the acceptability for deriving AWQC under General Notes. Expand table to include measured concentration data for each media type (*i.e.*, muscle, liver, blood, etc.).

Mortality Effects: Summarize mortality results (*if any*). Comment on concentration response relationship and slope of response if provided under *General Notes*.

Growth Effects: Summarize growth results (*if any*). Comment on concentration response relationship and slope of response if provided under *General Notes*.

Reproductive Effects: Summarize reproductive endpoint results (*if any*). For multi-generational studies, copy and paste Table A.II.5 for each generation with reproductive endpoint data. Comment on concentration response relationship and slope of response, if provided, under *General Notes*.

Other Sublethal Toxicity Effects: Summarize any other reported sublethal effect(s), including behavioral abnormalities or other signs of toxicity. Copy Table A.II.6 for each additional sublethal effect observed. Comment on concentration response relationship and slope of response if provided under *General Notes*.

Reported Statistics: Briefly summarize statistical analysis conducted by study authors or copy and paste statistical section from article.

Part B: Detailed Review *Do not* complete for studies marked "*Not Acceptable for Use*"

I. Materials and Methods: In text citations for test guidance and recommendations provided brackets.

Protocol/Guidance Followed: Indicate Protocol/Guidance *(e.g., U.S. EPA, ASTM, Environment Canada, European Union)* followed if identified by study authors, otherwise complete with relative information (*e.g.*, not provided).

Deviation from Protocol/Guidance: Indicate deviations from protocol/guidance as described by study authors, otherwise complete with relative information (*e.g.*, not provided).

Study Design and Methods: Briefly describe the experimental design or copy and paste related information from appropriate section of the article.

Test Organism Matrix: Complete for both Controlled Experiments and Field Studies/Observations. Complete information under *Details* column as noted in the study and add any pertinent notes under the *Remarks* column.

Study Parameters Matrix: Complete for both Controlled Experiments and Field Studies/Observations. Complete designated information in the *Details* column, paying particular attention to any relevant guidance information in the bulleted lists under the *Parameter* column. Summarize any pertinent information or deficiencies in the *Remarks* column.

Controlled Experiment Study Parameters Matrix: Complete designated information in the *Details* column, paying particular attention to any relevant guidance information in the bulleted lists under the *Parameter* column. Summarize any pertinent information or deficiencies in the *Remarks* column. Complete this Controlled Experiment Study Parameters Matrix for Controlled Experiments only. Leave blank for field studies/observations.

Study Design/Methods Classification: Place *X* by one classification. Provide details of major and minor deficiencies/concerns with study design under the associated sections of Part A of the DER paying attention to designations of study use classification noted on the DER (*i.e.*, items indicated under the Major Deficiencies section classify the study as *"Not Acceptable for Use*" and items indicated under the Minor Deficiencies section may make the study classification as "*Acceptable for Qualitative Use*"). This study design/methods classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A (*e.g.*, if the study design classification is "*Not Acceptable for Use*" the study classification in Part A should be consistent and not "*Acceptable for Quantitative Use*").

Additional Notes: Provide additional considerations related to the study design/methods, including details of particular study design parameters that may influence the use of measured study results or treatment groups.

II. Observations

Observations Matrix: Complete designated information in the *Details* column, paying particular attention to any relevant guidance information in the bulleted lists under the *Parameter* column. Summarize any relevant information or deficiencies in the *Remarks* column. Complete this Observations Matrix for both Controlled Experiments and Field Observations. This information should be consistent with the *Results* section in *Part A.*

Available Concentration-Response Data: Answer all questions related to data availability. The EPA project lead should stipulate who is responsible for contacting study authors and will identify the software that should be used to estimate concentration-response data from graphs.

Part C. Statistical Verification of Results Complete for all studies marked "***Acceptable for Quantitative Use***" and only for the five most sensitive genera and sensitive apical endpoint. If multiple sensitive apical endpoints were measured copy Sections I and II of Part C for each endpoint as needed. Completion of Part C should be designated by the EPA project lead.

I. Statistical Verification Information: Complete all information as provided.
Statistical Reviewer Information: Complete reviewer information. Primary and secondary reviewer should be designated by the EPA project lead and will typically consist of a secondary reviewer for studies marked for "*Quantitative Use*" and that are used to derive the criteria (*e.g.*, five most sensitive genera).

Endpoint(s) Verified: List all endpoints verified. Verification of endpoints should be designated by the EPA project lead and will typically focus on apical endpoints only.

Additional Calculated Endpoint(s): List all endpoints calculated. Statistical verification of additional calculated endpoints should be designated by the EPA project lead.

Statistical Method: Report statistical methods (*e.g.*, R, EPA TRAP, BMDS or other statistical packages) used to verify the test results and/or those used to calculate toxicity value point estimates, including for tests where toxicity values were not provided.

II. Toxicity Values: Provide the statistically verified toxicity values identified on the DER. If the values for the LC_{50}, $LT_{50,}$ and/or NOEC are greater than the highest test concentration, use the " > " symbol. Include confidence intervals if applicable.

Response-Curve Classification: Place *X* by one classification. This response-curve classification should be taken into consideration for the overall study classification for aquatic life criteria development in Part A (*e.g.*, if the response-curve classification is "*Not Acceptable for Use*" the study classification in Part A should be consistent and not "*Acceptable for Quantitative Use*"). The response-curve classifications should be based on model performance as follows:

- Acceptable for Deriving Criterion – Model performs well on all statistical metrics.
- Acceptable for Supporting Information – Model presents some metrics that may call estimates into question.
- Not Acceptable for Deriving Criterion – Model does not perform well to fit data and should not be used.

Summary of Statistical Verification: Provide summary of methods used in statistical verification, including pertinent information for drafting study summaries.

Additional Notes: Add notes related to the statistical verification not captured elsewhere in Part C, including pertinent information for drafting study summaries.

Attachments: Provide the attachments listed below as follows:

- **Concentration-Response Data:** Provide attachments to ensure that all data used in Part C is captured. This includes:
  - Study results reported in the publication (including supplemental materials), which are captured in Results section of Part A of DER above.
  - Additional data requested and provided by study authors, which are captured in Table C.II.1 below and include the original correspondence with study authors as an attachment to the DER.
- **Model Assessment:** Include all model figures and tables associated with the statistical verification.
- **Statistical Code:** Provide statistical code used to fit dose-response curve. This code can be saved in one file and referenced by file name in DER.

Additional Data Used in Response-Curve: Provide all data used to fit response-curve not already captured in the Results section of Part A of the DER in *Table C.II.1*. This data would typically involve replicate and/or raw data provided by study authors. Add rows as needed. First row in italicized text is an example. Edit pre-tabulated information designated by brackets and/or add columns for additional parameters (*e.g.*, non-detect concentrations, DOC, pH, hardness) to be consistent with the data used to fit the dose-response curve. Note: It should be anticipated that this data table will be copied and pasted into broader database with all studies and endpoints used in the criterion derivation.

Attachment E Fish Data Evaluation Record (DER) Template (September 2024)

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

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

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., Slonim, A.R. 1973. Acute toxicity of beryllium sulfate to the common guppy. J. Wat. Pollut. Contr. Fed. 45(10): 2110-2122)

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 these 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).*

EPA OW DER INSTRUCTIONS

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 DER.*

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)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration[b] (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)	Method[a]	Test Duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L or µg/g)	Verified Chronic Value[b] (mg/L or µg/g)	Chronic Value Endpoint	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 indicate 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., LC_{50}, EC_{20}, 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 (℃)	*[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 measured concentration data for each media type (i.e., water, diet, 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-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-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 relationship and slope of response if provided. Compare mortality in treatments with control group and/or the reference chemical.*

-

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

Mean percent mortality [or number of immobilized, 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 (units)	[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 relationship and slope of response if provided. Compare growth endpoints in treatments with control group 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 relationship and slope of response if provided. Compare reproductive endpoints in treatments with control group 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 Percent Hatch]	Sample Size	[Standard Deviation or Standard Error]	[Mean Percent Survival Post Hatch]	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:
• Wild caught from unpolluted areas [4]
  ○ Quarantine for at least 14 days or until they are disease free, before acclimation [2,4]
  ○ Quarantine at least 7 days before holding, which should be at least 12 days [1]
• Must originate from same source and population [1,2,4]
• Salmon and trout should be obtained from a hatchery certified disease free [1]
• Should not be used:
  ○ If appeared stressed, diseased, have physical abnormalities, or show unusual behavior [2,4]
  ○ If more than 5% die or show signs of stress during the 48 hours before test initiation [1,4]
  ○ If they were used in previous test treatments or controls [1,5]
  ○ If collected by electroshocking, chemical treatments, or gill netting [1,2]
• No treatments of diseases may be administered:
  ○ Within 16 hours of field collection [4]
  ○ Within 48 hours of testing or during testing [1]
  ○ Within 10 days of testing or during testing [4]
  ○ Embryos should not be obtained from fish treated for disease within past 14 days [2]
  ○ Embryos should not be treated for diseases during testing [2]		

Age at Study Initiation:
Acute:
• Juvenile stages preferred [1,4]
  ○ Should be less than 3 g weight and actively feeding [1]
Chronic:
• Life-cycle test:
  ○ Embryos or newly hatched young < 48 hours old [5]
• Partial life-cycle test:
  ○ Immature juveniles at least 2 months prior to active gonad development [5]
• Early life-stage test:
  ○ Shortly after fertilization [2,5]
  ▪ ≤24 hours post fertilization preferred, ≤ hours encouraged [2]		

Parameter	Details	Remarks
Was body weight or length recorded at test initiation?	____ Yes ____ No	
Was body weight or length recorded at regular intervals?	____ Yes ____ No *If yes, describe regular intervals:*	

Data Evaluation Record on the Effects of [Chemical] on Fish [Species]

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

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	Number of Replicates per Treatment Group: • Generally, at least 2 replicates/treatment recommended for acute [1,4] and chronic [6] tests. • At least 4 replicates/treatment recommended for early life stage (ELS) test [2]	Control(s): Treatment(s):	
	Number of Organisms per Replicate/ Treatment Group: • At least 10 organisms/treatment recommended [1,6] • At least 7 organisms/treatment acceptable [1,7] • At least 20 organisms/replicate (80 organisms/concentration) recommended for ELS test [2]	Control(s): Treatment(s):	
	Exposure Pathway: *(i.e., water, sediment, gavage, or diet).* *Note: all other pathways (e.g., dermal, single dose via gavage, and injection) are unacceptable.*		
	Exposure Duration: Acute • Should be at least 96 hours [1] • Should be 96 hours [5] Chronic • Life-cycle tests: o Ensure that all life stages and life processes are exposed [5] o Begin with embryos (or newly hatched young), continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation [5] • Partial life-cycle tests: o Allowed with species that require >1 year to reach sexual maturity, so that all major life stages can be exposed to the test material in <15 months [5] o Begin with immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation [5] • Early life-cycle tests: o 28 to 32 days (60 day post hatch for salmonids) exposures from shortly after fertilization through embryonic, larval, and early juvenile development [2,5]	____ Acute ____ Partial Life Cycle ____ Early Life Stage ____ Full Life Cycle ____ Other *(please remark):*	
	Observation Intervals: Should be an appropriate number of observations over the study to ensure water quality is being properly maintained [7]		
	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* [6]	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 o If culture water (or other source water, e.g. wild caught organisms) differs from test water, should be changed gradually to 100% test dilution water (usually 2 or more days) [1,2,4] o For wild-caught animals, test water temperature should be within 5°C of collection water temperature [4] o Temperature change rate should not exceed 3°C within 72 hours [4] • To avoid unnecessary stress and promote good health: o Organisms should not be crowded [4] ▪ See "Biomass/Loading Rate" for guidance on holding densities o Water temperature variation should be limited [4] o Dissolved oxygen: ▪ Maintain between 60 - 100% saturation [4] ▪ Continuous gentle aeration if needed [4] o Unionized ammonia concentration in holding and acclimation waters should be < 35 µg/L [4] o Mortality during the week preceding the test (following a 48 hour adjustment period) must be ≤ 10%, or the batch should be rejected [1] ▪ If between 5-10%, holding should be extended an additional 7 days [1]	Duration: Feeding: Water type: Temperature (°C): Dissolved Oxygen (mg/L): Health (*any mortality observed?*):	*Identify number of individuals excluded from testing and/or analysis (if any):*
Acclimation followed published guidance? *Describe, if any*	____ Yes ____ No *If yes, indicate which guidance:*	
Test Vessel: • Test chambers should be loosely covered [4] • Test chamber material: o Should minimize sorption of test chemical from water [4] o Should not contain substances that can be leached or dissolved in solution and are free of substances that could react with exposure chemical [4] o Glass, No. 316 stainless steel, nylon screen and perfluorocarbon (e.g. Teflon) are acceptable for most chemicals [3,4] ▪ Other materials recommended for specific chemicals and should be used when appropriate (e.g., polyethylene for PFAS chemicals [8] o Rubber, copper, brass, galvanized metal, epoxy glues, lead and flexible tubing should not come into contact with test solution, dil. water, or stock [3,4] • Size/volume should maintain acceptable biomass loading rates (see Biomass Loading Rate below) [4]	Material: Size: Fill Volume:	*Briefly describe the test vessel:*

	Parameter	Details	Remarks

For Controlled Experiments Only	**Test Solution Delivery System/Method:**
	• Flow-through preferred for some highly volatile, hydrolyzable or degradable materials [5]
	  o Concentrations should be measured often enough using acceptable analytical methods [5]
	• Chronic exposures:
	  o Flow-through, measured tests required for tests with fish [5]
		Test Concentrations Measured
		____ Yes ____ No
		
		Test Solution Delivery System:
		____ Static
		____ Renewal
		Indicate Interval:
		
		____ Flow-through
		Indicate Type of Diluter:	

	Dilution Water Source & Characteristics:
	• Dilution water must be characterized (natural surface water, well water, etc.) [6]
	  o Clean surface water, ground water, reconstituted water, or natural or artificial seawater (for saltwater species) are acceptable [1,2]
	  o Dechlorinated tap water should not be used as some forms of chlorination difficult to adequately remove [1,2]
	  o Distilled/deionized water without the addition of appropriate salts should not be used [5]
	• Freshwater hardness range should be < 5 mg/L or < 10% of the average (whichever is greater) [4]
	  o Recommended hardness <250 mg/L (preferably <180 mg/L); or 40-50 mg/L for metals [1,2]
	  o 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) [4]
	  o Recommended salinity 15-25 ‰ [1,2]
	  o Unless study is examining effects of salinity on toxicity.
	• Dissolved oxygen in dilution water at start of test recommended to be 90-100% of saturation [1,2]
	• pH should be between 6-8.5 for freshwater species and 7.5-8.5 for saltwater species [1,2]
	• Dilution water in which total organic carbon (TOC) > 2 mg/L [OCSPP Guidance – 1,2] should not be used (U.S. EPA Guidelines recommends limit of >5 mg/L – 5)
	  o Unless data show that TOC or particulate matter do not affect toxicity [5], or the study is examining effects of TOC on toxicity		

	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 $CaCO_3$): Salinity (ppt): Total Organic Carbon (mg/L): Dissolved Organic Carbon (mg/L):	

	Parameter	Details	Remarks
For Controlled Experiments Only	**Aeration:** • Acceptable to maintain dissolved oxygen at 60 - 100% saturation at all times [1,2,4] • Avoid aeration when testing highly oxidizable, reducible and volatile materials [4] • Turbulence should be minimized to prevent stress on test organisms and/or re-suspend fecal matter [1,2,4] • Aeration should be the same in all test chambers at all times [4] • Generally not recommended. Only permitted when D.O. levels are in danger of falling below 60% saturation [1,2]	____ 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)*		
	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 (Water or Dietary):
	• When used, a carrier solvent should be kept to a minimum concentration [4]
	  ○ Should be restricted to situations where no other acceptable method of media preparation is available [3]
	• 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 [6]
	• Should not exceed 0.1 mL/L [1-3]
	  ○ Solvent concentration as low as 0.02 mL/L recommended [1-3]
	• 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:*

	Parameter	Details	Remarks
For Controlled Experiments Only	**Other Control:** *If any (e.g. solvent control)*		
	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. [4] • This maximum loading would 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: o Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [4,9] o Unionized ammonia does not exceed 35 µg/L [4] o Uptake by test organisms does not lower test material concentration by > 20% [4] o 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: o Static tests: > 0.8 g/L (lower temperatures); > 0.5 g/L (higher temperatures) [1,4] o 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) [4] • > 0.5 g/L/day or > 5 g/L at any time (all temperatures) [1,2] • Lower temperatures are defined as the lower of 17˚C or the optimal test temperature for that species [6]		

	Parameter	Details	Remarks

For Controlled Experiments Only	**Feeding:**
	• Unacceptable for acute tests [1,5]
	  o Should not be fed for 24-48 hours before test initiation [1]
	  o Exceptions:
	  ▪ Data indicate that the food did not affect the toxicity of the test material [5]
	  ▪ Test material is very soluble and does not sorb or complex readily (e.g., ammonia) [5]
	• Feeding during chronic tests should be appropriate to the species and size of the test organisms [2]
	  o Should be adjusted during the test to account for size and number of individuals per chamber [2]
	  o Feeding levels should be identical across treatment levels [2]
	  o Should observe food consumption and any bacterial development, which should be avoided [2]
	  o Fish should not be fed during the final 24 hours of a test [2]
		_____ Yes _____ No	

	Lighting: • Depends on the type of test (acute or chronic) and endpoint (e.g., reproduction) of interest. • Light levels between 540-1080 lux (50-100 foot candles) that are constant throughout the test are recommended [1,2] • Constant photoperiod between 12 light: 12 dark and 16 light: 8 dark recommended [1,2] • Newly hatched larvae should be kept in the dark (except for inspection) for one week [2] • Artificial light cycles should have a 15 – 30-minute transition period to avoid stress due to rapid increases in light intensity [1,2,4]		

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** • EC_{50} based on percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus percentage of organisms killed [5] o If not available, the 96-hr LC_{50} should be used [5] **Chronic** • Life-cycle/Partial Life-cycle test: o Survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability [5] • Early life-cycle test: o Survival and growth [5]	*List parameters:*	
Was control survival acceptable? **Acute** • ≥ 90% control survival at test termination [5] **Chronic** • ≥ 80% control survival at test termination [5]	____ Yes ____ No Control survival (%):	
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* • Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)* • Were concentration-response data estimated from graphs study publication or supplemental materials? • Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete* ***Verification of Statistical Results (Part C)*** *for sensitive*	____ Yes ____ No ____ Yes ____ No ____ Tables ____ Graphs ____ Supplemental Files ____ Yes ____ No *If yes, indicate software used:* ____ Yes ____ No Requested by: Request date: Date additional data received:	

Parameter	Details	Remarks
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 LC_{50}, LT_{50} 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:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

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. Add rows as needed. First row in italicized text is an example.*

Table C.II.1 Additional Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[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 850.1075: Freshwater and saltwater fish acute toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-007. October 2016.
2. U.S. EPA. 2016b. OCSPP 850.1400: Fish early life stage toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-008. October 2016.
3. U.S. EPA. 2016c. 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 729, 1980. 2002. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. ASTM International, West Conshohocken, PA.
5. 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.
6. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
7. OECD 203. 1992. Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264069961-en.
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.

Attachment F Aquatic Invertebrate Data Evaluation Record (DER) Template (September 2024)

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

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

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 these 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)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration[b] (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)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L or µg/g)	Verified Chronic Value[b] (mg/L or µg/g)	Chronic Value Endpoint	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., LC_{50}, EC_{20}, 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-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-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]			
[LC_x]			
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						
[EC_x]						
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]		

Parameter	Details	Remarks
• *Hyalella azteca* (chronic exposure) o Generally, 7 - 8 days old [11] • Freshwater mussels (chronic exposure) o Generally, 2 month old juveniles [12] • Oysters (C. virginica) o 30-50mm valve height and as similar in size as possible (<20% coefficient of variation) [3] • Mysids o < 24-hr post release [4,9] • Penaeid shrimp o 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.*

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	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] o Longer durations acceptable if test species not fed and had acceptable controls [10] • Freshwater mussel glochidia should be a maximum of 24 hours [12] o 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] o 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 o 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):*	

	Parameter	Details	Remarks
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.*

	Parameter	Details	Remarks
For Controlled Experiments Only	**Acclimation/Holding:** • Should be placed in a tank along with the water in which they were transported [9] o Water should be changed gradually to 100% dilution water (usually 2 or more days) [5,9] o For wild-caught animals, test water temperature should be within 5°C of collection water temperature [9] o Temperature change rate should not exceed 3°C within 72 hours [9] • Test specific recommendations: o Mysids: should be maintained in dilution water 48 hours before test, with < 1°C/day variation in water temperature [4] o Gammarids: should be acclimated to dilution water conditions over 48 hours, then held an additional 7 days [2] o Bivalves: Brood adults should be held for at least 14 days in test dilution water prior to spawning [6] o *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: o Organisms should not be crowded [9] ▪ See "Biomass/Loading Rate" for guidance on holding densities o Water temperature variation should be limited o Dissolved oxygen: ▪ Maintain between 60 - 100% saturation [9] ▪ Continuous gentle aeration if needed [9] o Unionized ammonia concentration in holding and acclimation waters should be < 35 µg/L [9] o 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] o If between 5-10%, holding should be extended an additional 7 days [2,3,5,6]	Duration: Feeding: Water: Temperature (°C): Dissolved Oxygen (mg/L): Health (*any mortality observed?*):	*Identify number of individuals excluded from testing and/or analysis (if any):*
	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: o Should minimize sorption of test chemical from water [9] o Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [9] o 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] o 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: o Required for some species (e.g., *Hyalella azteca*) [11] o Common types: stainless steel screen, nylon screen, quartz sand, cotton gauze and maple leaves [11] o More inert substances preferred over plant material, since plants may break down during testing and promote bacterial growth [11] o 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] o 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: Size: Fill Volume: Substrate Used *(if applicable)*:	*Briefly describe the test vessel here*
	Test Solution Delivery System/Method: • Flow-through preferred for some highly volatile, hydrolyzable or degradable materials [10] o Concentrations should be measured often enough using acceptable analytical methods [10] • Acute *C. virginica* shell deposition test must be flow through [3] • Chronic exposures: o Flow-through, measured tests required [10] o 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]
	  o Clean surface water, ground water, reconstituted water, or natural or artificial seawater (for saltwater species) are acceptable [1-7]
	  o Dechlorinated tap water should not be used as some forms of chlorination difficult to adequately remove [1,2,7,8]
	  o 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]
	  o Recommended hardness <250 mg/L (preferably <180 mg/L); or 40-50 mg/L for metals [1,2,7]
	  o 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]
	  o Recommended salinity 20±2 ‰ [3-6]
	  ▪ For *C. virginica* test with unfiltered seawater, recommended salinity >12±5‰ [3]
	  o 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)
	  o 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*
	  o Reconstituted waters should have at least 0.02 mg bromide/L; natural ground or surface water presumed to have sufficient bromide [11]
	  o 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 $CaCO_3$): 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] o 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	

	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]
	  o 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]
	  o 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: o Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [9,15] o Unionized ammonia does not exceed 35 µg/L [9] o Uptake by test organisms does not lower test material concentration by > 20% [9] o 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: o Static or renewal tests: > 0.8 g/L (lower temperatures); > 0.5 g/L (higher temperatures) [9] o >1 Daphnid / 20 mL [1] o >1 Daphnid / 40 mL for chronic renewal tests [7] o >30 Mysids / L [4] o >30 bivalve embryos/mL [6] o >0.8 g/L [2,5] o 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] o > 0.5 g/L/day or > 5 g/L [1,2,4,5,7] o 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] o 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] o 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: o EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed [10] • Embryo/larva (bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones): o EC_{50} based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed [10] ▪ If available, the 96 hour EC_{50} based on the percentage of organisms with incompletely developed shells and the 96-hr LC_{50} should also be reported separately [2] • Freshwater mussel (glochidia and juveniles): o Glochidia: EC_{50} 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] o Juvenile: EC_{50} based on percentage exhibiting foot movement within a 5-min observation period [12] • All other species and older life stages: o EC_{50} 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 LC_{50} should be used [10] **Chronic** • Daphnid: o Survival and young per female [10] • Mysids: o Survival, growth and young per female [10]	*List parameters:*	
Were controls acceptable? **Acute** • ≥ 90% control survival at test termination [10] o Glochidia 90% after 24 hours, or, the next longest duration less than 24 hours that had at least 90% survival [12] o ≥70% normal oyster embryos, or ≥60% normal hard clam or mussel larvae at the end of the test [6] o ≥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] o ≥80% in 42 day test with *Hyalella azteca*, slightly lower in tests substantially longer than 42 days [11] o ≥ 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 (%):	

Data Evaluation Record on the Effects of [Chemical] on Aquatic Invertebrate [Species]

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 LC_{50}, LT_{50} 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:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

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 Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[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

10. 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.
11. U.S. EPA. 2016b. OCSPP 850.1020: Gammarid amphipod acute toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-012. October 2016.
12. U.S. EPA. 2016c. OCSPP 850.1025: Oyster acute toxicity test (Shell deposition). Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-010. October 2016.
13. U.S. EPA. 2016d. OCSPP 850.1035: Mysid acute toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-011. October 2016.
14. U.S. EPA. 2016e. OCSPP 850.1045: Penaeid acute toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-009. October 2016.
15. U.S. EPA. 2016f. OCSPP 850.1055: Bivalve acute toxicity test (Embryo-larval). Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-006. October 2016.
16. U.S. EPA. 2016g. OCSPP 850.1300: Daphnid chronic toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-16-005. October 2016.
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Attachment G Aquatic Nonvascular Plant Data Evaluation Record (DER) Template (September 2024)

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

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

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 these 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)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration[b] (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)	Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L)	Verified Chronic Value[b] (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., LC_{50}, EC_{20}, 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-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-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]			
[LC_x]			
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.*

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	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.*

	Parameter	Details	Remarks

For Controlled Experiments Only	**Acclimation/Culturing:**
	• Should be incubated under test conditions [1]
	• Should be used when still growing exponentially [1]
	  o Water should be changed gradually to 100% dilution water (usually 2 or more days) [4]
	  o Temperature change rate should not exceed 2°C during acclimation or testing [1]
	• To avoid unnecessary stress and promote good health:
	  o Organisms should not be crowded [1]
	  o 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]
	  o 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]
	  o Stock algal cultures should be shaken to prevent clumping (at least daily) [1]
	  o 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]
	  o 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:
		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?*):
			Identify number of individuals excluded from testing and/or analysis (if any):

	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]
	  o Acceptable covers include foam plugs, stainless steel, glass, or plastic screw caps [1]
	  o 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:
	  o Should minimize sorption of test chemical from water [1,9]
	  o Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical
	  o May not contain substances that inhibit the growth of test organisms [1]
	  o 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]
	  o 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:
		
		Size:
		
		Fill Volume:
			Briefly describe the test vessel here

	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]
	  o 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]
	  o 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 o ~ 7.5 for most freshwater algal species [1] o ~8 for *Skeletonema* spp. [1] o Recommend measuring at start and end of test [1] • Temperature o 24-25 ºC for most species [1] o 20ºC for *Skeletonema* spp. [1] o 22-24 ºC for *Champia parvula* reproduction test [7] • Salinity o 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 $CaCO_3$): 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] o 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 10^4 cells/mL for *R. subcapita* (formerly *S. capricornatum*), *N. pelliculosa*, *A. flos-aquae*; and 7.7 x 10^4 cells/mL for *S. costatum* [2] • Recommended ~5 x 10^3 - 10^4 cells/mL for *R. subcapita* , 2-5 x 10^3 *S. subspicatus*, 5 x 10^4 – 10^5 *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/m^2) for *Raphidocelis, Navicula, and Skeletonema* [2] • ~2.2 K lx for *Anabaena* [2] • Light should have PAR of ~60-70 µE/m^2/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** • EC_x, IC_x 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* • Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • • If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)* • Were concentration-response data estimated from graphs study publication or supplemental materials? Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete* ***Verification of Statistical Results (Part C)*** *for sensitive species*.	____ Yes ____ No ____ Yes ____ No ____ Tables ____ Graphs ____ Supplemental Files ____ Yes ____ No *If yes, indicate software used:* ____ Yes ____ No *If yes, requested by:* *Request date:* *Date additional data received::*	

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 LC_{50}, LT_{50} 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:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

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 Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[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

25. 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.
26. 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.
27. 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.
28. 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.
29. 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.
30. 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.
31. ASTM Standard E 1498, 1992. 2012. Standard guide for conducting sexual reproduction tests with seaweeds. ASTM International, West Conshohocken, PA.
32. 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.
33. 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.
34. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.

Attachment H Aquatic Vascular Plant Data Evaluation Record (DER) Template (September 2024)

Data Evaluation Record on the Effects of [Chemical] on Aquatic Vascular Plant [Species or Grouping]

Part A: Overview

I. Test Information

Chemical name:

CAS name: not provided CAS Number: not provided
Purity: not provided Storage conditions: not provided
Solubility in Water (units):

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

Primary Reviewer: _____ Date: _____ ____ EPA ____ Contractor (*Place X by One*)

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

CITATION: *Indicate: author(s), year, study title, journal, volume, and pages.*
(e.g., Antunes, P.M.C., M.L. Scornaienchi, H.D. Roshon. 2012. Copper toxicity to Lemna minor modelled using humic acid as a surrogate for the plant root. Chemosphere. 88 (4):389-394.)

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

-

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

-

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

_____ Acceptable for Quantitative Use
_____ Acceptable for Qualitative Use
_____ Not Acceptable for Use/Unused

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

-

- **Major Deficiencies (note any stated exclusions)**: *Check all that apply. Checking any of these items make the study "**Not Acceptable for Use**"*

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

-

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

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

General Notes:

-

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

-

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

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

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

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

General Notes:

-

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

ABSTRACT: *Copy and paste abstract from publication.*

SUMMARY*: Fill out and modify as needed.*

Acute:

Species (life stage[a])	Method[b]	Category[c]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration (mg/L)	Classification
												Quantitative / Qualitative / Unused

[a] e.g., seed, seedling, adult
[b] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved
[c] B=benthic, E=emergent, Sub=submerged

Chronic:

Species (life stage[a])	Method[b]	Category[c]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Chronic Limits	Reported Chronic Value (mg/L)	Verified Chronic Value (mg/L)	Chronic Value Endpoint	Classification
													Quantitative / Qualitative / Unused

[a] e.g., seed, seedling, adult
[b] S=static, R=renewal, F=flow-through, U=unmeasured, M=measured, T=total, D=dissolved
[c] B=benthic, E=emergent, Sub=submerged

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

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

General Notes: *For aquatic life criteria development, measured water quality parameters in the treatments nearest the toxicity test endpoint(s), e.g., LC_{50}, EC_{20}, 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 (℃)	*[1]*		
	[2]		
	j		
	j		
pH	*[1]*		
	[2]		
	j		
	j		
Other (e.g., hardness, salinity, DOC)	*[1]*		
	[2]		
	j		
	j		

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

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

-

Table A.II.2. Measured (and Nominal) Chemical Concentrations in Test Solutions/Media.
[Analytical Method] verification of test and control concentrations during an [X]-day exposure of [test organism] to [test substance] under [static renewal/flow-through] conditions.

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

[a]Non-Detect: 0 = measured and detected; 1=measured and not detected; if not measured or reported enter as such

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

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

-

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

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

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

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

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

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

-

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

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

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

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

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

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

-

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

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

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

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

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

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

-

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

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

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

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

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

I. Materials and Methods

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

-

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

-

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

Data Evaluation Record on the Effects of [Chemical] on Aquatic Vascular Plant [Species or Grouping]

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

Parameter	Details	Remarks
Species: Useful sites include: • https://www.itis.gov/ • https://www.fws.gov/endangered/ • https://www.fisheries.noaa.gov/find-species	Common or Group Name: Scientific Name:	North American species? ____ Surrogate for North American Taxon? ____ FIFRA 5 Species? ____ Is this species Threatened or Endangered? ____ *(Place X if applicable)*

Strain/Source:
• Obtained from laboratory culture or commercial source. [1]
  ○ Identification of clone is desirable [1]
• Obtained from unpolluted areas in the wild
  ○ If collected from the field, plants should be maintained in culture in the same medium as used for testing for a minimum of eight weeks prior to use. [2]
• *Oryza sativa* (rice) are obtained as seeds and can be kept in a cool area for one year [5]
• Must originate from same source and population [4, 5]
• Should not be used:
  ○ If contaminated by other organisms such as algae and protozoa [2]
  ○ If visible lesions or discoloration (chlorosis) [2]
  ○ If large number of plants with single fronds [2]		

Age of inoculum at Study Initiation: • EPA recommends 7-12 day old cultures for *Lemma spp.* [1] • *Oryza sativa* (rice) approximately 8-10 cm tall [5]		
Was growth recorded at regular intervals?	____ Yes ____ No *If yes, describe regular intervals:*	

Data Evaluation Record on the Effects of [Chemical] on Aquatic Vascular Plant [Species or Grouping]

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

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

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

For Controlled Experiments Only

Parameter	Details	Remarks

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

Acclimation followed published guidance? *Describe, if any*	____ Yes ____ No *If yes, indicate which guidance:*	
Test Type:	____ Acute ____ Partial Life Cycle ____ Chronic ____ Germination ____ Other *(please remark):*	

Data Evaluation Record on the Effects of [Chemical] on Aquatic Vascular Plant [Species or Grouping]

	Parameter	Details	Remarks

For Controlled Experiments Only	**Test Vessel:**
	• Test chambers should be loosely covered [1]
	  o Test vessels/covers should not create shadows or otherwise affect light levels. [1]
	• Test chamber material:
	  o Should minimize sorption of test chemical from water [1]
	  o Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [1]
	  o May not contain substances that inhibit the growth of test organisms. [1]
	• Test chamber type:
	  o Erlenmeyer flasks, crystallizing dishes, glass petri plates, or other container can be suitable.
	  ▪ Sizes between 250-1,000 mL are suggested. All vessels should be the same size. [1]
	  ▪ Vessels for *Lemna* tests should be ≥ 20 mm depth and ≥ 100 mL volume. [1]
	  ▪ Should be wide enough so that fronds in control vessel do not overlap (*Lemna* tests). [1]
	  o Plastic pots with drainage holes in the bottom are used for culturing and exposing other macrophytes. [5]
	• Size/volume should maintain acceptable biomass loading rates (see below)
		Material:
		
		Size:
		
		Fill Volume:
			Briefly describe the test vessel here

	Test Solution Delivery System/Method:	__Static __Renewal; Interval: __Flow-through: __Delivered to water or sediment? __Test concentrations measured?	
	Sediment Used (For Rooted Plants): • Origin (e.g., natural, artificial, field collected, reference) • Textural Classification (% sand, silt, clay) • Organic Carbon (%) • Geographic Location • Chemical quality confirmed?		

	Source of Dilution Water:
	• Freshwater hardness range should be <5 mg/L or <10% of the average (whichever is greater) [4]
	• Saltwater salinity range should be <2 g/kg or <20% of the average (whichever is greater) [4]
	• Dilution water must be characterized (natural surface water, well water, etc.) [8]
	  o Distilled/deionized water without the addition of appropriate salts should not be used. [7]
	• Dilution water in which total organic carbon or particulate matter exceed 5 mg/L should not be used [7]
	  o Unless data show that organic carbon or particulate matter do not affect toxicity. [7]		

	Dilution Series (*e.g., 0.5x, 0.6x, etc.*): • 0.667x or 0.5x is recommended. [1] • <0.25x not recommended. [1]		
	Water Pretreatment	____ Yes ____ No	
	Intervals of water quality measurement:		

	Parameter	Details	Remarks
For Controlled Experiments Only	**Dilution Water Parameters:** *Measured at the beginning of the experiment or averaged over the duration of the experiment (details of water quality parameters measured in test solutions should be included under the results section)* Recommendations: • pH o 6.5 for *L. minor* tests and 7.5 for *L. gibba* tests. [1] o Recommend measuring at beginning and end of test. [1] • Temperature o EPA and OECD recommend 24-25 ºC (±2 ºC) for *Lemna spp.* tests ▪ Can be measured in growth medium of extra chambers during test. o ASTM recommends 20-30°C for other macrophytes [5]	Dissolved Oxygen (mg/L): Temperature (°C): Light:dark cycle: pH (test initiation): pH (test termination): Hardness (mg/L as $CaCO_3$): Salinity (for marine plants, ppt): Total Organic Carbon (mg/L): Dissolved Organic Carbon (mg/L): Chelator used: Carbon source:	
	Aeration or Agitation: (Describe if yes) • Aeration not recommended unless appropriate for the test substance [7]	____ Yes ____ No	
	Describe Preparation of Test Concentrations: (Indicate how test material was added to the growth medium (e.g., added directly or used stock solution)		
	Test Chemical Solubility in Water: List units and conditions (e.g., 0.01% at 20ºC)		
	Were concentrations in water or nutrient medium verified by chemical analysis? *Measured test concentrations should be reported in* Table A.II.2 *above.*	____ Yes ____ No *Indicate media:*	
	Were test concentrations verified by chemical analysis in tissue? *Measured test concentrations can be verified in test organism tissue alone if a dose-response relationship is observed.* *Measured test concentrations should be reported in* Table A.II.2 *above.*	____ Yes ____ No *Indicate tissue type:*	*If test concentrations were verified in test organism tissue, was a dose-response relationship observed?*
	Were stability and homogeneity of test material in water/nutrient medium determined?	____ Yes ____ No	

	Parameter	Details	Remarks
For Controlled Experiments Only	**Solvent/Vehicle Type**: • When used, a carrier solvent should be kept to a minimum concentration. [4] • Should not affect either survival or growth of test organisms (e.g., N, N-dimethyl-formamide recommended for *Lemma* spp. instead of acetone). [1] • Should be reagent grade or better [4] • Should not exceed 0.1 ml/L, unless it was shown that higher concentrations do not affect toxicity [1]		
	Negative Control:	____ Yes ____ No	
	Reference Toxicant Testing:	____ Yes ____ No *If Yes, identify substance:*	
	Other Control: *If any (e.g. solvent control)*		
	Biomass Loading Rate:		
	Feeding: • Nutrient medium added during renewal tests?	____ Yes ____ No	
	Lighting: • Lighting conditions should be consistent with conditions during culturing/acclimation [1, 2] • Should be measured in the growth chamber at the same distance from light source as test plants [1] • Should be measured before, after, and at least once during test [1] • Day:night period should be appropriate to the test species (e.g., continuous lighting recommended for *Lemna spp.*) [1] • Fluorescent lighting recommended [1, 2] • OECD recommends 6,500-10,000 lux (85-125 $\mu E/m^2/s$) for *Lemna spp*. test. [2] • EPA recommends 4,200-6,700 lux (57-90 $\mu E/m^2/s$) for *Lemna spp*. test. [1] • Should not change by more than 15% during test [2] ▪ Measure daily if suspected		

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

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

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

_____ Study Design Acceptable for Quantitative Use

_____ Study Design Acceptable for Qualitative Use

_____ Study Design Not Acceptable for Use

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

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

Parameter	Details	Remarks
Parameters measured including sublethal effects/toxicity symptoms: **Growth** • EC_x, IC_x based on growth inhibition (e.g., biomass, frond reduction, etc.) relative to control. [1, 5] Reproduction • Seed germination, seedling production, etc. [5] **Other Endpoints** o Chlorophyll a, pigment content, etc. [5]	*List parameters:*	
Was control growth acceptable? • Change in frond number, area, size (e.g., *Lemna*)? • How was acceptable control growth determined?	____ Yes ____ No	
Were controls acceptable? • Relative increase in frond number or size (e.g., *Lemna*) [1] • Growth rate, etc.	____ Yes ____ No *If yes, describe justification provided:*	
Were individuals excluded from the analysis?	____ Yes ____ No *If yes, describe justification provided:*	
Additional observations • Unusual colors [1] • Differences in chloroplast morphology [1] • Changes in test solution appearance (e.g., clarity, films, precipitates, etc.) [1] • Flocculation, clumping, adhering to test containers [1] • Was crowding observed? [1]		
Was water quality in test chambers acceptable? • If appropriate, describe any water quality issues (e.g., EPA and OECD recommend temperature of 24-25 ºC (±2 ºC) for *Lemna spp.* tests) [1, 2]	____ Yes ____ No	
Availability of concentration-response data: • Were treatment level concentration-response data included? (specify endpoints in remarks) • Were replicate level concentration-response data included? (specify endpoints in remarks) • How was concentration-response data presented? (check all that apply) • Were concentration-response data estimated from graphs study publication or supplemental materials? • Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete **Verification of Statistical Results (Part C)**.*	____ Yes ____ No ____ Yes ____ No ____ Tables ____ Graphs ____ Supplemental Files ____ Yes ____ No *If yes, indicate software used:* __Yes __No Requested by: Request date: Date additional data received:	

Part C: Statistical Verification of Results

I. Statistical Verification Information: *Report the statistical methods (e.g., EPA TRAP, BMDS, R, other) used to verify the reported study or test results for the five (5) most sensitive genera and sensitive apical endpoints. Report the statistical methods (e.g., EPA TRAP, BMDS, R, other) used to verify the reported study or test results and calculate point estimates (including for tests where such estimates were not provided). If concentration-response data were provided, include here along with all figures and tables associated with the statistical verification of the results and toxicity values. If values for the LC_{50}, LT_{50} and NOEC are greater than the highest test concentration, use the ">" symbol.*

Primary Statistical Reviewer: **Date**: **___ EPA** **___ Contractor** (*Place X*)

Secondary Statistical Reviewer: **Date**: **___ EPA** **___ Contractor** (*Place X*)
(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:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

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:

- *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*
- *Model assessment output (including all model figures, tables, and fit metrics)*
- *Statistical code used for curve fitting*

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

Table C.II.1 Additional Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[a] N = number of individuals per treatment; k = number of replicates per treatment level; n = number of individuals per replicate

35. U.S. EPA. 2012. OCSPP 850.4400: Aquatic plant toxicity test using *Lemna spp*. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-008. January 2012.
36. OECD. 2002. Test No. 221. *Lemna sp*. Growth Inhibition Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. 22 pp.
37. 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.
38. 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.
39. ASTM Standard E 1841-04. 2012. Standard Guide for Conducting Renewal Phytotoxicity Tests with Freshwater Emergent Macrophytes. ASTM International, West Conshohocken, PA. 10 pp.
40. U.S. EPA. 2012. OCSPP 850.4600: Rhizobium-legume toxicity. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-004. January 2012.
41. 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.
42. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.

Attachment I Amphibian Data Evaluation Record (DER) Template (September 2024)

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

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

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 these 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 Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Relative Humidity	Effect	Reported Effect Concentration (mg/L)	Verified Effect Concentration[b] (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 Method[a]	Test duration	Chemical / Purity	pH	Temp. (°C)	Hardness (mg/L as $CaCO_3$) or Salinity (ppt)	DOC (mg/L)	Relative Humidity	Chronic Limits	Reported Chronic Value (mg/Lor µg/g)	Verified Chronic Value[b] (mg/L or µg/g)	Chronic Value Endpoint	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

U.S. EPA OW AMPHIBIAN DER

Part A: Overview

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., LC_{50}, EC_{20}, 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 (℃)	*[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-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
j							

[a]Non-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 Stage[b]]	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: o 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)*		

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	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] o For wild-caught animals, test water temperature should be within 5˚C of collection water temperature [2] o Temperature change rate should not exceed 3°C within 72 hours [2] • To avoid unnecessary stress and promote good health: o Organisms should not be crowded [2] o Water temperature variation should be limited (e.g., <3°C in any 12 hour period) [2] o Water dissolved oxygen: ▪ Maintain between 60-100% saturation [2] ▪ Continuous gentle aeration if needed [2] o 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: o Should minimize sorption of test chemical from water [2] o Should not contain substances that can be leached or dissolved in solution and free of substances that could react with exposure chemical [2] o 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] o 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: Size: Fill Volume:	*Briefly describe the test vessel here*

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 $CaCO_3$): 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]
	  o 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]
	  o 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: o Dissolved oxygen is at least 60% of saturation (40% for warm-water species) [2,9] o Unionized ammonia does not exceed 35 µg/L o Uptake by test organisms does not lower test material concentration by >20% [2] o 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: o Static tests: >0.8 g/L (lower temperatures); >0.5 g/L (higher temperatures) [2] o 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] o 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]
	  o 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
	  o Embryos should be incubated under dim incandescent lighting (≤20 fc) or total darkness during early life-stage toxicity testing
	  o 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/m^2) 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
• EC_{50} 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 LC_{50} 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:* ____ Yes ____ No	
• Should additional concentration-response data be requested from study authors?	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 LC_{50}, LT_{50} 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:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

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 Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[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

43. 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.
44. 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.
45. 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.
46. 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.
47. 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.
48. 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.
49. Stephan, C.E. 1995. Review of results of toxicity tests with aquatic organisms. Draft. U.S. EPA, MED. Duluth, MN. 13 pp.
50. 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.
51. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.

Attachment J Avian Data Evaluation Record (DER) Template (September 2024)

Part A: Overview

I. Test Information

Chemical name:

CAS name: CAS Number:
Purity: Storage conditions:
Solubility in Water (units):

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

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., Heinz, G. H. 1979. Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. J. Wildl. Manage. 43(2): 394 – 401.)

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 these items make the study "**Not Acceptable for Use**"*

_____ Mixture (for controlled experiments only)
_____ No Controls (for controlled experiments only)
_____ Excessive Control Mortality (dependent on test type and species)
_____ Diet not adequately characterized
_____ Bioaccumulation: steady state not reached
_____ Dermal or Injection Exposure Pathway
_____ Review paper or previously published without modification
_____ Other: *(if an, list here)*

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.*

Species (lifestage)	Duration	Exposure Media[a]	Measured–M; Unmeasured–U; Form Measured[b]	Chemical Form Exposure	WW / DW / FWW[c]	Moisture Content (%)	Test Endpoint and Effect[d]	Reported Effect Concentration (µg/g or ppm)	Verified Effect Concentration[e] (units)	Classification
										Quantitative / Qualitative

[a] Diet, tissue type, etc.
[b] In addition, note if maternal transfer (MT)
[c] WW=wet weight, DW=dry weight, FWW=fresh wet weight.
[d] Where Test Endpoint = ECx, NOEC, LOEC, MATC, etc., and Effect = growth, survival, reproduction, etc.
[e] 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**".*

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

General Notes:

-

Table A.II.1. Measured Test Condition Parameters.

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
Photoperiod	*[1]*		
	[2]		
	j		
	j		
Temperature (°C)	*[1]*		
	[2]		
	j		
	j		
Humidity (%)	*[1]*		
	[2]		
	j		
	j		
Other (e.g., ventilation, lighting)	*[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-Detect[a]	Number of Samples Below Non-Detect	[Standard Deviation or Standard Error]	Range
Control							
[1]							
[2]							
[3]							
[4]							
[5]							
[6]							
J							

[a]Non-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 [Adult/Offspring] [Weight] (units)	Sample Size	[Standard Deviation or Standard Error]	Mean Growth [Adult/Offspring] [Length] (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 Clutches]	Sample Size	[Standard Deviation or Standard Error]	[Mean Clutch Size]	Sample Size	[Standard Deviation or Standard Error]	[Mean Number of Hatchlings]	Sample Size	[Standard Deviation or Standard Error]	[Mean Number of Fledglings]	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] Per EPA's Ecological Effects Test Guidelines - OCSPP 850.2300: Avian Reproduction Test, the following general requirements apply to controls.

(ii) For a satisfactory test, the following values for response variables in controls should be met or at least approached at test termination. There is likely to be a problem with test procedures or conditions that should be investigated and corrected when these values are not met.

(A) **Eggs lai**d - Normal values for both northern bobwhite and mallards are 29 to 61 eggs per hen for a 10 week egg laying period.

(B) **Eggs cracked** - Normal values for northern bobwhite are 0 to 7.0% of eggs laid. Normal values for mallards are 0 to 4.0% of eggs laid.

(C) **Fertility (viable embryos)** - Normal fertility values for northern bobwhite and mallards are 80 to 100% of eggs set.

(D) **Live 18-d or 21-d northern bobwhite and mallard embryos, respectively (as a percentage of viable embryos**) - Normal values for northern bobwhite are 97 to 100%. Normal values for mallards are 94 to 100%.

(E) **Hatchability (percentage of 18-d or 21-d northern bobwhite and mallard embryos, respectively that hatch**) - Normal values for northern bobwhite are 85 to 100%. Normal values for mallards are 52 to 100%.

(F) **Percentage of eggs set that hatch** - Normal values for northern bobwhite are 71 to 95%. Normal values for mallards are 44 to 92%.

(G) **14-day-old survivors of eggs hatched** - Normal values for northern bobwhite are 77 to 100%. Normal values for mallards are 94 to 100%.

(H) **Eggshell thickness** - Normal average values for northern bobwhite are 0.20 to 0.24 mm. Normal values for mallards are 0.316 to 0.372 mm.

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: • May be laboratory-reared or purchased from a breeder [1-3] • All birds should be from the same source and breeding population [1-3] • Test birds should be phenotypically indistinguishable (except for size) from wild stock [1-3]		
Age at Study Initiation: • Acute test: Young adults of both sexes, not mated, at least 16 weeks old [1] • Dietary test: Not too old to be able to avoid eating (e.g., mallard – 5 days old, bobwhite quail – 10-14 days old [2]) • Reproduction test: approaching first breeding season, at least 16 weeks old, all within 1 month age [3]		
Was body weight or length recorded at test initiation and/or at regular intervals? • For field observations, was body weight measured in a consistent manner (e.g., during blood sample collection)	____ Yes ____ No	
Was body weight or length recorded at regular intervals?	____ Yes ____ No *If yes, describe regular intervals:*	

Data Evaluation Record on the Chronic Effects of [Chemical] on Avian [Species]

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

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	Number of Replicates per Treatment Group: • Acute and Dietary tests: 1-2 per treatment level [1,2] • Reproductive test: 16 per treatment level [3]	Control(s): Treatment(s):	
	Number of Birds per Replicate/Test Condition: • Acute and Dietary tests: at least 10 per treatment level (equal numbers from each sex) [1,2] • Reproductive test: one pair (1 M, 1 F) [3]		
	Body Condition: • Birds should be healthy without excess mortality [1-3] • Deformed, abnormal, sick, of injured birds should not be used [1-3] • Birds used in a previous test, or offspring of birds used in a test treatment group, should not be used [3]	Good: Poor: Number of individuals excluded from analysis:	
	Exposure Pathway: • Should be dietary exposure [3]		
	Exposure Duration:		
	Exposure Time:	____ Breeding ____ Non-breeding ____ Year round	
	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 test conditions are being properly maintained [4]		
	Test Concentrations (remember units): *Recommended test concentrations include at least two concentrations other than the control; three 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?		

EGG COLLECTION AND INCUBATION (*if applicable*):

	Parameter	Details	Remarks
For Both Controlled Experiments and Field Observations	Collection Interval: • Recommend daily [3]		
	Egg Storage Conditions: • Temperature recommend 13-16ºC (55-61ºF) [3] • Relative humidity recommend 55-80% [3]	Temperature: Relative humidity:	
	Were eggs candled for cracks prior to setting for incubation?	____ Yes ____ No	
	Were eggs set weekly?	____ Yes ____ No	
	When was candling done to check for fertility?		
	When were eggs transferred to the hatcher?		
	Hatching Conditions: • Temperature recommend 37.5-39ºC (100-102ºF) [2,3] • Relative humidity recommend ~70% [2,3]	Temperature: Relative humidity:	
	What day was hatched eggs removed and counted? *(e.g., removed on day 27)*		

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

	Parameter	Details	Remarks
For Controlled Experiments Only	**Acclimation Period:** • Recommend at least 2 weeks [1,3] • Dietary test: 3 days – mallard, 7 days – bobwhite quail [2] • Acute test: Should not be if mortality during acclimation >5% (lab, breeder) or 10% (wild) [1] • Dietary test: Should not be used if >5% mortality during acclimation • Reproduction test: Should not be used if >3% dead or debilitated during acclimation [3]		
	Acclimation followed published guidance? *Describe, if any*	____ Yes ____ No	
	Food Type: • Recommend commercial feed or nutritional equivalent [1-3]		
	Test Chemical Solubility in Water: • List units and conditions (e.g., 0.01% at 20ºC)		
	Solvent/vehicle Type: • Recommended solvents include (acetone, methylene chloride, table grade corn oil, propylene glycol, gum arabic) [3] • Should not comprise more than 2% of diet by weight [3] • Should be completely evaporated before feeding [3] • Equivalent amount should be added to control diets [3]		
	Negative Control:	____ Yes ____ No	
	Reference Toxicant Testing:	____ Yes ____ No *If Yes, identify substance:*	
	Other Control: *If any (e.g. solvent control)*		
	Describe preparation of test diet:		
	Were concentrations in diet verified by chemical analysis?	____ Yes ____ No	
	Indicate whether stability and homogeneity of test material in diet determined:	____ Yes ____ No	
	Indicate if the test material was regurgitated/avoided:	____ Yes ____ No	
	Pen Size: • Acute test: At least 75 in.^{2} / bird (quail) or 150 in.^{2} / bird (mallard) surface area [1] ○ Should be at least 9.5 in. height (quail) or 12.5 in. height (mallard) [1] • Dietary test: At least 50 in.^{2} / bird (quail) or 100 in.^{2} / bird (mallard) surface area, and pens should be arranged to prevent cross contamination [2] • Reproductive test: should be of sufficient size to prevent stress, and pens should be arranged to prevent cross-contamination [3] • Outdoor pens should only be used during breeding season.[3]		

	Parameter	Details	Remarks
For Controlled Experiments Only	**Number of Birds per Pen:** • Acute and Dietary tests: at least 10 per pen (equal numbers from each sex) [1,2] • Reproductive test: one pair (1 M, 1 F) [3]	Male: Female:	
	Number of Pens per Group/Treatment: • Acute and Dietary tests: 1 or 2 per treatment 1,2] • Reproductive test: 16 per treatment level [3]	Control: Treatment:	
	Test Conditions: • Recommended temperature: o Adults: ▪ 15-27ºC (59-81ºF) [1] ▪ 15-30ºC (59-86ºF) [3] o Hatchlings: ▪ 22-38˚C (72-100ºF) [2] ▪ 22-35˚C (72-95ºF) [1,3] • Relative humidity recommend 45-70% [1-3]	Temperature: Relative humidity: Photoperiod:	
	Feeding: • Should be administered *ad libitum* throughout the study [1-3]		
	Lighting: • All pens should receive equal illumination [1-3] • Acute and Dietary tests: indoor lighting recommended [1,2] o Incandescent of fluorescent acceptable [1,2] o 14 Light: 10 Dark photoperiod recommended for most species [1,2] ▪ Should be adjusted as appropriate for test species [1] o Light intensity not specified [1,2] • Reproductive test. o Should be full spectrum simulating daylight (avoid shorter wavelength "cool-white" fluorescent) [3] o Photoperiod should be acceptable for the test and species [3] o Recommended illumination (10-65 lux) [3] o Outdoor lighting acceptable but not recommended [3]		

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
Parental: *(e.g., mortality, body weight, mean feed consumption)* [1-3]	*List parameters:*	
Reproductive Success: *(e.g., eggs laid/pen, nestlings produced, juvenile body weight)* [3]	*List parameters:*	
Was control survival acceptable? • Unacceptable if >10% control birds dead or moribund [1-3]	____ Yes ____ No Control survival (%):	
Were individuals excluded from the analysis?	____ Yes ____ No *If yes, describe justification provided:*	
Was test condition parameters acceptable? ***(see notes under Reproductive Effects of Results Section for test validity considerations) [1-3]***	____ 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* • Were replicate level concentration-response data included in study publication (can be from tables, graphs, or supplemental materials)? *specify endpoints in remarks* • If treatment and/or replicate level concentration-response data were included, how was data presented? *(check all that apply)* • Were concentration-response data estimated from graphs study publication or supplemental materials? • Should additional concentration-response data be requested from study authors? *If concentration-response data are available, complete* ***Verification of Statistical Results (Part C)*** *for sensitive species*.	____ Yes ____ No ____ Yes ____ No ____ Tables ____ Graphs ____ Supplemental Files ____ Yes ____ No *If yes, indicate software used:* ____ Yes ____ No Requested by: Request date: Date additional data received:	

Part C: Statistical Verification of Results

I. Statistical Verification Information: *Report the statistical methods (e.g., EPA TRAP, BMDS, R, 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 LC_{50}, LT_{50} 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:

EC_5:
EC_{10}:
EC_{20}:
EC_{50} or LC_{50}

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 Data Used in Dose-Response Curve.

Curve ID	Species	Endpoint	Treatment	Replicate	[Standard Deviation or Standard Error]	# of Survivors	N[a]	k[a]	n[a]	Response	Response Unit	Conc	Conc units
Alchronic1	*Ceriodaphnia dubia*	*# of young/female*	*0*	*6*			*10*	*10*	*1*	*18*	*count*	*0.03*	*mg/L*

[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

52. U.S. EPA. 2012a. OCSPP 850.2300: Avian acute oral toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-025. January 2012
53. U.S. EPA. 2012b. OCSPP 850.2300: Avian dietary toxicity test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-024. January 2012
54. U.S. EPA. 2012c. OCSPP 850.2300: Avian reproduction test. Ecological effects test guidelines. Office of Chemical Safety and Pollution Prevention. EPA 712-C-023. January 2012
55. American Public Health Association (APHA). 2012. Standard methods for the examination of water and wastewater. Part 8000 - Toxicity. APHA. Washington, DC.