Baseline Testing for *Ceriodaphnia dubia* Toxicity Testing Laboratory Standardization

Study Plan and Logistics

May 16, 2022

First Draft

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1. BACKGROUND

The *Ceriodaphnia dubia (C. dubia)* chronic reproduction toxicity test is an established whole effluent toxicity (WET) test method (U.S. EPA 2002a, b, c; U.S. EPA 2016), commonly used in regulatory programs including the Toxicity Provisions recently adopted by the State of California. However, regulators and stakeholders have recognized that some laboratories may need to improve their implementation of the *C. dubia* method to reduce intra-laboratory (within-laboratory) variability and increase inter-laboratory (amongst-laboratory) comparability. The present study commissioned by the California State Water Resources Control Board, in collaboration with stakeholders and laboratories, aims to (1) evaluate laboratory performance among those accredited by the State of California Environmental Laboratory Accreditation Program, (2) investigate factors that can lead to test variability and decrease confidence in assessments of toxicity, and (3) provide revised laboratory technique guidance to improve laboratory performance and reduce intra- and inter-variability.

To standardize test methods and parameters that may contribute to intra- and inter-laboratory variability, the Expert Science Panel and Stakeholder Advisory Committee have recommended that an intercomparison exercise be conducted by all California-accredited laboratories.

Two key questions were identified:

- Which laboratory technique(s) should be standardized to reduce intra- and inter-laboratory variability?
- Does standardizing laboratory techniques improve consistency and comparability in *C. dubia* test results?

2. GENERAL APPROACH

To address these questions, a three-step approach was proposed. During Step 1, all laboratories will participate in an intercomparison exercise using their current protocols and provide additional data that may not be routinely collected/reported by all laboratories. Based on the results of Step 1 and discussions among the Expert Science Panel and the Stakeholder Advisory Committee, Step 2 will aim to standardize select *C. dubia* test parameters. Finally, Step 3 will consist of another intercomparison exercise amongst all laboratories using split samples and the standardized *C. dubia* toxicity testing protocol. This document describes the approach, overall methodology and logistics that will be used to conduct Step 1 baseline testing intercomparison exercise. Detailed description of the subsequent steps will depend on the analyses and group discussions of the results of Step 1 baseline testing.

The document below aims to describe the key elements and steps for the baseline study. A separate quality assurance project plan (QAPP) will be produced to provide all the detailed instructions for the laboratories.

3. BASELINE TESTING PROCEDURE

California-accredited laboratories will participate in a round-robin split sample exercise and each laboratory will test split water samples in multiple batches within a 3-week window. Two testing options are proposed to ensure that a minimum of seven (7) test control datasets are generated (Table 1). This sample size was determined based on a power analysis conducted by the project biostatistician.

Testing option #1: Each laboratory will analyze three sample types in three different test batches. Sample types will include (a) one unspiked sample, consisting of dilution water to be tested at full strength (i.e., 100%); (b) one spiked sample, using sodium chloride, to be tested at 5 different concentrations, and (c) one duplicate sample to be determined by SCCWRP. A total of 336 split-samples will be tested. This testing option will generate nine test control datasets, three spiked sample datasets, three duplicate datasets, as well as three laboratory control and three reference toxicant datasets.

Testing option #2: Each laboratory will analyze five sample types in two different test batches. Sample types will include (a) three unspiked samples, consisting of different dilution water recipes to be tested at full strength; (b) one spiked sample, using sodium chloride, to be tested at 5 different concentrations, and (c) one duplicate sample, to be determined by SCCWRP. A total of 288 split-samples will be tested. This testing option will generate 10 test control datasets, two spiked sample datasets, two duplicate datasets, as well as two laboratory control and two reference toxicant datasets.

Standard Operating Procedures

Participating laboratories will perform three test batches within a 3-week window using their own standard operating procedures for the *C. dubia* chronic toxicity test. A summary of standard operating procedures, test acceptability criteria and measurements expectations are provided in Table 2. However, all laboratories will be required to do the following specifications:

- All tests will be carried out to 8 days (i.e., 192 hours).
- Each sample will be tested with a separate laboratory control.
- A concurrent reference toxicant will be run with each test batch using the laboratory's own reference chemical.
- Each sample/dilution will be tested using 10 replicate chambers.
- Test set-up will be randomized using blocking by known parentage.

Additionally, participating laboratories will be required to report data that may not be currently documented/reported including:

- Number of males, unhealthy and dead adults, and dead neonates in the brood board
- Specific beginning and end time window for age of neonates at test initiation
- Daily neonate production in test chambers, twice at 3-4 hours intervals
- Water chemistry including ionic composition at test initiation. Note that the ionic composition samples will be shipped to SCCWRP, and all samples will be analyzed by the same laboratory.
- Water quality parameters (temperature, pH, conductivity, alkalinity, hardness) before and after daily renewal, measured in surrogate test chambers

- Measured concentration of the laboratory reference toxicant stock solution
- Light intensity and air temperature within the testing area at the time of the experiments

Sample Preparation and Distribution

All split-samples will be prepared in the SCCWRP laboratories using large sample containers and thoroughly mixed on a large-capacity stirrer to ensure that the samples are homogenous. Subsampling will be conducted while continuously mixing the samples. Subsample cubitainers (volume TBD (L) per sample per laboratory) will be filled using a peristaltic pump and pre-cleaned (inside and outside) sampling hose kept in constant motion within the large sample container. The laboratory technician responsible for handling the sampling hose will ensure that the hose remains between 30 and 80 percent of the depth of the water column and does not touch the bottom of the water container. All samples will be kept in the walking fridge at 4 ℃ up to [TBD] days before shipping them to the participating laboratories.

To ensure that all subsamples are representative of the original test samples, each cubitainer will be subsampled to measure conductivity, alkalinity and hardness in triplicate. A subset will be sent for ion composition analyses and another subset of samples will be archived in the SCCWRP laboratories.

A total of [TBD based on testing option selected] samples will be shipped to each laboratory (see Table 1) according to the schedule agreed upon with the participating laboratories. Samples will be shipped on wet ice using priority overnight (OnTrac or FedEx) service to the laboratories to the addresses in Table 3. 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, hardness, alkalinity, temperature, pH and dissolved oxygen must be measured and recorded for each sample. The cubitainers must be kept at 4℃ up to 48 hours before first use. For laboratories unable to run all test batches concurrently, the maximum holding time allowed is [TBD] hours.

Data Submission

SCCWRP will provide an Excel data submittal form and culture/bench sheet templates to the participating laboratories. All test data in electronic format and scanned copies of the culture/bench sheets must be submitted to the SCCWRP data portal no later than [date TBD]. Data required include:

- Laboratory information
- Sample information upon receipt
- Testing conditions including dilution water and food recipe
- Brood board health data
- Bench water quality, survival and reproduction counts
- Control charts for reference toxicant tests for the last 12 months

4. COMMUNICATION AND SCHEDULE

Coordination with Participating Laboratories

Participating laboratories 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.

The first meeting, to be held remotely on May 24, 2022, and attended by the stakeholders will aim to review the first draft of the testing approach (including sample preparation and shipping, test measurements and data reporting) and discuss the timeline for testing and data submission. The second meeting held on [date TBD] among members of the Expert Science Panel and laboratories will aim to finalize the study design, review the QAPP and the logistics. The third meeting will focus on providing training for data collection and data submission.

Schedule

- **May 17:** Draft study plan sent to all stakeholders for review
- **May 24:** Stakeholder Committee meeting, held via Zoom, to discuss the first draft of the study plan
- **June 10:** SCCWRP will send the revised study plan to the Expert Science Panel along with the draft QAPP
- June (week of 27): Public meeting with Expert Science Panel and participating laboratories to finalize the study plan and approve the QAPP
- July date TBD: Meeting with participating laboratories to provide training on data collection and submission
- July dates TBD Split samples prepared by SCCWRP
- July date TBD: Cubitainers containing split samples shipped to the laboratories.
- Dates TBD: *C. dubia* toxicity tests
- August date TBD: Deadline for data submission

5. CONTINGENCIES

Lost Sample

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 hrs to ensure that holding times are comparable to other laboratories.

Failed Test Acceptability Criteria

A laboratory will be given the opportunity to retest up to two test batches if acceptability criteria are not met. A laboratory planning to retest must contact SCCWRP within 24 hrs of knowing that a test failed the acceptability criteria. Laboratories are encouraged to retest with 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 [date TBD]. If a laboratory experiences some delays, SCCWRP must be contacted no later than 48 hours before the deadline. Laboratories will be granted an additional TBD days to submit all their data. Past this new deadline, SCCWRP cannot guarantee that the data will be used in subsequent data analyses.

6. REFERENCES

U.S. EPA. 2002a. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. EPA-821-R02-013. U.S. Environmental Protection Agency. Washington DC.

U.S. EPA. 2002b. Guidelines establishing test procedures for the analysis of pollutants: Whole effluent toxicity test methods; Final rule. Environmental Protection Agency. 40 CFR Part l36 [FRL-7408-6].

U.S. EPA. 2002c. Guidelines establishing test procedures for the analysis of pollutants: Whole effluent toxicity test methods; Final rule. 67 Fed. Reg. 69952-69972 (November 19, 2002).

U.S. EPA. 2016. Whole effluent toxicity methods errata sheet. Office of Water, Environmental Protection Agency. 821-R-02-012-ES.

Table 1. Total number of spit-samples based on testing option. Note that each test batch will include a laboratory control and reference toxicant.

Sample Type	No. of Test Samples	No. of Dilutions to Test	No. Labs	No. of Test Batches	Total No. of Samples to Test
Testing Option #1					
Unspiked sample	1	1	16	3	336
Spiked sample	1	5			
Duplicate sample	1	1			
Testing Option #2					
Unspiked sample	3	1	16	2	288
Spiked sample	1	5			
Duplicate sample	1	1			

Table 2. Summary of test conditions and acceptability criteria for the *Ceriodaphnia dubia* survival and reproduction test.

Parameter	**Description**
Test organism	*Ceriodaphnia dubia*
Protocol	EPA/821/R-02-013, EPA 2002 Chronic Manual
Exposure	Static, daily renewal
No. replicate test chambers	10 replicates per sample/dilution
Sample holding time	Up to [TBD] hrs before test initiation
Test duration	8 days, i.e., 192 hours
Endpoints	Survival and reproduction
Water quality measurements	Temperature (℃) and pH shall be reported with 0.1 precision Hardness and alkalinity shall be measured in mg/L $CaCo_3$, light intensity in foot-candles, conductivity in µS/cm, dissolved oxygen in mg/L
Test Acceptability Criteria	80% or greater survival and an average of 15 or more neonates per surviving female in the controls

Table 3. Laboratory contact information and shipping address. **DRAFT TABLE BASED ON LABS THAT HAVE PROVIDED HISTORICAL DATA. NO LABS HAVE FORMALLY COMMITTED TO PARTICIPATING YET**.

Laboratory	Name	Contact information	Shipping Address
49er	Shane Burr	209-418-3175 Shane@49erwaterlab.com	245 New York Ranch Rd, Ste A, Jackson, CA 95642
Aquatic Bioassay & Consulting Labs, Inc	Joe Freas	805-643-5621 x18 joe@aquaticbioassay.com	29 N Olive St., Ventura, CA 93001
Aquatic Testing Laboratory	Joe LeMay	805 650-0546 jlemay12@pacbell.net	4350 Transport Street, Unit 107 Ventura, CA 93003
Aquatic Toxicity Lab (UCD)	Marie Stillway	530-752-0772 Mstillway@ucdavis.edu	UC Davis AHP. Institute of Ecology CABA, Bldg. #5. Garrod Road West, Davis, CA 95616
AquaScience	Kimberly Miller	530-753-5456 Kimberley@aqua-science.com	630 Cantrill Dr., Davis, CA 95618
City of Los Angeles	Stacee Karnya	310-648-5923 stacee.karnya@lacity.org	12000 Vista del Mar, Playa del Rey, CA 90293
EcoAnalysts	Brian Hester	360-297-6040 x6045 bhester@ecoanalysts.com	4770 NE View Dr., Port Gamble, WA 98364
Enthalpy	Peter Arth	858-587-7333 ext. 214 Peter.arth@enthalpy.com	4340 Vandever Avenue, San Diego, CA 92120
GEI	Natalie Love	303-264-1070 Nlove@geiconsultants.com	4601 DTC Boulevard, Suite 900, Denver, CO, 80237
Inland Empire	Sushmitha Reddy	909-993-1813 Sreddy@ieua.org	Water Quality Laboratory, Building C, 6075 Kimball Ave., Chino 91708

McCampbell	Drew Gantner	925-252-9262 Drew.gantner@mccampbell.com	1534 Willow Pass Road Pittsburg, CA 94565-1701
MBC Applied Environmental Sciences	Sonja Beck	714-850-4830 x225 Smbeck@mbcaquatic.com	MBC is 3000 Redhill Ave., Costa Mesa CA, 92626
Pacific Ecorisk	Stephen Clark	707-207-7760 Slclark@pacificecorisk.com	2250 Cordelia Road. Fairfield, CA 94534
Sanitation Districts of Los Angeles County	Josh Westfall	562-908-4288 x2815 Jwestfall@lacsd.org	San Jose Creek Biology Lab. 1965 Workman Mill Rd. Whittier, CA 90601
TetraTech	Marcus Bowersox	410-902-3142 Marcus.Bowersox@tetratech.com	10711 Red Run Blvd., Suite 105, Owings Mills, MD 21117
Wood	Steve Carlson	858-299-5368 Steve.carlson@woodplc.com	4905 Morena Blvd. Ste. 1304, San Diego, CA 92117