

Quality Assurance Project Plan (QAPP) for the State Water Resources Control Board *Ceriodaphnia dubia* Study - Second Interlaboratory Comparison Study

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Title and Approval Sheet

Title: Quality Assurance Project Plan (QAPP) for the State Water Resources Control Board *Ceriodaphnia dubia* – Second Interlaboratory Comparison Study

Prepared by: the Southern California Coastal Watershed Research Project

QAPP Approval Date: _____

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1 PROJECT MANAGEMENT

1.1 TITLE AND APPROVAL SHEET (QA/R-5 ELEMENT A1)

See page i.

1.2 TABLE OF CONTENTS (QA/R-5 ELEMENT A2)

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1.3 DISTRIBUTION LIST (QA/R-5 ELEMENT A3)

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1.4 INTRODUCTION AND PROJECT ORGANIZATION (QA/R-5 ELEMENT A4)

This Quality Assurance Project Plan (QAPP) was developed according to United States Environmental Protection Agency (EPA 2002a, 2016) guidance to inform the implementation of the Interlaboratory Study (ILS) component of the California State Water Resources Control Board (SWB) study titled *Development of Quality Assurance Recommendations for the Ceriodaphnia Toxicity Test* (SWB *C. dubia* study). The ILS was informed by Southern California Coastal Watershed Research Project (SCCWRP) discussions with an Expert Science Panel (ESP) and a Stakeholder Advisory Committee (SAC), ASTM (1996) *Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method*, and the EPA (2001a,b) *Interlaboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods*.

Roles and responsibilities for individuals needed to implement task planning, management and oversight, sampling/sample preparation, sample analysis, and data management for the ILS are summarized below. SCCWRP is implementing this work on behalf of the SWB in coordination with commercial and municipal laboratories.

Co-Project Manager /Technical Coordinator – Dr. Alvina Mehinto is the SCCWRP project manager responsible for overall project approval and the lead coordinator responsible for the distribution of this QAPP, providing updates to all participants and corresponding with the Stakeholder Advisory Committee and Expert Science Panel.

Co-Project Manager – Ken Schiff is responsible for SCCWRP’s oversight of the project to ensure that the needed resources are available to complete this project.

Quality Assurance (QA) Officer – Darrin Greenstein will oversee the preparation and shipping of all samples and conduct quality assurance oversight of the tests performed by the laboratories to ensure submitted data are accurately reported and meet quality criteria described in this QAPP.

Biostatistician – Drs David Gillett (SCCWRP) and Jing Zhang (Miami University) are the biostatisticians knowledgeable regarding statistical procedures needed to meet the goals of this study and with the materials being tested in order to ensure that data to be collected will meet the statistical needs to achieve the study goals. The biostatisticians will also assist in interpreting the results of data analysis.

Data Manager – Paul Smith will coordinate data compilation and will perform calculations to evaluate data.

Sixteen laboratories participated in the SCCWRP’s historical data and SOPs request. Among them, ten (10) laboratories have committed to participating in the ILS (**Appendix A**). These laboratories represent current and former Environmental Laboratory Accreditation Program (ELAP) accredited laboratories; thus, they are a reasonable representation of qualified laboratories applicable to toxicity testing in California (i.e., they are or recently were ELAP accredited for the test method) and this subset can adequately characterize test reproducibility even if a few do not participate. The ILS is not restricted to a group of laboratories judged to be exceptionally qualified and equipped for the ILS (ASTM 1996).

Laboratory Coordinators – Each participating laboratory will identify a laboratory coordinator to oversee their implementation of testing associated with this ILS. They will also coordinate communication between the laboratory and the SCCWRP Technical Coordinator and Quality Assurance Officer.

Laboratory QA Manager – Each participating laboratory will identify a QA manager to ensure that testing is conducted in accordance with laboratory SOPs and testing protocols described in this QAPP. They also perform QA reviews of submitted data to confirm that results are accurately reported and meet quality criteria described in this QAPP.

1.5 PROBLEM DEFINITION AND BACKGROUND (QA/R-5 ELEMENT A5)

This section describes the rationale for this project and relevant background information.

1.5.1 SWB *C. dubia* Study

The SWB adopted Resolution No. 2020-0044¹ in 2020 to establish the *Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California and Adopting Toxicity Provisions* (Toxicity Provisions). Public comments at the SWB adoption hearing on December 1, 2020, communicated that dischargers were primarily concerned with interlaboratory variability for the chronic *C. dubia* test (achieving the same test result among multiple laboratories testing the same sample) and incorrect indications of toxicity on non-toxic samples.

To address some of the stakeholders' concerns, the SWB is funding a *C. dubia* QA evaluation study, facilitated by SCCWRP, that aims to provide laboratory technique guidance to: (a) improve the consistency of the execution of the *C. dubia* test method within each testing laboratory; and (b) improve the consistency and comparability of *C. dubia* test results among testing laboratories, while retaining the necessary flexibility for environmental relevance. The ILS represents one of the tasks being conducted as part of the SWB *C. dubia* study.

The SWB *C. dubia* study is divided into four primary tasks. These are described in the Conceptual Workplan (SCCWRP 2021b) and summarized below. The second ILS described by this QAPP is Task 4.

The QAPP describes the approach, overall methodology, and logistics that will be used to conduct the study.

- Task 1 – Establish a governance structure for the study (completed).
- Task 2.1 and 2.2 – Inventory of protocols and historical control data for the *C. dubia* chronic WET test from ELAP accredited laboratories (completed).
- Task 2.3 – A baseline ILS was conducted using split samples to assess inter- and intra-laboratory variability (described in this QAPP). The split sample analysis data was used to supplement historical data analyses and develop the approach for Task 3 (completed).

¹ In 2021, the State Water Board adopted Resolution No. 2021-0044 to rescind the December 1, 2020, establishment of water quality control plan for inland surface waters, enclosed bays, and estuaries of California and confirmed that the “Toxicity Provisions” were adopted as state policy for water quality control for all waters of the state.

- Task 3 – Training and education was conducted in the form of: (1) Visit to four laboratories by members of the Science Panel. These visits served to further explore the differences in culturing, feeding, and testing that exist among the participating labs (completed). (2) Roundtable workshop attended by the participating laboratories and members of the Science Panel to discuss specific techniques in culturing, feeding, testing and documentation. The workshop resulted in a list of standardized laboratory techniques to implement in a second interlaboratory testing exercise (completed).
- Task 4 – A second round of split-sample interlaboratory testing will be conducted to determine if recommendations developed in Task 3 are successful in reducing inter- and/or intra-laboratory variability among a wide range of laboratories.
- Task 5 – A final report will be prepared that describes study results and recommendations for reducing inter- and intra-laboratory variability.

1.6 PROJECT DESCRIPTION (QA/R-5 ELEMENT A6)

This section summarizes the work to be performed and any decision(s) to be made or outcomes expected from information to be obtained.

1.6.1 Study Goals and Objectives

The overall goal of this study is to build on previous efforts and investigate a variety of possible sources of variability in the *C. dubia* reproduction test conducted by testing laboratories currently or recently accredited for the *C. dubia* test method. The primary objectives of the SWB *C. dubia* study, in collaboration with stakeholders and laboratories, are as follows.

- 1) Evaluate laboratory performance among those currently and recently accredited by the state of California (i.e., ELAP).
- 2) Investigate factors that can lead to inter- and intra-laboratory test variability and decrease confidence in assessments of toxicity.
- 3) Recommend specific guidance for laboratory techniques to improve laboratory performance reduce intra- and inter- laboratory variability (SWB 2020a, SCCWRP 2021a, 2022).

The ILS is intended to address the following question from the ESP and SAC.

Does standardizing lab practices improve consistency and comparability in *C. dubia* test results?

1.6.2 Approach

The second ILS testing is summarized in this section to provide an overview and rationale for the planned approach. A detailed study plan and data quality objectives (DQO) are provided in **Appendix B and C**. The study will assess the toxicity of different samples provided by SCCWRP using the chronic *C. dubia* WET test (EPA 2002b) and compare the results with those of the baseline ILS. The study design, developed by the ESP and previously used in the baseline ILS, focuses on *C. dubia* reproductive endpoints (i.e., 25 and 50% inhibition concentrations, IC 25 and IC50). Test samples were selected to assess interlaboratory agreement in estimated IC values and the impact of different dilution water recipes on test outcome. Ten out of the 12 laboratories that participated in the baseline ILS will participate in this exercise.

Samples

The three sets of samples to be tested as part of this ILS are described below and in **Table 1**.

- Sample 1: Moderately hard dilution water recipe #1 (EPA MH) to be tested at full strength (i.e., 100%). This sample shall be tested along with one (1) laboratory control consisting of the lab's own dilution water recipe.

Sample 1 will be prepared using EPA MH water following the protocol described in the EPA manual. This recipe is currently used by two (2) of the 10 participating labs. The other laboratories are either supplementing the EPA MH water with selenium and/or vitamins in various quantities, using the modified EPA method or using mineral water. Due to the issues documented with the use of EPA MH alone by several laboratories (i.e., low reproduction and poor culture viability), this recipe was not selected for the dilution series. This sample will be used to compare intra- and inter-laboratory performance.

- Sample 2A: Moderately hard dilution water recipe #2 (Perrier) to be tested at full strength (i.e., 100%). This sample shall be tested along with one (1) laboratory control consisting of the lab's own dilution water recipe.
- Samples 2B, 2C, 2D, 2E, 2F: 5 concentrations of sodium chloride (NaCl; 2000, 1000, 500, 250, 125 mg/L) diluted in moderately hard water recipe #2, Perrier water. All dilutions will be prepared at SCCWRP. Therefore, the samples shall be tested as is (i.e., no additional sample dilution allowed) along with one (1) laboratory control consisting of the lab's own dilution water recipe.

Samples 2A-F will use Perrier water, a recipe used by four (4) of 10 participating laboratories to prepare the dilution series with NaCl. The concentrations of NaCl were determined based on previous work from the EPA and historical data from the participating laboratories using this salt as their reference toxicant. Sample 2A will be

tested with a separate laboratory control than will samples 2B-F. This is to ensure that individual laboratories generate sufficient control data to assess intra-laboratory variability. However, laboratories will treat samples 2A-F as one test to be conducted simultaneously using blocked randomization from the same known parentage. Samples 2A-F will be used to assess interlaboratory agreement for *C. dubia* reproductive endpoints IC25 and IC50.

- Sample 3: NaCl will be provided (14 g as a solid) to each lab with instructions to prepare 5 dilutions using the lab’s own dilution water. This serial dilution will be tested along with one (1) laboratory control consisting of the lab’s own dilution water recipe.

Participating laboratories are currently using different types and concentrations of reference toxicant, limiting our ability for inter-laboratory comparison across a large number of laboratories. Here, Sample 3 will replace the reference toxicant and laboratories will prepare a dilution series with NaCl (i.e., 0, 125, 250, 500, 1000 and 2000 mg/L) using their own control water. Data generated will be used to evaluate the impact of dilution water and lab techniques on reproductive endpoints.

Table 1. Second Interlaboratory study tests to be conducted by participating laboratories.

Sample Type	Number of Samples	Dilution Series	Number of Rounds	Number of Labs
Sample 1- dilution water recipe #1 (EPA MH)	1	No	3	10
Sample 2A- dilution water recipe #2 (Perrier)	1	No	3	10
Sample 2B-F- NaCl in dilution water recipe #2 (Perrier)	5	No	3	10
Sample 3- NaCl solid sample (to be diluted by the labs using their own dilution water)	1	Yes	3	10

The second ILS testing will be conducted with three rounds of tests (i.e., repeat testing with newly prepared samples in each of three rounds of testing). This approach will yield up to 120 test results (4 tests per batch x 3 batches x 10 participating laboratories), as shown in **Table 1** above.

Sample Preparation and Testing

Sample preparation and testing will be conducted as follows.

- New samples will be prepared by SCCWRP for each round of testing and allowed to equilibrate for 48 hours. Samples will be prepared with the same procedures and equipment used for the baseline ILS.

- The water samples will be split into cubitainers for distribution to the laboratories. The original bulk sample and each split sample will be analyzed for alkalinity, conductivity, hardness, and pH prior to shipment to document consistency with the parent sample and among the splits.
- Samples will be maintained at $4^{\circ}\text{C} \pm 2$ prior to use. Samples will be stored with headspace minimized.
- Laboratory testing will be conducted promptly (i.e., within 24 hours of receiving the split samples) so that sample holding times are not exceeded.
- The ILS will be conducted over a seven-week period.
- Chronic *C. dubia* testing will include:
 - Static exposures with daily renewal using the single cubitainer supplied by SCCWRP.
 - 10 replicate chambers per sample/dilution concentration.
 - Survival and reproduction (i.e., neonates per female) will be documented daily.
 - **Each sample will be tested with a separate laboratory control** (i.e., the standard control water used by each laboratory). One control will be used for samples tested as a dilution series.
 - Laboratories will collect and archive a sample of their standard control water at test initiation.

Additionally, all laboratories will follow the standardized test parameters and lab techniques described below:

- Limit the age of adults used to start the test to 6-10 days old.
- Use < 24 hr old neonates produced within an 8-hr window. Record which brood number (e.g., 3rd, 4th, or 5th) is used to start the test.
- Independently quantify initial food density (both algae and YCT) and document density in test chambers to ensure that it meets EPA protocols requirements (i.e., do not use concentrations reported by the supplier). Use **Appendix D** to record all information requested including method for YCT total suspended solids and algal density. For accurate volumes, use a repeat pipettor for direct feeding in test cups or a volumetric pipette if feeding in test solutions.
- Follow holding times of YCT ≤ 8 days after thawing and algae ≤ 21 days (from time of receipt if purchased or from production if cultured in-house). Use one batch for an entire test round.
- Evaluate brood board health for 2 weeks prior to testing using a common set of health criteria provided by SCCWRP (see **Appendix E**). Organisms should be out of mass culture for at least two weeks prior to testing.

- Conduct the tests for 8 days (i.e., 192 hours).
- Use randomization by blocking of known parentage AND randomization of cups on the test board. Test set-up must use the randomized blocking by known parentage, using 10 randomly selected brood board chambers with a minimum of 8 neonates from the adult on test initiation day. Each test will be treated as independent for blocking by known parentage except for samples 2A and 2B-F. These two tests will be treated as one for the purposes of blocking by known parentage. For randomization on the test board, follow the guidance provided in **Appendix F**.
- Renew or terminate test boards daily at 24 hours within a 2-hr window (i.e., plus or minus one hour) from test initiation.
- Document split broods on bench sheets at the time of observation is required (see **Appendix G**). Labs may rely on the final evaluation of combining broods by the laboratory QA officer or Supervisor.
- All reagents must be stored in a desiccator from time of receipt.

Laboratory Documentation

The following information will be collected and submitted by each laboratory to SCCWRP in electronic format.

- Measured alkalinity, conductivity, dissolved oxygen (DO), hardness, pH, and temperature for each sample upon receipt.
- Counts of males, unhealthy (including description of the abnormalities) and dead adults, and dead neonates in the brood boards used to set up the tests (see **Appendix E** for guidance).
- Specific beginning and end time window for age of neonates at test initiation (hours and minutes).
- The specific time of daily test solution renewal (hours and minutes).
- Water quality parameters (dissolved oxygen, air and water temperature, pH, and conductivity,) measured before and after daily renewal, following the laboratories' routine procedures. The laboratories will document their procedures including lab techniques used for sample collection (surrogate cup or pooled sample from multiple test cups), analysis (including measurement devices), and volume of sample needed. It is also recommended that the laboratories measure water temperature continuously.
- Detailed observations supporting the determination of split broods, including number and size of neonates, and females' movement and presence of eggs in pouch (see **Appendix F**).
- Record of when the tests would have ended if the lab was using their own SOP.

- Dilution water recipe and preparation dates will be reported.
- Water treatment system. Fill in the checklist provided in **Appendix H**.
- Food source (in-house or purchased), recipe and creation date for each food batch, as well as feeding procedure (in individual cups or in dilution water).
- Vendors for all reagents shall be documented.
- Light intensity measured once at test initiation within the location of each test.
- Air temperature measured twice daily at the location of the testing chambers.
- Control charts for reference toxicant testing over the last 6 months (i.e., October 2022 to March 2023). For laboratories conducting multiple reference toxicant tests per month, the first test of the month will be provided. Laboratories who have less than one test per month will submit whatever reference toxicant data they have during the last 6 months. Charts will include IC50 and IC25 data and a tabular listing of the individual IC values and confidence intervals.
- Scanned copies of the bench sheets. SCCWRP will provide bench sheets templates, however, laboratories may use their own bench sheets as long as they include all data categories found in the SCCWRP templates.

1.6.3 Possible Outcomes and Decisions

The ILS will produce a second dataset that can be compared to the baseline ILS data. A possible outcome is that standardization of the lab techniques (listed on page 7) reduces intra- and/or inter-laboratory variability. Such outcome will lead to recommendations to implement these standardized lab techniques to reduce sources of variability and improve consistency and comparability in *C. dubia* chronic toxicity test results. The SWB will make decisions regarding how to use any recommendations developed from this study.

1.7 QUALITY OBJECTIVES AND CRITERIA (QA/R-5 ELEMENT A7)

This section describes how data of known and acceptable quality will be developed to satisfy the study objectives.

1.7.1 Data Quality Objectives

A systematic project planning process, the seven-step Data Quality Objectives (DQOs) process, was used to determine study boundaries and specifications needed to develop a study that will support the qualitative and quantitative derivation of data, based on EPA (2006) guidance. DQOs are based on a seven-step process.

- Step 1: State the Problem

- Step 2: Identify the Goal of the Study
- Step 3: Identify Information Inputs
- Step 4: Define the Boundaries of the Study
- Step 5: Develop the Analytical Approach
- Step 6: Specify Performance or Acceptance Criteria
- Step 7: Develop the Plan for Obtaining Data

DQOs specify the data types, quality, quantity, and uses, as needed to make decisions, and are the basis for designing data and sample collection activities. These minimum standards are developed to provide data of known quality to address the study objectives. **Appendix C** presents DQOs for this ILS.

1.7.2 Data Quality Indicators

Performance and acceptance criteria are described in terms of Data Quality Indicators (DQIs). DQIs for this project consist of qualitative and quantitative indicators, including precision, accuracy, representativeness, completeness, and comparability.

Precision

Tests performed on presumably identical materials in presumably identical circumstances do not typically yield identical results. An indication of a test method's consistency is its precision – a measure of agreement for repeated analyses under similar conditions over a relatively short period of time. The CV is a simple statistic to describe precision by comparing the results of replicate analyses within a single lab (i.e., intra-laboratory precision or *repeatability*) or among different laboratories (i.e., inter-laboratory precision or *reproducibility*).

$$\text{Coefficient of Variation (CV)} = \text{standard deviation} / \text{mean}$$

The use of a CV removes units from the measurement and allows comparisons of variability among different types of test methods or toxicity endpoints (i.e., neonates per female and analytical chemistry). However, there is no common agreement on an acceptable standard deviation (EPA 2000).

The percent difference (RPD) is another measure of inter- or intra-laboratory variability among repeated measurements.

$$\text{Relative Percent Difference (RPD)} = ((M - MD) / \text{mean}) * 100$$

Where:

M: Measured value associated with the primary sample

MD: Measured value associated with the duplicate or replicate analysis

Mean: the mean of the two measurements $((M + MD)/2)$.

The percent minimum significant difference (PMSD) is a measure of test precision that quantifies within-test variability.

$$\text{Percent Minimum Significant Difference (PMSD)} = (100 \times (d \sqrt{\text{EMS}} \sqrt{2/r}) / \text{control mean})$$

Where:

d: Critical value of Dunnett's statistic when comparing "k" treatments to a control

EMS: Error mean square from the analysis of variance of the endpoint responses

r: Number of replicates at each concentration

Precision estimates should be obtained through the efforts of qualified laboratories and personnel operating under prevailing conditions when the test method is used in practice (ASTM 1996).

Accuracy

Accuracy is the degree of agreement between a measured value and the "true" or expected value. As such, it represents an estimate of total error from a single measurement, including both systematic error ("bias") and random error that may reflect variability because of imprecision. Accuracy is typically expressed in terms of percent recoveries determined from results of matrix spike/matrix spike duplicates, laboratory control standards, and ongoing evaluation of calibration verification information. Such measurements are appropriate for chemical analyses but not for toxicity testing, which is method dependent and, thus, is subject to the variability inherent among test organisms (EPA 2001a,b).

Representativeness

Representativeness is the degree to which sample data accurately expresses the characteristics of a population of samples, parameter variations at a sampling point, or an environmental condition. It is a qualitative parameter achieved through proper sampling program design and use of appropriate sampling strategies and techniques. For example, holding time requirements are intended to ensure the representativeness of conditions at the time the samples are collected. The use of quality control (QC) samples that are similar in composition to samples being measured

provides a means of estimating precision and accuracy that are representative of sample measurements. In addition to sample holding times, a factor that affects representativeness in the ILS study is split-sample homogeneity. This will be evaluated by comparing water quality measurements among split samples and comparisons with the parent sample.

Completeness

Completeness can be defined both qualitatively and quantitatively. Qualitative completeness is determined as a function of all factors that contribute to sampling. Quantitative completeness is calculated as the percentage of measurements that are judged to be valid compared to the total number of measurements planned. Effectively, it measures the amount of data available for valid measurements compared to the amount lost or destroyed, and it is strictly defined as the ratio of the number of usable data points over the possible number of data points, by method/matrix. A completeness goal for the second ILS is 100 percent.

Comparability

Comparability is a qualitative indicator of the confidence with which one data set can be compared to another. Confidence is achieved by maintaining standard techniques and procedures for collecting and analyzing representative samples and reporting the analytical results in standard units.

1.7.3 Measurement Quality Objectives

Laboratory QC results must meet the performance/acceptance limits detailed in the applicable measurement quality objectives (MQOs), or acceptance thresholds and goals, for chemical analyses and toxicity testing. These specify how much error is acceptable and are often expressed in terms of DQIs. Chronic *C. dubia* toxicity test conditions and test acceptability criteria (TAC) from EPA (2002b) are summarized in **Table 2**. DQIs for water quality measurements and methods are presented in **Table 3**. MQOs for chemical analyses conducted by SCCWRP, are presented in **Table 4**.

Table 2. Summary of test conditions and acceptability criteria for conducting chronic *C. dubia* toxicity tests.

Parameter	Measurement Quality Objective
Test type	Static renewal (required)
Test temperature (°C)	25.0 ± 1.0°C (recommended) Test temperatures must not deviate (i.e., maximum minus minimum temperature) by more than 3.0°C during the test (required)
Light quality	Ambient laboratory illumination (recommended)
Light intensity	10-20 µE/m ² /s or 50-100 ft-c (ambient laboratory levels) (recommended)
Photoperiod	16 h light, 8 h dark (recommended)
Test chamber size	30 mL (recommended minimum)
Test solution volume	15 mL (recommended minimum)
Renewals	Daily (required)
Age of test organisms	Less than 24 h; and all released within an 8-h period (required)
Number of organisms per replicate	One (1) per replicate. Assigned using blocking by known parentage (required)
Number of replicate test chambers per concentration	10 (required)
Feeding regime	Feed 0.1 mL each of YCT and algal suspension per test chamber daily (recommended)
Cleaning	Use freshly cleaned glass beakers or new plastic cups daily (recommended)
Control water	Uncontaminated source of receiving or other natural water, synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or DMW
Test Concentrations	All split water samples provided for this ILS will be tested without dilution by the laboratories. Sample 3 (provided as solid) will be tested in 5 dilutions (2000, 1000, 500, 250 and 125 mg/L)
Dilution Factor	
Test Duration	8 days (required)* Note that EPA (2002a) defines the test duration as “Until 60% or more of surviving control females have three broods (maximum test duration 8 days) (required)”
Endpoints	Survival and reproduction (required)
Test acceptability criteria	80% or greater survival of all control organisms and an average of 15 or more live young per surviving female in the control solutions. 60% of surviving control females must produce three broods (required)
Sampling requirements	A single split sample will be provided for testing.
Sample volume required	One (1) L/day (recommended)
Water quality measurements	Initial: DO, pH, temperature, conductivity, alkalinity, and hardness (required)

Table 2. Summary of test conditions and acceptability criteria for conducting chronic *C. dubia* toxicity tests.

Parameter	Measurement Quality Objective
	Daily before and after renewal: DO, pH, air and water temperature, and conductivity (required); continuous water temperature measure (recommended)
	Final: DO, pH, temperature, conductivity, alkalinity, and hardness (required)
Aeration	None; unless DO falls below 4.0 mg/L or the sample has high likelihood to have substantial BOD
<p>Notes: Source: EPA 2002b (Method 1002.0) Each test condition listed above is identified as required or recommended for the purposes of reviewing test data submitted under NPDES permits. These requirements are applicable to the routine test procedures that would apply under the Toxicity Provisions and will be followed for the ILS. *ILS specific requirement BOD – Biological Oxygen Demand DO – dissolved oxygen DMW – demineralized water ILS – Interlaboratory study NPDES – National Pollutant Discharge Elimination System EPA – United States Environmental Protection Agency YCT – Yeast, Cerophyl®, trout chow</p>	

Table 3. Water quality measurements and methods to be used by the participating testing laboratories.

Parameter	Method ¹	Units	Accuracy	Precision ²	Resolution ³
Dissolved Oxygen	Meter or probe	mg/L	± 0.5	± 0.5 or 10%	± 0.1
pH	Meter or probe	pH units	± 0.2	± 0.2	± 0.1
Conductivity	Meter or probe	µS/cm @25°C	± 2	± 2 or 10%	± 1
Temperature	Meter, probe, or HOBO	°C	± 1	± 1 or 10%	± 0.1
Alkalinity	Titration or spectrophotometric	mg/L CaCO ₃	± 2	± 2 or 10%	± 1
Hardness	Titration or spectrophotometric	mg/L CaCO ₃	± 2	± 2 or 10%	± 1
<p>Notes: Source: Conventional Parameters in Fresh and Marine Water https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/mqo/conventional_parameters_in_fresh_water_and_marine_water.pdf Notes: Readings should be recorded with significant figures as shown in the resolution column. ¹ Method used must be documented in the data submission form. ² Precision is defined by the Relative Percent Difference (RPD). This is the difference between two repeated measurements expressed as a percentage. %RPD = (sample result – duplicate result) / sample result x 100. ³ Resolution refers to the capability of a method or instrument to recognize small differences between values. This term is often used to assess if an instrument or method is useful to a study and is provided by the manufacturer.</p>					

Table 4. Chemical measurements and methods for analyses of aqueous samples by the analytical lab.

Parameter	Method	Units	Method Detection Limit	Reporting Limit
Alkalinity, Total	SM2320B	mg/L	1.0	5.0
Ammonia, Total	SM 4500-NH3	mg/L	0.03	1.0
Hardness, Total	EPA 200.7	mg/L	0.19	1.0

All samples will be held at 4°C in the dark until analyzed. No additional preservation is needed.

1.8 TRAINING AND CERTIFICATION REQUIREMENTS (QA/R-5 ELEMENT A8)

ILS participants will have appropriate training and experience conducting their tasks, including conducting the short-term chronic *C. dubia* toxicity test, data collection, data analysis, and reporting. Toxicity testing laboratory staff will be current on all training protocols required for implementing the *C. dubia* test, conducting water quality measurements, and record keeping. These activities will be performed according to their own laboratory SOPs except for select standardized lab techniques and additional data collection required by this QAPP (e.g., extending the test duration through day-8 may differ from standard laboratory practices, counting live and dead young, etc.). Toxicity testing laboratories will be currently or recently accredited by ELAP to conduct all testing procedures they are assigned to perform.

1.9 DOCUMENTATION AND RECORDS (QA/R-5 ELEMENT A9)

Laboratory documentation and data reporting requirements required by participating laboratories are detailed in *Toxicity Data Submittal Instructions* (**Appendix I**).

All data, reports, and documents submitted to SCCWRP will be stored in electronic format as scans of hard copies and Microsoft Excel files saved in a database that can be made publicly available after completion of the project. Similar to the policy used for the historical data collected and baseline ILS, the identity of individual laboratories will be kept anonymous in the database and proprietary information will not be shared with the public.

The SCCWRP Co-Principal Investigator/Project Manager will also ensure that each participating laboratory and SCCWRP personnel participating in this ILS are provided with any updated versions of this QAPP to ensure that ILS protocols and data reporting requirements are consistently followed.

2 DATA GENERATION AND ACQUISITION

2.1 EXPERIMENTAL DESIGN (QA/R-5 ELEMENT B1)

This section will refer to SCCWRP's *Second ILS Study Plan and Logistics* (**Appendix B**) to describe the approach for ILS testing that was summarized in Section A6 – Project Description.

2.2 SAMPLING METHODS (QA/R-5 ELEMENT B2)

This section describes the methods for preparing the samples, dilution waters, and dilution series; subsampling for chemical analysis; splitting and distributing samples.

2.2.1 Production of Waters for Testing and Sampling

Two types of water will be produced for this testing. Water made using the EPA moderately hard (EPAMH, sample 1) formula will be used as one of the unspiked dilution water recipes. The EPA MH water will be produced by a contracted laboratory. The water will be made using the formula in the EPA manual without addition of selenium. SCCWRP will pick up the water for each testing round in a 20-gallon plastic carboy. A carboy will be delivered to the laboratory ahead of each round of testing. The total volume needed will be 20 gallons for each round. Once the water is transported to SCCWRP it will be allowed to equilibrate at 4°C in the walk-in fridge for 2 days before being transferred to cubitainers for the individual laboratories.

A second dilution water recipe will be prepared at SCCWRP using the “Perrier” method. This method will be used to produce sample 2A (unspiked) and to prepare a dilution series using sodium chloride as the spiking agent (samples 2B-F). The Perrier water will be made in a large plastic tank with a 20:80 mix of Perrier to MilliQ water. The water will be stirred on a large vortex mixer and aerated overnight. The water will then be equilibrated in the 4° C walk-in fridge for an additional day before being spiked with sodium chloride. The total needed for all samples using this recipe will be about 100 gallons. This will require approximately 20 gallons of Perrier.

2.2.2 Spiking

The most concentrated sample of the dilution series (Sample 2B; 2000 mg/L NaCl) will be created by dissolving NaCl (VWR Life Science, High Purity Grade, CAS 7647-14-5) into the “Perrier” water. The spiking will be done in 20-gallon carboys fitted with a spigot. Four additional concentrations will be produced (samples 2C-F; 1000, 500, 250, 125 mg/L NaCl) using a 1:1 dilution (i.e., 50%). The samples will be mixed for an hour on a large vortex mixer

and then allowed to equilibrate in the $4^{\circ}\text{C} \pm 2$ in the walk-in fridge for ~72 hours before being transferred to cubitainers for the individual laboratories.

For sample 3, 14.00 g of NaCl (same source as above) will be weighed and placed in 100 mL HDPE containers. Each laboratory will receive one container per round. Laboratories will be instructed to dilute the supplied NaCl in 7.0 L with their control dilution water and perform a 50% dilution series to generate a total of 5 dilutions (2000, 1000, 500, 250, and 125 mg/L).

2.3 SAMPLE HANDLING AND CUSTODY (QA/R-5 ELEMENT B3)

This section describes sample handling procedures and considerations.

Split test samples will be held at SCCWRP at $4^{\circ}\text{C} \pm 2$ for up to 72 hours before shipping. Samples will be shipped to each laboratory (see Appendix A) according to the schedule agreed upon with the participating laboratories. Samples will be shipped on wet ice using priority overnight courier service (i.e., OnTrac or FedEx Priority Overnight). The shipments will also include chain-of-custody (COC) forms completed by SCCWRP, which will reference the study plan and testing instructions. SCCWRP will notify the laboratories via email once the samples are in transit and provide a tracking number.

The cubitainers must be maintained at $4^{\circ}\text{C} \pm 2$ prior to use and may be held for up to 48 hours after being collected from the parent sample before first use.

If a sample is not delivered to a laboratory by 2 p.m. on the expected arrival date or if the sample has spilled during shipment, the laboratory must contact SCCWRP promptly. SCCWRP will ship new samples that same day. These replacement samples must be used to begin tests within 24 hours of receipt (i.e., within 48 hours of when the initial samples were collected from the original bulk sample).

A laboratory will be given an opportunity to retest a sample if acceptability criteria are not met. However, both the failed test and retest data must be submitted to SCCWRP. A laboratory planning to retest must contact SCCWRP within 24 hours of knowing that a test failed acceptability criterion. Laboratories are encouraged to retest with remaining sample. However, arrangements could be made to retest with the archived sample provided by SCCWRP, if possible.

2.4 ANALYTICAL METHODS (QA/R-5 ELEMENT B4)

2.4.1 Toxicity Tests

C. dubia toxicity testing will be performed according to EPA (2002b, 2016) guidance for test method 1002.0. Specific test conditions are described in **Table 2** with the exceptions described in Section 1.5.3 and **Appendix B**. Note if any SOPs have been updated since June 2022, laboratories shall provide the updated documents to SCCWRP. Study-specific procedures are intended only to collect additional data (e.g., extending the test through day-8 regardless of when the typical test termination criterion is met) and should not result in any changes to the normal test implementation. Labs must count and report the live young daily and may report any dead young separately as well. Any dead young that are noted should be reported in the comments field of data submission template. Labs should also document when split broods are detected and report their observations in the comments field of data submission template. An animal that dies before producing young, if not identified as a male, will be included in the analysis with zero (0) to represent the number of neonates. Thus, the resulting number of live neonates per female combines the survival and reproduction endpoints. Split broods may occur if the adult when transferred has not released all her young. Young should be compared for size for potential of split broods and data recorded as such in the event a split brood is detected. Outlier analyses will also be performed as described by EPA guidance, and test results may be reported with and without the outlier.

Toxicity test results will be described by SCCWRP as the percent survival and neonates per female. Concentration response relationships may also be described for dilution series by determining the no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC), and a point estimates to determine the IC25 and other relevant percent effect levels as described by EPA (2002b in Appendix M). The type of concentration response, such as those describe by EPA (2000b), will also be identified, and reported.

2.4.2 Analyses of Water

Water quality measurements (i.e., conductivity, DO, pH, and temperature) by each toxicity testing laboratory will be conducted according to each laboratory's SOPs and conform with the quality criteria described in Table 3. Water samples submitted to an analytical laboratory will be analyzed according to the methods, target MDLs, and target RLs listed in **Table 4**.

2.5 QUALITY CONTROL (QA/R-5 ELEMENT B5)

This section describes QC sample requirements and their frequency of collection. **Table 5** presents laboratory quality control samples for *C. dubia* toxicity testing. **Table 6** presents quality control samples for chemical analyses.

Table 5. Quality control samples for the second interlaboratory *C. dubia* toxicity tests.

Quality Control Sample	Description	Required	Sampling Frequency / Rationale
Negative Control	Laboratory controls will be prepared by each toxicity laboratory to (1) assess acceptable performance of the organism in the absence of a contaminant and (2) compare to organism performance in test samples.	Yes	Concurrent testing with each batch at each toxicity laboratory is required by the EPA (2002b) method.
Positive Control	A reference toxicant test is a toxicity test with a dilution series with a consistent toxicant (e.g., NaCl) in standard dilution water. The test is repeated over time to assess the consistency or organism responses.	No	Participating laboratories are using different chemicals at different concentrations. Instead of running their reference toxicant, all laboratories will provide their control charts for the last 6 months (i.e., Oct 2022 to March 2023).

Table 6. Quality control (QC) samples for chemical analyses.

QC Sample	Description	Required	Sampling Frequency / Rationale
Duplicates	Samples collected to monitor the precision of sampling.	No	A duplicate for chemical analyses will be collected and analyzed at a frequency of 5% of the total project sample count.
Equipment Blank	Samples of deionized water passed through and/or over the surface of decontaminated sampling equipment to evaluate the potential for sample contamination from equipment.	No	No equipment is needed. Samples for chemical analysis should be decanted directly in the sample containers.
Laboratory Blank	To assess potential ambient sources of contamination associated with decontamination and sampling procedures	Yes	Laboratory blanks will be analyzed by the analytical lab where appropriate.

2.5.1 Toxicity Test

A laboratory control for toxicity testing is a negative control that describes *C. dubia* performance absent chemical stressors. This is required with each toxicity test to evaluate test acceptability criteria, as a basis for determining the relative percent effect in a sample, and for determining if there are statistically significant effects in a sample. The control also provides information on stock organism health and the normal variability in survival and reproduction of those stock organisms (SWRCB 2020b).

Each test will be run for eight days with daily neonate production, including split broods, reported by the laboratories. After QA evaluation of the data submitted, SCCWRP will determine the production of three broods for each test in a consistent manner. Total neonate production for each female will then be calculated from these three broods and used in subsequent analysis.

Variability of toxicity test results will be evaluated using repeated analyses of the same samples within a single laboratory to assess intra-laboratory precision (i.e., repeatability). This will be accomplished by repeated testing among multiple batches. Likewise, repeated analyses of the same samples among laboratories will be compared to assess inter-laboratory precision (i.e., reproducibility). The mean and CV among replicate analyses will be used to describe variability.

2.5.2 Chemical Analysis

Duplicate samples and blanks will be submitted for chemical analyses as indicated in **Table 6**. Equipment blanks are not required because no equipment will be needed to collect samples.

2.6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE (QA/R-5 ELEMENT B6)

Analytical instrument testing, inspection, maintenance, setup, and calibration will be conducted by the laboratories in accordance with the requirements identified in the laboratory's SOPs and manufacturer instructions. Each analytical method provides protocols for proper instrument setup, tuning, and critical operating parameters that will be followed. Instrument maintenance and repair will be documented by each laboratory in maintenance logs or record books to be available upon request.

2.7 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY (QA/R-5 ELEMENT B7)

Laboratory instruments will be properly calibrated, and the calibration will be verified with appropriate check standards and calibration blanks for each parameter before beginning each analysis. Instrument calibration procedures and schedules will conform to analytical protocol requirements and descriptions in the SOP of each participating laboratory.

Calibration standards will be obtained from a commercial vendor traceable back to the National Institute of Standards and Technology (NIST) where available and appropriate. Stock standards will be used to establish intermediate standards and calibration standards. Special attention will be given to expiration dates, proper labeling, proper refrigeration and storage, and prevention of contamination. Documentation relating to the receipt, mixing, and use of standards will be recorded in a laboratory logbook. All analytical laboratory calibration and spiking standards will

be confirmed against standards from another source, or as specified in the testing method and laboratory SOP. These data may be requested from the laboratories later.

2.8 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES (QA/R-5 ELEMENT B8)

The quality of supplies and consumables used during the ILS can affect data quality. All equipment that comes in contact with samples must be sufficiently clean and absent residues to prevent detectable contamination. Analyte concentrations must also be accurate in all standards used for calibration and quality control purposes.

Supplies and consumables needed to complete this second ILS include the following:

- Sufficiently large carboys are needed to hold the entire volume of each sample for all testing and analyses so that each bulk sample for each batch can be prepared in a single container.
- Deionized water or Milli-Q water® and reagent grade chemicals will be used to prepare synthetic dilution waters as described by EPA (2002b, 2016). These chemicals include NaHCO₃, CaSO₄*2H₂O, MgSO₄, KCl, NaCl, and Perrier® water (if demineralized water is required).
- New one (1) gallon cubitainers will be used to transport split samples to participating laboratories for toxicity testing.
- Coolers and double-bagged ice will be used to transport samples to each participating laboratory.
- *C. dubia* for toxicity testing will be provided from in-house cultures by each participating laboratory. Cultures producing fewer than 20 young/female (cumulative) or with adult mortality greater than 20% mortality will not be used (EPA 2002b, 2016).
- Control water and food recipes prepared according to each participating laboratory's SOPs, *C. dubia* food prepared according to each participating laboratory's SOPs will be provided by each participating laboratory.
- New sample containers for chemical analysis will be provided by SCCWRP to each participating laboratory.
- Laboratory water and cleaning solutions used for decontamination will adhere to standard SOPs for each laboratory and will be documented.

2.9 NON-DIRECT MEASUREMENTS (QA/R-5 ELEMENT B9)

Existing chemical and toxicity data from prior toxicity tests will be used for this study. Reference toxicity testing to determine the running mean and quality limits (i.e., 2 standard deviations from the running mean) from the last 12 months will be used to describe the normal range of *C. dubia* responses to a consistent stressor at each laboratory. Any existing data will be reviewed for quality assurance and acceptability prior to use in the SWB *C. dubia* study.

2.10 DATA MANAGEMENT (QA/R-5 ELEMENT B10)

Data for this ILS will be generated at analytical laboratories. Data developed by or provided to SCCWRP by participating laboratories will be maintained by SCCWRP electronically in a server location dedicated to the SWB *C. dubia* study. Files will include complete data reports provided by analytical and toxicity testing laboratories, copies of any sample preparation logs, COCs, toxicity testing bench sheets, photos, and communications among laboratory participants. Data files will be routinely backed-up and a copy of current data will be stored securely at a separate location maintained by SCCWRP.

3 ASSESSMENT AND OVERSIGHT

3.1 ASSESSMENT AND RESPONSE ACTIONS (QA/R-5 ELEMENT C1)

Laboratories will promptly report any deviations from this QAPP to SCCWRP. The need for corrective actions will then be determined by the SCCWRP Technical Coordinator, SCCWRP Project Manager, SCCWRP QA Officer, Laboratory Coordinator, and Laboratory QA Manager by evaluating the root cause of any deviations from this QAPP and determining the potential effects of the deviation on data quality. Corrective actions will provide specific guidance and/or requirements to remedy the cause of such deviations. The SCCWRP QA Officer will provide a summary of the deviation, root cause, and any needed corrective action to the SCCWRP project team before additional sampling is conducted. Corrective actions could include resampling and/or re-analyzing samples if, for example, hold times have not been exceeded, or if data quality indicators are not met.

3.2 REPORTING (QA/R-5 ELEMENT C2)

Data developed from this second ILS will be summarized and reported to the SAC and ESP and made available to the public. Initial toxicity and chemical analysis results from interlaboratory

testing will be summarized by SCCWRP and reported to the ESP and SAC within 60 business days following the complete submission of the testing data by the laboratories.

SCCWRP will provide a description of the tested samples, the range of toxicity test results (e.g., live neonates per female, percent difference from controls, IC25, etc.), and the range of test variables (e.g., water quality characteristics, dilution water type, and specific 8-hour age range of neonates within the <24-hour old test requirement) will be summarized and compared within and among laboratories. SCCWRP will describe factors associated with inter- and intra-laboratory test variability that could be the subject of targeted experiments to standardize select test parameters to minimize inter- and intra-laboratory variability, if any are identified. Any laboratory tests that do not meet test acceptability criteria or results that do not meet MQOs and are qualified or rejected will also be presented. Deviations from the QAPP by any laboratory, lessons learned, and any corrective actions will also be discussed in the report.

4 DATA REVIEW AND USABILITY

This section describes the data verification and validation procedures that occur after the data collection or generation phase of the project to ensure that data are sufficient to meet the project goals and objectives by complying with specified criteria.

4.1 DATA REVIEW, VERIFICATION, AND VALIDATION (QA/R-5 ELEMENT D1)

A review to verify and validate data quality will be performed by the laboratories after each testing event. These evaluations will identify any deviations from the QAPP and confirm that MQOs were met (e.g., COC forms were complete, sampling hold times were met, RLs were met). Corrective actions may be implemented if data requirements are not met.

4.2 VERIFICATION AND VALIDATION METHODS (QA/R-5 ELEMENT D3)

Data verification, confirming that the correct values are recorded and are transcribed accurately into EDFs, will be performed initially by each laboratory prior to transmitting any data packages to SCCWRP. Secondary data verification will be conducted by SCCWRP by spot checking data submittals at a rate of 5% of the toxicity results (i.e., 1 of every 20 electronic data records will be confirmed by checking the reported value with the toxicity bench sheet) to confirm that bench sheet data are correctly reflected in the electronic files or summary tables provided by testing laboratories. These checks will also confirm the following:

- Required analyses were conducted

- Expected methods were used
- Target RLs and MDLs were met
- Batch and sample identifications were reported correctly
- Units were reported correctly
- Quality control evaluation calculations were complete
- COCs were complete
- Data were reported according to the required format

Any data inconsistencies or questions identified by SCCWRP will be discussed with the laboratory and either a corrected data file or documentation of the explanation will be recorded in the project file.

Data validation will be performed by SCCWRP following the receipt of each batch of toxicity test data to ensure that the data developed meet data quality requirements and are adequate to meet the study goals. Validation will evaluate the following:

- Hold times were met
- Detections in lab control samples
- Exceedances of MQOs
- Toxicity test acceptability criteria were met
- Toxicity test requirements specific to this ILS (e.g., test set-up, duration, and data requirements) were met

Any toxicity test results where the controls do not meet test acceptability criteria defined in Table 2 will be rejected for use as valid tests. Although, these test results may still be used to identify test parameters that contribute to inter- and intra-laboratory variability and may be included in statistical analyses to meet the study goals. Likewise, toxicity data where all test requirements were not met shall be qualified but may still be included in analyses to identify test parameters that contribute to inter- and intra-laboratory variability. Causes of any failures to meet test acceptability criteria or test requirements will be reported to SCCWRP by the laboratory. The SCCWRP Technical Coordinator, SCCWRP Project Manager, SCCWRP QA Officer, Laboratory Coordinator, and Laboratory QA Manager will determine if and how qualified results may be used and SCCWRP will document any corrective actions that were implemented.

4.3 RECONCILIATION WITH USER REQUIREMENTS (QA/R-5 ELEMENT D3)

Data developed from this ILS will be evaluated to identify factors contributing to inter- and intra-laboratory test variability. Such factors, if identified, may inform targeted experiments to standardize test parameters with the intent of minimizing inter- and intra-laboratory variability as part of Task 3 of the SWB *C. dubia* study.

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Appendix A – Contact information for laboratories participating in inter-laboratory *C. dubia* chronic toxicity testing as part of the California State Water Resources Control Board *C. dubia* study.

ID#	Laboratory	Contact Name	Contact information	Shipping Address
1.	AQUA-Science	Kimberly Miller	530-753-5456 Kimberley@aqu-a-science.com	630 Cantrill Dr., Davis, CA 95618
2.	Aquatic Bioassay & Consulting Labs, Inc	Joe Freas	805-643-5621 x18 joe@aquaticbioassay.com	29 N Olive St., Ventura, CA 93001
3.	Aquatic Testing Laboratory	Joe LeMay	805 650-0546 jlemay12@pacbell.net	4350 Transport Street, Unit 107 Ventura, CA 93003
4.	Enthalpy	Peter Arth	858-587-7333 ext. 214 Peter.arth@enthalpy.com	4340 Vandever Avenue, San Diego, CA 92120
5*.	GEI	Natalie Love	303-264-1070 Nlove@geiconsultants.com	4601 DTC Boulevard, Suite 900, Denver, CO, 80237
6.	Inland Empire	Sushmitha Reddy	909-993-1813 Sreddy@ieua.org	IEUA Water Quality Laboratory, Building C, 6075 Kimbal Ave., Chino, CA 91708
7.	McC Campbell	Alex Anderson	925-252-9262 alexander.anderson@mcccampbell.com	1534 Willow Pass Road, Pittsburg, CA 94565-1701
8.	Pacific Ecorisk	Stephen Clark	707-207-7760 Slclark@pacificecorisk.com	2250 Cordelia Road. Fairfield, CA 94534
9*.	TetraTech	Marcus Bowersox	410-902-3142 Marcus.Bowersox@tetrattech.com	10711 Red Run Blvd., Suite 105, Owings Mills, MD 21117
10.	WSP Environmental Laboratory	Steve Carlson	858-299-5368 steve.carlson@wsp.com	4905 Morena Blvd, Ste. 1304, San Diego, CA 92117
Note: * Laboratory is not currently ELAP accredited				

Appendix B – Second ILS Study Plan and Logistics

GENERAL APPROACH

A three-step approach was proposed. During Step 1, all laboratories participated in an intercomparison exercise by testing a common set of split samples using their own protocols (as described in the 08/11/22 QAPP for the baseline ILS) and provide more detailed data that may not be routinely collected/reported by all laboratories. Based on the results of Step 1 and discussions between the Expert Science Panel, the Stakeholder Advisory Committee, and the participating laboratories, Step 2 aimed to develop a list of standardized *C. dubia* test parameters. Finally, Step 3 (described in the current QAPP) will consist of another intercomparison exercise among all laboratories using split samples and the standardized *C. dubia* toxicity testing protocol developed from Step 2. This appendix aims to provide an overview of the key study elements and logistics for Step 3 the second ILS testing.

OVERVIEW OF BASELINE TESTING PROCEDURE

The specific objective of the second ILS is to collect additional *C. dubia* chronic toxicity data and a more complete/consistent lab technique dataset across California-accredited laboratories. Ten (10) laboratories will participate in an intercomparison exercise consisting of several split samples tested in three separate testing batches. This testing design is proposed to generate a minimum of seven (7) control datasets per participating laboratory. This was determined statistically based on analyses of the width of the confidence interval to assess intra-laboratory precision. Our analyses indicated that the grand mean for control neonate production from 7 separate tests (each test performed with 10 replicates) would increase our confidence that such mean will fall within the historical control grand mean +/- 5 neonates.

Split samples to be tested include:

Sample 1: Moderately hard dilution water recipe #1 (EPA MH) to be tested at full strength (i.e., 100%).

This sample shall be tested along with one (1) laboratory control consisting of the lab's own dilution water recipe.

Sample 2A: Moderately hard dilution water recipe #2 (Perrier) to be tested at full strength (i.e., 100%).

This sample shall be tested along with one (1) laboratory control consisting of the lab's own dilution water recipe.

Sample 2B-F: 5 concentrations of sodium chloride (NaCl) diluted in moderately hard water recipe #2 (i.e., Perrier). All samples will be prepared at SCCWRP according to the procedure described earlier in the QAPP. These samples shall be tested as is (i.e., no additional sample dilution allowed) along with one (1) laboratory control consisting of the lab's own dilution water recipe.

Sample 3: NaCl will be provided (as a solid) to each lab with detailed instructions to prepare 5 dilutions using the lab's own dilution water. This serial dilution will be tested along with one (1) laboratory control consisting of the lab's own dilution water recipe. *Note that Sample 3 is now replacing the requirement for each lab to test their routine reference toxicant with each testing batch.*

SUMMARY OF STANDARD OPERATING PROCEDURES

Participating laboratories (n= 10) will analyze three separate test batches within a ~ 8-week window using their own standard operating procedures for the *C. dubia* chronic toxicity test. A summary of standard operating procedures (SOPs), test acceptability criteria (TAC) and measurement expectations are provided in **Table B1** and in the QAPP. All laboratories will be required to meet the following specifications:

- Limit the age of adults used to start the test to 6-10 days old.
- Use < 24 hr old neonates produced within an 8-hr window.
Record which brood number (e.g., 3rd, 4th, or 5th) is used to start the test.
Record the specific beginning and end time window for age of neonates at test initiation (hours and minutes).
- Evaluate brood board health for 2 weeks prior to testing using a common set of health criteria provided by SCCWRP (**Appendix E**).
- Use randomization by blocking of known parentage AND randomization of cups on the test board. Test set-up must use the randomized blocking by known parentage, using 10 randomly selected brood board chambers with a minimum of 8 neonates from the adult on test initiation day. Each test will be treated as independent for blocking by known parentage **except for samples 2A and 2B-F**. These two tests will be treated as one for the purposes of blocking by known parentage. For randomization on the test board, follow the guidance provided in **Appendix F**.
- Renew or terminate test boards daily at 24 hours within a 2-hr window from test initiation.
- Conduct the tests for 8 days (i.e., 192 hours). Record of when the tests would have ended if the lab was using their own SOP.
- Independently quantify initial food density (both algae and YCT, do not use numbers provided by the supplier) and describe feeding procedure (incl. quantification method and volume dispensed) to estimate food density in test cups and ensure that it meets EPA protocols requirements. Record all information and measurements in **Appendix D**. Use repeating pipettor or volumetric pipette for accurate volumes.
- Follow holding times of YCT \leq 8 days after thawing and algae \leq 21 days (from time of receipt if purchased or from production if cultured in-house).
- Document split broods on bench sheets at the time of observation including number and size of neonates, and observations of females' movement and presence of eggs in pouch (**Appendix G**).

Table B1. Summary of test conditions and test acceptability criteria (TAC) for the *Ceriodaphnia dubia* survival and reproduction test.

Parameter ¹	Description
Test organism	<i>Ceriodaphnia dubia</i>
Protocol	EPA/821/R-02-013, EPA 2002 Freshwater Chronic Manual, EPA, 2016 ErrataPA 821-R-02012-ES
Exposure	Static, daily renewal
No. replicate test chambers	10 replicates per sample/dilution
Sample holding time ²	Up to 48 hours before test initiation
Test duration	8 days, i.e., 192 hours
Endpoints	Survival and reproduction (number of neonates per female)
Laboratory control	One laboratory dilution water control per test sample
Water quality measurements	Daily: air and water temperature in °C, pH and dissolved oxygen in mg/L reported with 0.1 precision; conductivity in µS/cm. Continuous monitoring of water temperature, if possible. Upon receipt and test termination: hardness and alkalinity in mg/L CaCO ₃ Once during test in testing area: light intensity in foot-candles; air temperature in °C (0.1 °C precision)
Test Acceptability Criteria (TAC)	80% or greater survival and an average of 15 or more live neonates per surviving female in the controls at test termination (i.e., 8 days)

¹ Parameters and test conditions used in this study are suitable for investigative/non-compliance testing but may be different than those required for NPDES permit testing.

² This is a deviation from the promulgated method.

OVERVIEW OF SPLIT SAMPLE PREPARATION AND DISTRIBUTION

Bulk test water samples will be prepared in the SCCWRP laboratories as described in the QAPP using large sample containers with spigots and thoroughly mixed on a large-capacity stirrer to ensure that the samples are homogeneous. The number of samples to be tested by the participating laboratories are presented in **Table B2**. Bulk samples will be allowed to equilibrate for up to 48 hours before preparing the split-samples that will be shipped to the laboratories. Subsampling of the bulk test samples will be conducted using 3.8 L cubitainers filled to the top. All cubitainers will be filled at random in two steps. First, each cubitainer will be filled halfway. Then the cubitainers will be filled the rest of the way in no particular order. Each cubitainer will be labelled with a unique sample ID and stored in the dark in the walk-in fridge at 4 °C less than 48 hours before shipping them to the participating laboratories.

Table B2. Number of split-samples to be tested by the 12 participating laboratories for each round. Three testing rounds will be completed for this study.

Sample ID	Number of samples per lab per round	Number of sample dilutions to test	Number of lab control to include per sample
1	1	_*	1
2A	1	_*	1
2B-G	5	_*	1
3	1 [‡]	5	1

*Water samples DO NOT require further dilution before testing.

[‡] Sample 3 will be shipped as a powder with instructions to prepare the serial dilution for testing.

To ensure that all subsamples are representative of the original bulk test samples, two subsamples will be collected in separate vessels from each cubitainer before shipment. These samples will be used to measure conductivity, alkalinity, and hardness. The analysis will be completed within 30 days of sampling.

Split samples will be shipped to each laboratory starting on April 24, 2023, according to the schedule presented below. Samples will be shipped on wet ice using priority overnight (OnTrac or FedEx) service to the laboratories to the addresses in Appendix A of the QAPP. The shipments will also include chain-of-custody (COC) forms completed by SCCWRP and a copy of the study plan and testing instructions. SCCWRP will notify the laboratories via email once the samples are in transit and provide a tracking number. It is the responsibility of the laboratories to contact SCCWRP if they have not received the samples by the following day 2:00 pm.

Upon delivery, temperature, conductivity, pH, dissolved oxygen, hardness, and alkalinity, must be measured and recorded for each sample to document their stability before testing is initiated. These measurements shall be made from a small subsample poured into a clean secondary vessel. Probes and any other measuring equipment cannot be used in the cubitainer, and the subsample used for water quality must be discarded after use (subsample cannot be used for testing or as a chemistry or archived sample).

For sample 3, 14.00 g of NaCl will be weighed and placed in 100 mL HDPE containers. Each laboratory will receive one container with instructions to prepare the serial dilution using their own lab dilution water (i.e., dilute the supplied NaCl in 7.0 L of their own dilution water and perform a 50% dilution series to generate a total of 5 dilutions). Similar to the split-water samples, once the dilutions are prepared, the laboratories must record temperature, conductivity, pH, dissolved oxygen, hardness, and alkalinity for each dilution at test initiation. A 125-mL sample must also be collected and archived from each dilution at test initiation.

Note that approximately one (1) hour prior to test initiation and water changes, the volume of water needed to renew the test solutions should be adjusted/maintained at test temperature.

DATA SUBMISSION

SCCWRP will provide an Excel data submittal form and culture/bench sheet templates to the participating laboratories. **For each testing round, data in electronic format and scanned copies of**

the culture/bench sheets must be submitted to the SCCWRP data portal no later than 10 business days after test termination. Data required include:

- Laboratory information
- Sample information upon receipt (time, temperature, condition, and more as described above)
- Testing conditions including dilution water and food recipe
- Brood board health data
- Feeding density
- Bench water quality data for testing, survival, and reproduction counts
- Control charts for reference toxicant tests for the last 6 months

Detailed data requirements are provided in the QAPP. Note that continuous water temperature data shall be submitted separately by email to SCCWRP.

COMMUNICATION

Participating laboratories and other stakeholders will meet with SCCWRP, and the Expert Science Panel advising on this project to finalize the study plan, discuss logistics and review the results. A minimum of three remote meetings will be scheduled to provide a forum for discussion and clear communication among the project team and participants. Additional communication via email will be encouraged throughout the study. For more information on the overall study design and coordination meetings, please contact Alvina Mehinto alvinam@sccwrp.org. For questions regarding samples shipping from and to SCCWRP and data submission, please contact Darrin Greenstein darring@sccwrp.org.

SCHEDULE

- **April 7:** Draft study plan sent to all stakeholders for review
- **April 13:** Stakeholder Committee meeting, held via Zoom, to discuss the draft of the study plan
- **April 18:** Revised study plan and QAPP to Science Panel for approval.
- **April 19 and 20 Meeting** with participating laboratories. SCCWRP review testing requirements and provided training for data collection/submission.
- **April 21:** First batch of split sample prepared by SCCWRP
- **April 24:** Cubitainers containing first batch of split samples shipped to the laboratories.
- **April 25:** First batch of *C. dubia* toxicity tests performed
- **May 12:** Second batch of split samples prepared by SCCWRP
- **May 15:** Cubitainers containing second batch of split samples shipped to the laboratories
- **May 16:** Second batch of *C. dubia* toxicity tests performed
- **May 27:** Third batch of split samples prepared by SCCWRP
- **May 30:** Cubitainers containing third batch of split samples shipped to the laboratories.
- **May 31:** Third batch of *C. dubia* toxicity tests performed

NOTE: Data submission is required after each round of testing, **no later than 10 days after test termination.**

CONTINGENCIES

Lost Samples: If a sample is not delivered to a laboratory on the expected arrival date or if the sample has spilled during shipment, the laboratory must contact SCCWRP promptly. SCCWRP will ship new cubitainers that same day. However, this second batch of samples sent must be tested within 24 hours to ensure that holding times are comparable to other laboratories.

Failed Test Acceptability Criteria (TAC): A laboratory will be able to retest up to two test batches if the test conditions and TAC are not met (see **Table 1** and the QAPP for this study). A laboratory planning to retest must contact SCCWRP within 24 hours of knowing that a test failed the TAC. Laboratories are encouraged to retest with the remaining sample; however, arrangements might be made to re-test with archived samples.

Late Data Submission: All data must be submitted to the SCCWRP data portal and pass the QA checkers by **June 21, 2023**. Past this deadline, SCCWRP cannot guarantee that the data will be used in subsequent data analyses.

Appendix C – Data Quality Objectives for the SWB *C. dubia* Study Interlaboratory Testing.

Step	DQO Guidance of Purpose and Outputs of Step	<i>C. dubia</i> Interlaboratory Study
1. State the Problem	<p>Purpose</p> <ul style="list-style-type: none"> Define the problem that necessitates the study; identify the planning team, examine budget, and schedule. <p>Outputs from this step</p> <ul style="list-style-type: none"> A concise description of the problem. A list of the planning team members and identification of the decision makers. A description of overall approach for assessment. A summary of available resources, constraints, and relevant deadlines for the study. 	<p>Problem</p> <ul style="list-style-type: none"> Variability in the test outcome can occur when split samples are submitted to two or more laboratories for chronic toxicity testing with <i>C. dubia</i> so that effluent or blank spit samples have been reported to cause a significant effect at one laboratory and no significant effect at another laboratory. <p>Decision-makers and team members</p> <ul style="list-style-type: none"> The SWB is funding and managing a study titled Development of Quality Assurance Recommendations for the Ceriodaphnia Toxicity Test (SWB <i>C. dubia</i> Study) as part of implementing Resolution No. 2020-0044 to establish the Water Quality Control Plan for Inland Surface Waters, Enclosed Bays, and Estuaries of California and Adopting Toxicity Provisions (Toxicity Provisions). SCCWRP is implementing this work on behalf of the SWB in coordination with commercial and municipal laboratories. A Stakeholder Advisory Committee (SAC) and Expert Science Panel (ESP) provide review and input on the study design and reporting. <p>Overall approach</p> <ul style="list-style-type: none"> Interlaboratory testing with split samples (the interlaboratory study or ILS) is being conducted as part of the overall SWB <i>C. dubia</i> Study to assess factors contributing inter- and intra-laboratory variability. <p>Resources</p> <ul style="list-style-type: none"> The SWB is funding and managing the overall SWB <i>C. dubia</i> study. ILS testing performed by participating laboratories is being funded by CASA. <p>Constraints</p> <ul style="list-style-type: none"> The capacity of participating laboratories to perform ILS testing may be limited. Scheduling tests among participating laboratories will need to be coordinated. Available funding may limit the number of tests that can be performed.
Deadlines		

Appendix C – Data Quality Objectives for the SWB *C. dubia* Study Interlaboratory Testing.

Step	DQO Guidance of Purpose and Outputs of Step	<i>C. dubia</i> Interlaboratory Study
		<ul style="list-style-type: none"> The ILS results are to be reported in a technical memorandum by July 15, 2023. A Final Recommendations Report for the SWB <i>C. dubia</i> study is to be completed by September 30, 2023.
2. Identify the Goals of the Study	<p>Purpose</p> <ul style="list-style-type: none"> State how environmental data will be used in meeting objectives and solving the problem, identify study questions, define alternative outcomes. <p>Approach</p> <ul style="list-style-type: none"> Identify the key question that the study attempts to address and alternative outcomes or actions that may be taken, depending on the answer to the key study question; and develop decision statements. <p>Outputs from this step</p> <ul style="list-style-type: none"> A statement of the decision that must be resolved using data in order to address or solve the problem. A list of possible actions or outcomes that would result from each resolution of the decision statement. 	<p>Overall goal</p> <ul style="list-style-type: none"> Identify potential sources of variability within and among laboratories and make recommendations to the SWB regarding how the chronic <i>C. dubia</i> test method could be conducted to reduce sources of variability in the results (SWB 2020a, SCCWRP 2021b). <p>Key questions</p> <ul style="list-style-type: none"> Does standardizing lab practices improve consistency and comparability in <i>C. dubia</i> test results within and among laboratories? <p>Possible outcomes</p> <ul style="list-style-type: none"> One or more controllable variables are identified that are associated with reduced inter- and/or intra-laboratory variability and recommendations can be made to the SWB to reduce this source(s) of variability. One or more variables are identified that are associated with inter- and/or intra-laboratory variability but they are not controllable and recommendations cannot be made to the SWB to reduce this source(s) of variability. No consistent source(s) of variability associated with the method can be identified and recommendations cannot be made to the SWB to reduce source(s) of variability in test results.
3. Identify Information Inputs	<p>Purpose</p> <ul style="list-style-type: none"> Identify the data and information needed to answer study questions. <p>Activities</p> <ul style="list-style-type: none"> Identify the types and sources of information needed to resolve decisions or produce estimates. Identify the sources for each item of information identified. 	<p>Informational inputs</p> <ul style="list-style-type: none"> <i>C. dubia</i> chronic toxicity testing with splits of positive and negative control samples among multiple laboratories in California. Repeated analyses within participating laboratories (i.e., 3 batches of testing). Water quality data and other testing or culture data collected during split-sample testing. <p>Variables/characteristics to be measured</p>

Appendix C – Data Quality Objectives for the SWB *C. dubia* Study Interlaboratory Testing.

Step	DQO Guidance of Purpose and Outputs of Step	<i>C. dubia</i> Interlaboratory Study
	<ul style="list-style-type: none"> Select appropriate sampling and analysis methods to provide the necessary data. <p>Outputs from this step</p> <ul style="list-style-type: none"> A list of informational inputs (including sources and potential action levels) needed to resolve the decision. The list of environmental variables or characteristics that will be measured. 	<ul style="list-style-type: none"> Chemical analysis of split samples to document consistency or test materials (alkalinity, conductivity, hardness, major ion concentrations, pH). Water quality analysis of tested samples to document conditions during testing (alkalinity, conductivity, dissolved oxygen, hardness, pH, and temperature). Laboratories will report daily survival and neonates per female; number of males, unhealthy and dead adults, and any dead neonates in the brood board; specific beginning and end time window for age of neonates at test initiation; dilution water recipe and food recipe; and light intensity and air temperature within the testing area at the time of the testing.
<p>4. Define the Boundaries of the Study</p>	<p>Purpose</p> <ul style="list-style-type: none"> Specify the target population and characteristics of interest; define the spatial and temporal limits, and scale of inference. <p>Activities</p> <ul style="list-style-type: none"> Define the target populations/parameters (physical, chemical, and biological), as applicable. Specify the spatial and temporal boundaries. <p>Outputs from this step</p> <ul style="list-style-type: none"> Characteristics that define the domain of the assessment. A detailed description of the spatial and temporal boundaries of the characterization. 	<p>Study domain</p> <ul style="list-style-type: none"> Participating laboratories (n=11) are among those currently or recently accredited by ELAP to perform the <i>C. dubia</i> chronic toxicity test in California. Toxicity testing will be conducted consistent with EPA (2002) guidance and according to each laboratory's SOP. <p>Spatial domain</p> <ul style="list-style-type: none"> Participating laboratories are located throughout California (9 out of 11) and two laboratories are out of state in Colorado and Maryland. <p>Temporal domain</p> <ul style="list-style-type: none"> Testing is to be performed in 2022 over a seven-week period.
<p>5. Develop the Analytical Approach</p>	<p>Purpose</p> <ul style="list-style-type: none"> Define the parameter of interest, specify the type of inference, and develop the logic for drawing conclusions from findings. <p>Activities</p>	<p>Parameters of interest</p> <ul style="list-style-type: none"> Mean neonates per female in tested samples and controls as well as calculated IC25 and IC50 values will be compared to determine if they are significantly different within and among laboratories. Culturing information (e.g., culture water type and water quality parameters, food quality, culture age) and ancillary testing information (e.g., specific age of test organisms within an 8-hour window, specific time at test termination, etc.).

Appendix C – Data Quality Objectives for the SWB *C. dubia* Study Interlaboratory Testing.

Step	DQO Guidance of Purpose and Outputs of Step	<i>C. dubia</i> Interlaboratory Study
6. Specify Performance or Acceptance Criteria	<ul style="list-style-type: none"> • Specify the parameters (e.g., mean, median, percentile) that are considered important to characterize the population of interest. • Develop decision rules (“if ... then ...”) for the parameters of interest. <p>Outputs from this step</p> <ul style="list-style-type: none"> • Identify the most relevant population parameters for estimation. • Decision rules for interpretation of results. 	<ul style="list-style-type: none"> • Test data/factors will be evaluated to determine if it is associated with variability in <i>C. dubia</i> survival or reproduction within or among laboratories. <p>Decision rules</p> <ul style="list-style-type: none"> • Standard test method statistics (EPA 2002b, 2016) may be used to evaluate statistical differences between samples and control endpoints. • Percent effect may be calculated for the sample test treatment relative to the laboratory’s standard control water. • Inter- and intra-laboratory variability will be evaluated by direct comparisons of the IC values, CVs and PMSD among repeated analyses. • IC values, CVs and PMSD of this ILS will be directly compared to those calculated for the baseline ILS. • ASTM (1999) h and k statistics will be used to evaluate data consistency and identify potential outliers among laboratories and within a laboratory, respectively. • Multi-variate and other statistical analyses will be used to assess test factors contributing to the differences in inter- and intra-laboratory variability between the baseline ILS and this second ILS.
	<p>Purpose</p> <ul style="list-style-type: none"> • Develop the performance criteria for new data being collected or acceptable criteria for existing data being considered for use. <p>Activities</p> <ul style="list-style-type: none"> • Specify the performance metrics and acceptable levels of uncertainty. <p>Outputs from this step</p> <ul style="list-style-type: none"> • The primary output is a set of acceptance criteria that collected data should achieve in order to minimize the 	<p>Performance criteria</p> <ul style="list-style-type: none"> • Toxicity test results must meet test acceptability criteria described by the test method (EPA 2002b, 2016) to be considered valid for comparisons among valid test results. • Tests that do not meet test acceptability criteria and all test requirements will also be assessed to determine factors contributing to the failure, which may inform the analysis of variables causing or contributing to inter- and intra-laboratory variability. • Water quality (e.g., dissolved oxygen, pH, etc.) and sample chemistry (e.g., major ion concentration) data must meet measurement quality objectives defined in Section A7 of this QAPP.

Appendix C – Data Quality Objectives for the SWB *C. dubia* Study Interlaboratory Testing.

Step	DQO Guidance of Purpose and Outputs of Step	<i>C. dubia</i> Interlaboratory Study
	possibility of failing to keep uncertainty within acceptable limits.	
7. Develop the Plan for Obtaining Data	<p>Purpose</p> <ul style="list-style-type: none"> Select the resource-effective sampling and analysis plan that meets the performance criteria. <p>Activities</p> <ul style="list-style-type: none"> Compile information that we will need (generated in Steps 1 through 6) in an acceptable and efficient sampling and analysis design. Identify constraints that will affect the sampling and analysis design. Provide details on the sampling and analysis methods that we will use to generate the data. Prepare a resource-effective information collection plan that will meet our needs and requirements. <p>Outputs from this step</p> <ul style="list-style-type: none"> The most resource-effective design for the study that is expected to achieve the DQOs, selected from a group of alternative designs. 	<p>Approach</p> <ul style="list-style-type: none"> The sampling and analysis approach is described in this QAPP and relies on standardized methods for data collection and analysis. SCCWRP and the ESP are currently discussing the study design and this section will be updated with study design details once determined.

Notes: CASA – California Association of Sanitary Agencies; ELAP – Environmental Laboratory Accreditation Program; SP – Expert Science Panel; QAPP – Quality Assurance Project Plan; SAC – Stakeholder Advisory Committee; SCCWRP – Southern California Coastal Watershed Research Project; SOP – Standard Operating Procedure; SWB – California State Water Resources Control Board

Appendix D – Ceriodaphnia Food and Feeding Data Sheet for Second ILS

Test Start Date _____

Test Batch Numbers _____

Sheet ____ of ____ for this set of tests

Feeding Method: Direct or In Test Solution (Circle)

Each food individually Or Foods are Mixed and Fed (Circle)

YCT

Source of Prepared YCT: inhouse or purchased _____ (Circle; give supplier if purchased)

Is YCT filtered before use _____ or does the YCT have solids strained out _____

Production Date _____ YCT Batch ID _____

Laboratory Measured Solids _____ mg/ml

Concentration fed to tests _____ (x ml/x ml of _____ mg/L TSS YCT)

Concentration in each test cup is calculated to be: _____

Algae

Source of Prepared Algae: inhouse or purchased _____ (Circle; give supplier if purchased)

Production Date _____ Algae Batch ID _____

Supplier Cells/ml _____

Laboratory Measured Cells/ml _____ Measurement Method _____

Procedure used to determine cells/ml _____

Concentration fed to tests _____ (x ml/x ml) of _____ algae concentration, cells/ml)

Concentration in each test cup is calculated to be: _____

Appendix E – Guidance for Documenting Brood Board Health

This appendix is intended to help with the determination of abnormal occurrences in the brood board for the second interlaboratory study (ILS). If any of the notable observations illustrated below occur in the brood boards used in the second ILS, they should be noted with appropriate brood board health code (**Table E1**) on the daily observation sheets and included in the electronic data submission. Notes are given about how some of these appear to the naked eye. If any of these are suspected greater magnification should be used for verification.

Table E1. Health codes and descriptions that must be used for documenting brood board health for the second ILS.

Health Code	Parameter	Description of Parameter
A	Unhealthy adult	Lack of normal movement, not normal shape, smaller than normal, or atypical coloration. Presence of ephippia in brood pouch. Empty brood pouch, lack of clear gutline, and undersized brood based on age (i.e. only 3-6 eggs present 5 or more day post initiation).
D	Dead adult	Self-explanatory.
K	All OK	Nothing in any of the other categories to report.
M	Male	An animal that produces no young and is microscopically examined to determine sex. Triangular abdomen and fast/irregular swimming.
N	Dead neonates	Self-explanatory. Can be some or all of brood.
U	Unhealthy neonates	Lack of normal movement, not normal shape, ephippia or atypical coloration.
O	Other occurrences	Growth on adults or neonates, biofilm in brood cups, foreign species, aborted broods, flocculent material, etc. Describe in comments section.
Y	Neonates used to initiate a test	Neonates from this brood board chamber were used to initiate a test.



Abnormal, non-reproductive female. These will sometimes appear as having an empty and translucent brood pouch or contain a small number of eggs (A) that are aborted or do not become viable neonates. This would fall under health code A, as an unhealthy adult.

Photo courtesy of Alison Briden Pacific EcoRisk



Adult female with ephippia (A) (resting or diapause stage embryo). Ephippia are not normally observed in healthy cultures. This would receive health code A.

Photo courtesy of Alison Briden Pacific EcoRisk



Normal adult females will have visible eggs in the brood pouch (A) that are clearly differentiated, especially early in the brood cycle. As the organism gets closer to releasing the brood, unborn neonates should have visible eye spots. The adult should have a visible gut line and usually has a visible green coloration from feeding on the algal suspension. This would receive health code K.

Photo courtesy of Alison Briden Pacific EcoRisk



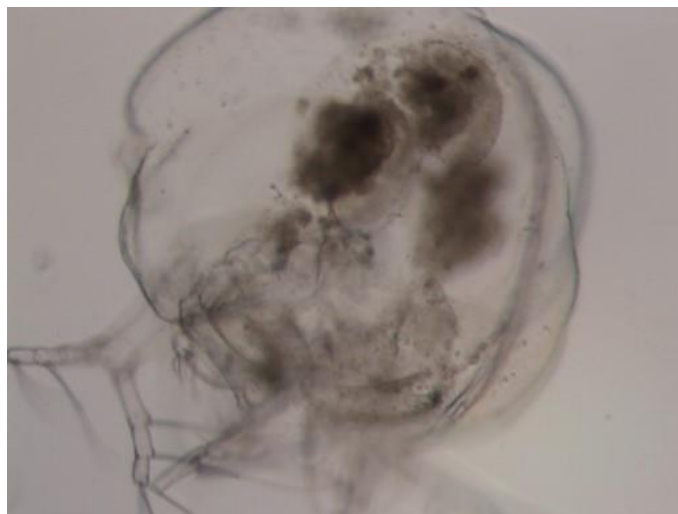
Male. Note the flatter back with absence of brood pouch (A) and longer antennules (B) than the female. Males are noticeably smaller than reproductive age females. Males also tend to have more erratic swimming behavior. This would be health code M.

Photo courtesy of Alison Briden Pacific EcoRisk



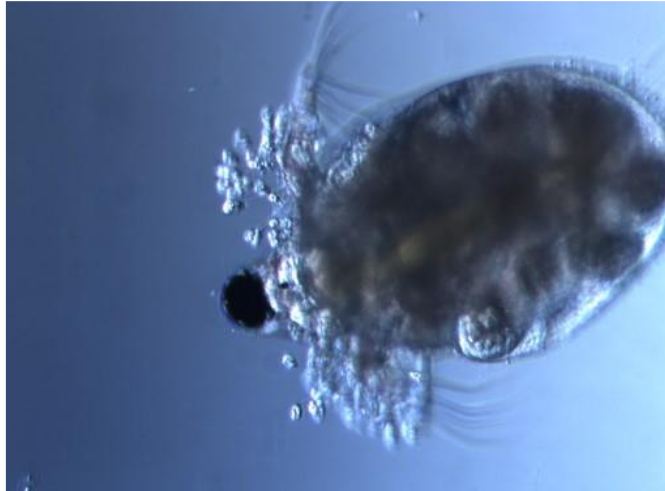
Male left and female right. Note the difference in size and shape (A) and difference in antennules (A) from the female. Health code K for the female, M for the male.

Photo courtesy of Alison Briden Pacific EcoRisk



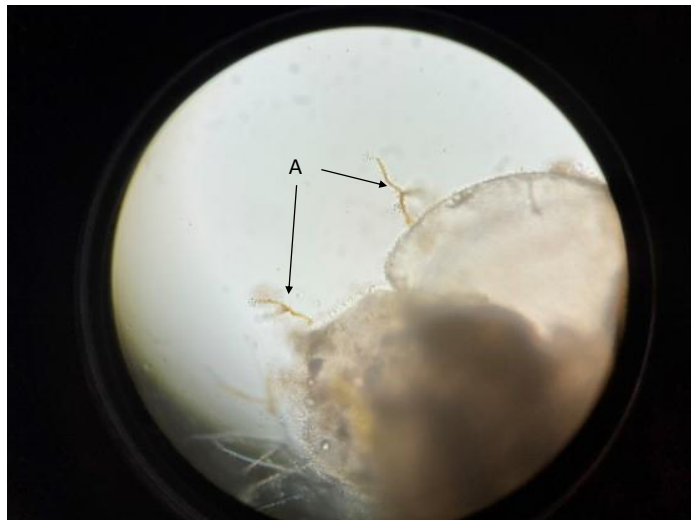
Partially aborted brood with eggs in molted carapace. The aborted brood was visible to the naked eye as a small black dot inside the molted carapace. Unhealthy females will sometimes partially or completely abort underdeveloped eggs without molting the carapace; if a female is observed with an empty brood pouch, the replicate should be examined for aborted eggs which appear as small black dots at the bottom of the chamber under a microscope. This would receive health code O.

Photo courtesy of Alison Briden Pacific EcoRisk



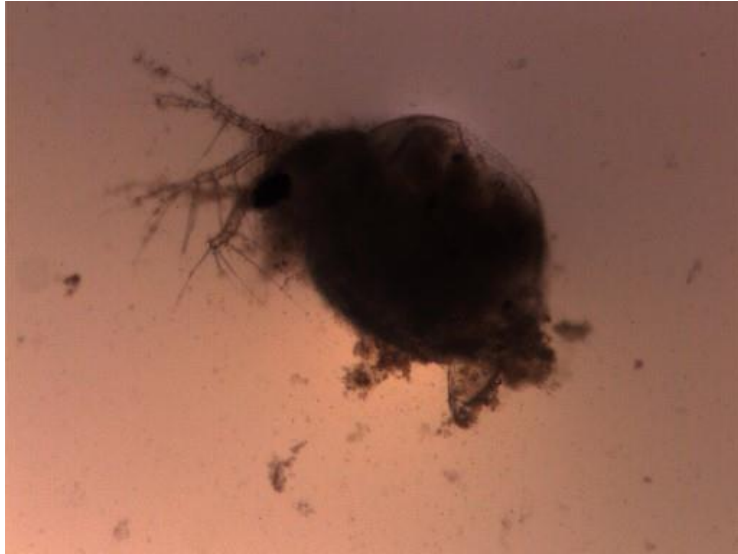
Female with stalked ciliate infection This infection affected reproduction but was not fatal. The ciliates were shed with the carapace but quickly returned This would receive health code Q

Photo courtesy of Alison Briden Pacific EcoRisk



Adult female with Peritrichs (stalked ciliates) on carapace. These appeared as reddish spots on the animal and bottom of cup to the naked eye. This infection was usually fatal. This would receive health code O.

Photo courtesy of Alison Briden Pacific EcoRisk

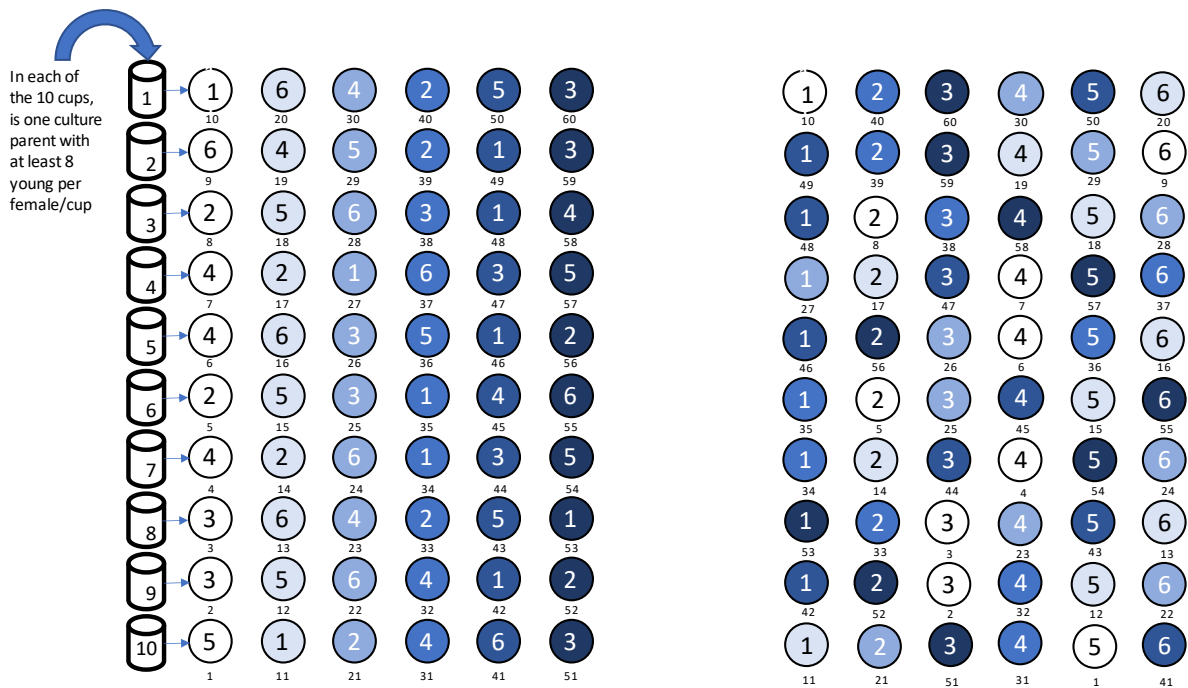


Dead adult covered with biofilm. This receive health code D,O.

Photo courtesy of Alison Briden Pacific EcoRisk

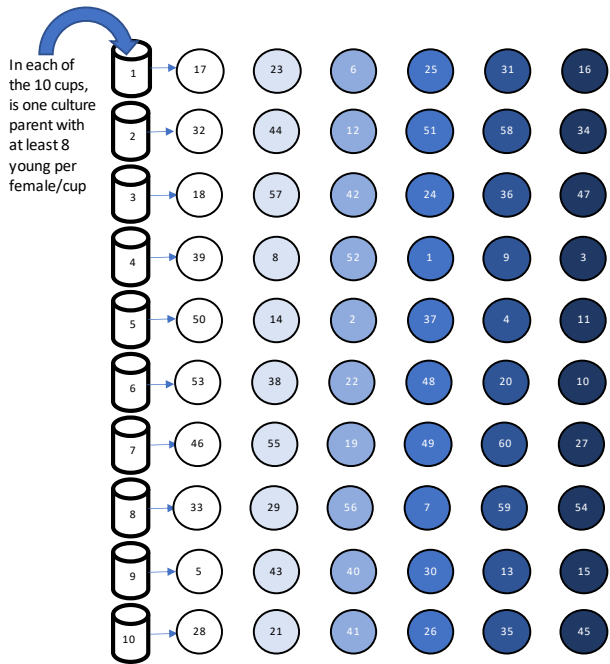
Appendix F – Guidance for Randomization of Test Chambers

Randomization of the test chambers is required for the second ILS. The manner of randomization is left to the individual laboratories. If a laboratory is currently randomizing their tests, they are encouraged to continue with their method. For laboratories not currently randomizing, below are two possible methods that can be used. Other acceptable methods may also be used. Either multiple sets of random numbers should be assigned for use in testing or new numbers can be chosen for each test performed. A single set of random numbers must not be used for each test.

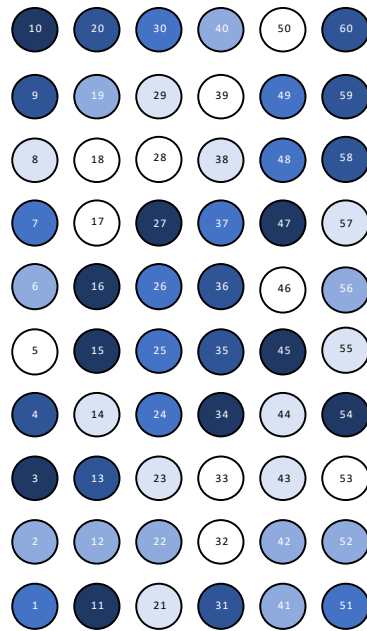


Test board with chambers in each row assigned random numbers. Each row represents a random block from a single female. Colors and columns represent separate samples or concentrations. The small numbers identify each chamber.

Test board adjusted using the random numbers assigned in the left graphic to randomize within each row. This method represents the minimum level of randomization required.



In this form of randomization, each chamber is assigned a random number. The chambers are arranged in treatment blocks for addition of the neonates.



After addition of the neonates, the chambers are arranged in numerical order which randomizes the entire test. The use of colored labels will help to keep the treatments straight during subsequent water changes.

Appendix G – Guidance for Identifying and Documenting Split Broods

Initial identification of possible split broods must be conducted at the time of each daily check. Notations of possible split broods must be made on the datasheets daily. Notes for indicators of split broods should be made on bench sheets or an observation recording sheet. Final determination of split broods may be made by a more senior employee, but again must be done on a daily basis and the data sheet initialed. Identification of split broods by bench analysts can be reviewed as part of the laboratory's data QA/QC process and any changes to a determination for split brood made by the reviewer should be done in conjunction with the analyst who performed the test and the notes that they took during testing. Determination of split broods cannot be conducted after the test has ended unless detailed bench notes are available from the daily observations.

The process for identifying split broods should involve multiple steps.

- When a female produces a small brood relative to other brood sizes on that test day (within the same test concentration), she should be examined to determine if there are remaining eyed neonates in her brood pouch which, in combination with observing the neonates present in the replicate look newly released, is an indication that the organism is actively releasing the brood during the changeover time. If so, this should be noted on the bench sheet. The presence of additional neonates can be seen with the naked eye and is easy to observe under a dissecting scope.
- On the day following a small brood, the size of the individual neonates should be compared to those released by other females. If the neonates are larger than those from other females, this would indicate that they are from the part of the previous day's brood.
- If a split brood is detected, it should be indicated on the bench sheet and the neonate counts from the two days must be circled to denote that they should be treated as a single brood.

Appendix H – Documentation of Water Treatment System

Laboratories shall fill in the questionnaire below to describe their water treatment system.

Source water

<input type="checkbox"/>	Municipal
<input type="checkbox"/>	Well water
<input type="checkbox"/>	Surface water
<input type="checkbox"/>	Other. Specify _____

Treatment train

Step 1

<input type="checkbox"/>	Carbon cartridge
<input type="checkbox"/>	Ion exchange
<input type="checkbox"/>	Reverse osmosis
<input type="checkbox"/>	Other organic clean-up
<input type="checkbox"/>	Filtration
<input type="checkbox"/>	MilliQ
<input type="checkbox"/>	Disinfection. Specify _____

Step 4

<input type="checkbox"/>	Carbon cartridge
<input type="checkbox"/>	Ion exchange
<input type="checkbox"/>	Reverse osmosis
<input type="checkbox"/>	Other organic clean-up
<input type="checkbox"/>	Filtration
<input type="checkbox"/>	MilliQ
<input type="checkbox"/>	Disinfection. Specify _____

Step 2

<input type="checkbox"/>	Carbon cartridge
<input type="checkbox"/>	Ion exchange
<input type="checkbox"/>	Reverse osmosis
<input type="checkbox"/>	Other organic clean-up
<input type="checkbox"/>	Filtration
<input type="checkbox"/>	MilliQ
<input type="checkbox"/>	Disinfection. Specify _____

Step 5

<input type="checkbox"/>	Carbon cartridge
<input type="checkbox"/>	Ion exchange
<input type="checkbox"/>	Reverse osmosis
<input type="checkbox"/>	Other organic clean-up
<input type="checkbox"/>	Filtration
<input type="checkbox"/>	MilliQ
<input type="checkbox"/>	Disinfection. Specify _____

Step 3

<input type="checkbox"/>	Carbon cartridge
<input type="checkbox"/>	Ion exchange
<input type="checkbox"/>	Reverse osmosis
<input type="checkbox"/>	Other organic clean-up
<input type="checkbox"/>	Filtration
<input type="checkbox"/>	MilliQ
<input type="checkbox"/>	Disinfection. Specify _____

Final de-ionized water

<input type="checkbox"/>	Type 1
<input type="checkbox"/>	Type 2

Appendix I – Toxicity Data Submittal Instructions

This section provides guidance for the use of the data submission template provided by SCCWRP to submit the results of this ILS. The template is an Excel file with seven tabs corresponding to different data types. Each tab and the fields within are described below. No fields can be left blank. In cases where no data is available, a default value will be provided.

Once filled out, the template must be submitted through the SCCWRP data portal. The portal will perform automatic checks for business rules and verify that the data is complete and meet study specific requirements. Submissions not meeting the rules will be returned automatically with descriptions of the problems with the file. The file can then be corrected and resubmitted. Once the data passes the checking process a receipt will be issued confirming success.

For any questions or issues with data entry and submission, please contact Darrin Greenstein at darring@sccwrp.org.

LabTestInfo

Per the laboratories request, this tab will be filled as a survey form. A separate link will be sent to the laboratories. The instructions below pertain to the survey form. LabTestInfo provides information on a wide range of test conditions that are expected to be the same for all tests conducted within a given laboratory. If any of the parameters in this tab/survey change during the course of the study, the laboratory must contact SCCWRP for instructions on how to proceed.

LabCode-Enter the code from the list below. During the data submission process the system will anonymize each lab to the code they were assigned for the historic data assessment.

Lab Name	LabCode
Aquatic Bioassay	ABC
AquaScience	AQSC
Aquatic Testing Laboratories	ATL
Enthalpy	ENPY
GEI	GEI

Lab Name	LabCode
Inland Empire	IEUA
McC Campbell	MCBL
Pacific EcoRisk	PCR
TetraTech	TRTH
WSP (formerly Wood)	WOOD

Contact-First name and last initial of person to contact with regards to data questions.

DilutionWaterRecipe-Code for the method used to make dilution water used for laboratory controls and dilution of sample 3. Choose from the list below.

Description	Code
EPA Moderately Hard	EPAMH
EPA Moderately Hard + Se	EPAMH+Se
EPA Moderately Hard + Vitamins	EPAMH+V
EPA Moderately Hard + Se + Vitamins	EPAMH+Se+V
Mineral Water + DIW. Code is mineral water type followed by the ratio of mineral water to DIW (e.g. Perrier 20:80)	XXXX YY:ZZ
Other water type not mentioned above	Contact SCCWRP

SourceWater-Brief description of type of water used as the base for dilution water (e.g., Millipore, RO, Resin).

YCTSource-Is the YCT made in-house or purchased? Field can be “In-house”, “ARO”, “ABS”, or other. If other, please contact SCCWRP for a code.

YCTRecipe-Brief description of each component used to make the YCT (e.g., Fleischmans Yeast+Trader Joes Wheatgrass+Purina Trout Chow).

AlgaeSource-Is the YCT made in-house or purchased? Field can be “In-house”, “ARO”, “ABS”, or other. If other, please contact SCCWRP for a code.

TestChamberMaterial-Brief description of material that the test chambers are made from (e.g., Polyethylene, Glass)

TestChamberVolume-Volume of test chambers expressed in milliliters.

VolumeTestSolution-Volume of sample in the test chambers expressed in milliliters.

FeedingFrequency-Number times per day that the test chambers are fed.

FeedingMethod-Code for feeding procedure; either directly into the chambers or into solutions before addition to the chambers. “Direct” for the former and “Solution” for the latter.

YCTConcentration-Concentration of YCT expressed in milliliters of YCT per milliliter of test solution.

AlgaeConcentration-Concentration of algae expressed as number cells per milliliter of test solution.

AlkalinityMethod-What specific method is used to measure alkalinity?

HardnessMethod- What specific method is used to measure hardness?

ConductivityMethod- What specific method is used to measure conductivity? Provide specific device.

pHMethod- What specific method is used to measure pH? Provide specific device.

WQSampleCollectionMethod-What method is used to collect the “before” sample for water quality measurement? Is it surrogate, composite of all cups or something else?

WQSampleMinimumVol-What is the minimum volume of sample needed to measure all water quality parameters, expressed in milliliters.

Comments-A place for any comments or clarifications to any of the fields in this tabs.

ToxBatchInfo

This tab contains information for each individual test. The definition of a “batch” is a sample or samples (dilution series) and its associated dilution water control. The expected number of lines in this tab should be equal to the number of samples tested in the study for each laboratory. Some fields are repeated in subsequent tabs to facilitate downstream analyses and merging.

ToxBatch-Unique identifier assigned by the laboratory. Each grouping of samples and controls must be assigned its own identifier. The identifier can be alphanumeric.

LabCode-Same as for the LabTestInfo.

SampleHoldTime-Amount of time between the receipt of the sample and the start of the test in hours or decimal days.

SampleHoldTimeUnits-Units for sample hold time. Either “hours” or “days”.

TestStartDateTime-Date and time of the start of the test.

TestEndDateTime-Date and time of the end of the test.

Matrix-Designation for a test as water samples (W) or (S) for the solid NaCl sample.

ActualTestDuration-Duration of the test expressed in hours or decimal days.

ActualTestDurationUnits-Units for the test duration. Either “hours” or “days”.

TestAcceptability-Code for the overall acceptability of the test. See table below. Contact SCCWRP if it appears that a condition occurred that is not covered by a code in the table.

Acceptability Code	Test Condition Description
A	Test meets all acceptability criteria
C	Reduced number of replicates
D	Control performance criteria not met
E	Sample tested outside of specified holding time
H	Water quality data incomplete
J	Minor deviation in test conditions
K	Incoming sample temperature exceeded limits

ReferenceBatch-ToxBatch-Not used for this study. Enter NR for all rows.

LightIntensity-Measured light intensity at the location of the experiment.

LightIntensityUnits-Units for the light intensity measurement. Footcandles or microEinsteins/square meter/second (uE/m²/s).

NeonateMinAge-Minimum age of neonates used to start the test expressed in hours.

NeonateMaxAge-Maximum age of neonates used to start the test expressed in hours.

SampleStoreCond-Brief description of how sample was stored. Include temperature and light conditions (e.g., 4°C, dark).

DilWaterPrepDate-Date that dilution water was prepared.

YCTPrepDate-Date that the YCT used in the test was prepared. For purchased YCT, use the date of arrival for non-frozen or the date thawed for frozen. Note in comments if arrival frozen or not.

AlgaePrepDate-Date that the algae was prepared. For purchased algae, use the date of arrival.

Comments- A place for any comments or clarifications to any of the fields in this tab. Comments should pertain to the specific ToxBatch on each line.

ToxResults

This is the main data tab where survival and reproduction data are entered. Each line is a combination of replicate, day, and time of observation.

SampleID-The ID provided by SCCWRP for each sample. For all laboratory control samples, enter 0000 as the sample ID.

SampleArrivalDateTime-The date and time that the sample arrived at the laboratory.

ToxBatch-Same value as from the ToxBatchInfo tab.

LabCode-Same value as from the LabTestInfo tab.

Dilution-Strength of the sample. Full strength sample will be 100. For laboratory control, enter -88.

Concentration-Not used for this study. Enter -88 for all sample types.

ConcentrationUnits- Not used for this study. Enter NR for all sample types.

EndPoint-Test endpoints. Either Reproduction for neonate production or Survival.

LabRep-Replicate number for each sample.

Day-Test day.

Time-The time of the observation.

Result-Data for reproduction or survival. Reproduction data must be entered as counts of neonates at each observation point. For survival, enter 1 for live and 0 for dead adult. After an adult dies, enter 0 for all subsequent days and -88 for reproduction.

BroodGroup-A value indicating the brood number as identified by the laboratory. For split broods, the values will be identical for consecutive days. On days when no reproduction occurs, enter 0.

ResultUnits-Unit of measurement for the result. For both reproduction and survival, the value must be Count.

QA Code-Similar to AcceptabilityCode in ToxBatchInfo, but at the individual observation level. Choose from the table below. Field cannot be blank. Use comments for any explanation of a code that is not A. Contact SCCWRP for any situation that does not fit one of the descriptions.

Acceptability Code	Test Condition Description
A	Test meets all acceptability criteria
C	Replicate lost or observation not made.
E	Sample tested outside of specified holding time
J	Minor deviation in test conditions

SampleTypeCode-Code for one of three sample types referred to on a given line. For laboratory controls, the code should be CNEG. For any of the test samples provided by SCCWRP, the enter Result.

Comments-Place for any explanations that are needed at the observation level.

ToxWQ

This tab is for the entry of all periodic water quality measurements related to conducting the test.

SampleID-Same as for the ToxResults ta.

ToxBatch-Same as for the ToxResults tab.

Dilution-Same as for the ToxResults tab.

Concentration-Same as for the ToxResults tab.

ConcentrationUnits-Same as for the ToxResults tab.

Day-Day number of the test exposure. For measurements made before test initiation, enter -1. For measurements made on test initiation day, enter 0.

TimePoint-Code for which of the two daily measurements the line pertains. For samples that have been in contact with the animals for a day, enter Before. For water that is to be added to the test chambers, enter After. For Days 0 and -1, enter NA.

Parameter-Water quality parameter name. Choose from list below.

Code	Water Quality Parameter	Units	Number of Decimal Digits Required
COND	Conductivity	µS/cm	0
DO	Dissolved Oxygen	mg/L	1
PH	pH	pH	2
Hardness	Hardness	mg/L CaCO3	0
Alkalinity	Alkalinity	mg/L CaCO3	0
AirTemp	Air Temperature	C	1
WaterTemp	Water Temperature	C	1

Qualifier-Modifier to the value in the Result field. The expectation for all samples to be tested for this study is that the value should be =, meaning that the value is unmodified. If a situation arises that another modifier is needed, please contact SCCWRP.

Result-Value for the analysis for each water quality parameter.

ResultUnits-Units for the water quality parameters from the table above.

LabRep-Replicate from which water quality sample was taken. If measurement is taken from a surrogate or composite sample, enter 0.

LabCode-Same as from the LabTestInfo ta.

SampleTypeCode-Same as from the ToxResults tab.

Comments- Place for any explanations that are needed for the water quality measurement on the line of data.

BroodBoardInfo

Information on the brood boards used to initiate the tests for the study. Daily neonate counts and health assessment codes are data types of importance must be recorded in this tab.

BroodBoardID-Identification number used by the laboratory

LabCode-Same as from the LabTestInfo tab.

DateInitiated-Date the brood board was started.

CupNo-Chamber number for the brood board.

Day-Day number of brood board.

NumNeonates-Number of neonates for the combination of CupNo and Day.

HealthCode-Code for the assessment of the combination of CupNo and Day. Codes from the table below. A more detailed description and photographic examples are provided in **Appendix E**.

Health Code	Description
A	Unhealthy adult
D	Dead adult
K	All OK
M	Male
N	Dead neonates
O	Other occurrences
U	Unhealthy neonates
Y	Neonates used to initiate test

Comments-Comments associated with each combination of CupNo and Day. Include descriptions of any combinations with a HealthCode of “O”.

BBtoTestInfo

This tab has information on brood boards and cup numbers used to initiate the tests.

BroodBoardID-Same as from the BroodBoardInfo tab.

LabCode-Same as from the LabTestInfo tab.

ToxBatch-The test batch identifier for the test that the brood board was used to initiate.

CupNo-Cup numbers used to initiate the test.

Day-The brood board day when the test was initiated.

BroodBoardWQ

This tab is for water quality data from the brood boards.

BroodBoardID-Same as from the BroodBoardInfo tab.

LabCode-Same as from the LabTestInfo tab.

CupNo- Same as from the BroodBoardInfo tab.

Day-Same as from the BroodBoardInfo tab.

TimePoint-Same data type as for ToxWQ tab, but for the brood board.

BBWQParameter-Same data type as the Parameter from the ToxWQ tab and using the same codes.

BBWQResult-Brood board water quality measurement values.

BBWQUnits- Same data type as the ResultsUnits from the ToxWQ tab and using the same codes.

Comments-Any comments pertaining to the brood board water quality data.