

DEVELOPMENT OF QUALITY ASSURANCE RECOMMENDATIONS FOR THE *CERIODAPHNIA DUBIA* TOXICITY TEST

Stakeholder Advisory Committee for *Ceriodaphnia dubia* Quality Assurance Study

Minutes of Meeting #5

Held remotely on Wednesday, February 23, 2022, 1:00 PM to 3:30 PM

List of Participants:

Facilitators:

- Alvina Mehinto and Ken Schiff (SCCWRP)

Stakeholder Committee members:

- State Water Board - John Wheeler and Katie Fong (SWRCB)
- USEPA – Amelia Whitson (EPA Region IX)
- Regional Water Quality Control Boards - Veronica Cuevas (RWQCB4)
- Wastewater Agencies - Mitch Mysliwicz (Larry Walker Assoc/CASA)
- Stormwater Agencies - Jian Peng (Orange County Public Works/CASQA)
- Agriculture Organizations - Sarah Lopez (Central Coast Water Quality Preservation Inc)
- Private Laboratories - Jeff Miller (Aqua-Science Laboratories)
- Public Laboratories - Josh Westfall (Los Angeles County Sanitation Districts)
- Non-Governmental Agencies - Annelisa Moe (Heal the Bay)

There were 46 online attendees.

Agenda Item #1 – Opening remarks, self-introductions, and review of the agenda

Alvina Mehinto of SCCWRP called the meeting to order at 1:03 PM and welcomed the attendees. The Stakeholder Committee members provided roll-call attendance. Alvina welcomed two new member of the Stakeholder Committee, Katie Fong who will be replacing John Wheeler and Annelisa Moe who replaced Kaitlyn Kalua, each of whom provided self-introductions. Minutes from the previous meeting were approved electronically after the last meeting.

Alvina spent a few moments commenting on the recent closed session by the Expert Science Panel. These closed sessions were at the request of the Expert Science Panel for them to

conduct a working meeting, diving into details about specific laboratories. She has been providing meeting summaries following each meeting to maintain transparency and keeping the Stakeholder Committee and the public informed.

Agenda Item #2 – Inventory of the historical and lab information collected

Alvina provided a review of the inventory of test data (from controls and reference toxicant) and laboratory methods. The inventory – now one of the largest of its kind - was created from laboratory data requests, laboratory interviews, plus reviewing Standard Operating Procedures and/or Quality Assurance Plans. In summary, all 17 laboratories accredited in California for toxicity testing were contacted, generating a database of over 1,000 tests and more than 10,000 data points. The inventory met or exceeded all of the data quality objective goals set for this task in the Workplan.

Alvina provided some example information from the inventory, highlighting the differences among laboratories. The examples focused on dilution water make-up, food preparation, and the trigger for test completion. It was noted that, despite the differences, most all of these lab techniques are allowable under the currently adopted method manual.

Agenda Item #3 – Exploratory and statistical data analyses

Alvina presented a summary of the exploratory data analysis, some of which was a review from the previous Stakeholder Committee meeting. There were three basic conclusions from the exploratory data analysis: 1) no two laboratories in the database are conducting the test in exactly the same manner, 2) large intra- and inter-laboratory variability was observed for both water quality and biological response data, and 3) confounding factors such as lab practices reduced the statistical power to identify key test variables for improving testing.

Alvina provided several examples of exploratory data analysis prompting a number of clarifying questions from the Stakeholder Committee members and from the public. The discussion led to specific issues such as the potential aim of using split samples for disentangling confounding factors in both laboratory methods and water quality parameters.

Alvina presented the primary study questions followed by a description of the statistical methods utilized for analyzing data, including random forest, Generalized linear regression, and logistic regression. Alvina showed results using the random forest techniques, the primary data analysis described in the study Workplan, acknowledging that the other statistical methods produced similar conclusions.

Results did not clearly indicate the water quality variable(s) responsible for the variability in control responses from the data inventory. Instead, the variable importance plots from the random forest analysis revealed that the variables differed from lab to lab, and varied within a lab based on the biological response evaluated (e.g., mean neonates per female vs coefficient of variation in mean neonates per female).

The Stakeholder Committee discussed a variety of aspects of the data analysis including the proper biological response variables, the relative importance of different variables, dissecting the random model results, and what additional data would be useful to help interpret the results. Some examples of additional information included major ion concentrations and brood board health data.

Public comment was largely focused on clarifying data analysis.

Agenda Item #4 - Summary of discussions from the Science Panel's closed sessions

Alvina Mehinto summarized the Expert Science Panel recommendations:

- Statistical analysis alone will not identify key test parameters to optimize
- Combination of narrative information (from the interviews), data analysis output, and best professional judgement is necessary
- Any additional testing should address both lab techniques and water quality test parameters

The Stakeholder Committee had relatively little discussion about these recommendations having reached a similar conclusion independently during the previous agenda item.

Agenda Item #5 – Split sample testing design

Alvina facilitated a discussion to plan the split sample study design. Her goal was to get the Stakeholder Committee to list potential factors they think should be tested in the split sample testing, then prioritize their highest ranked factors.

Alvina listed two categories of factors mirroring the Expert Science Panel recommendations. The first category of factors were options to evaluate the influence of water chemistry. This included factors such as conductivity hardness, alkalinity, pH, and/or mineral content. The Stakeholder Committee had a robust discussion of the pros and cons of several issues including the correlation between these factors, the potential contribution of particulates in a sample, samples in clean dilution water vs in receiving water of effluent matrix, concentrations and concentration ratios on major ions, using toxic vs non-toxic samples, and age of dilution water.

The second category of factors to be identified included laboratory techniques. Alvina described testing these factors by having labs do some lab techniques exactly the same, then retesting using their existing lab techniques, and looking for differences. Alvina provided five potential lab techniques to evaluate: test duration, narrowing the age of neonates at test initiation, reduced maximum age of neonates at test initiation, specific time windows for daily neonate counts, and/or defined age window for brood board. Once again, the Stakeholder engaged in a robust discussion of alternatives. Ideas included: test duration, age of neonates, purchased vs in-house cultured specimens, specimens from different vendors, how labs define different broods, and test temperature. One Stakeholder member recommended reaching out to the laboratories for ideas.

Much of the public comment reinforced many of the ideas discussed by the Stakeholder Committee, with one public member emphasizing the need for a clearly articulated and documented study plan.

Alvina summarized the Stakeholder Committee's recommendations:

- Water chemistry parameters to consider include conductivity, spiked chemical, turbidity and hardness
- Laboratory techniques should be focused on test duration and age of neonates
- Additional data should be requested e.g. ionic composition of dilution and/or culture water

Agenda Item #6 – Public Comments

The public comments were addressed live during each of the individual agenda items.

Agenda Item #7 - Schedule and next steps

The next step for SCCWRP is to: 1) refine the study questions and design for split sample testing based on the input and recommendations from the Stakeholder Committee; 2) present this draft study design to the Expert Science Panel, then based on their approval; 3) create the foundation for implementing the split sample study including a Quality Assurance Plan, logistics for creating and shipping, defining information management, and communicating with the laboratories for outreach, training, timing, and contracting. The hope is to have samples distributed in late spring.

SCCWRP agreed with the Stakeholder Committee that the study design discussion with the Expert Science Panel should be conducted in a public meeting.

The meeting adjourned at 3:15 PM.