

***C. dubia* QA evaluation study**
Stakeholder Committee Meeting

June 29, 2023

Agenda

1. Opening Remarks and Introductions
2. Approval of Minutes of April 13 meeting
3. Update on Second Intercalibration
4. Development of Test Guidance by Expert Science Panel
5. Questions from the Public
6. Next Steps

Stakeholder Advisory Committee

- Katie Fong (SWRCB)
- Amelia Whitson (EPA Region IX)
- Veronica Cuevas (NPDES permits)
- Mitch Mysliwec (Wastewater)
 - Paul Bedore (alternate)
- Jian Peng (Stormwater)
- Sarah Lopez (Agriculture)
- Peter Arth (Private Laboratories)
- Josh Westfall (Public Laboratories)
- Annelisa Moe (NGO)
- Steven Boggs (ELAP)

Expert Science Panel

- Robert Brent (James Madison University)
- Teresa Norberg-King (Formerly US EPA)
- Howard Bailey (Nautilus Environmental)
- Leana Van der Vliet (Environment and Climate Change Canada)
- A. John Bailer (Miami University, Ohio)

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Project Tasks and Progress to Date

- Identify potential sources of variability within and among laboratories
 - Compile and analyze historical data
 - Conduct baseline intercalibration
- Conduct lab “training and education”
 - Lab visits
 - Roundtable workshop
- Evaluate efficacy of test method and QA refinements
 - Conduct second intercalibration
- Develop test guidance to reduce intra- and inter-lab variability

Study Design Overview

- Split samples provided by SCCWRP (same as used in the baseline ILS)
- 9 out of 11 labs that participated in the baseline ILS

| Sample Type | # of samples provided | Dilution series to prepare | # of rounds | # of labs |
|---|------------------------------|-----------------------------------|--------------------|------------------|
| Sample 1: EPA MH dilution water | 1 | No | 3 | 10 |
| Sample 2A: Perrier dilution water | 1 | No | 3 | 10 |
| Sample 2B-F: NaCl in Perrier dilution water | 5 | No | 3 | 10 |
| Sample 3: NaCl salts (diluted by labs using their dilution water) | 1 | Yes | 3 | 10 |

Standardized lab techniques implemented during the second ILS

- Conduct detailed evaluation of brood board health for 2 weeks prior testing
- Use [neonates from 6-10 days old females](#) to start the test.
- Run test for 8 days
- Renew or terminate test daily at 24 hrs within + or -1 hour
- Use randomization by blocking of known parentage AND randomization of cups on the board

Standardized lab techniques implemented during the second ILS

- Quantify initial food density and document feeding method to estimate density in test chambers
- Holding times of YCT ≤ 7 days after thawing and algae ≤ 21 days. Use same batch for an entire testing round
- Document split broods on bench sheets at the time of observation
- Store reagents appropriately (e.g., salts in desiccator)

Second Intercalibration Schedule

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 initiated

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 initiated

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 initiated

June 23: Deadline for data submission

Data Quality Objectives

Completeness:

- >95% of data expected

QA audit completed

- 15% random check for raw data entry errors (edata vs bench sheets)
- Any failures resulted in 100% data audit for that lab

Test acceptability criteria

- 96% met TAC of ≥ 15 neonates per surviving female in controls

Inventory of datasets collected

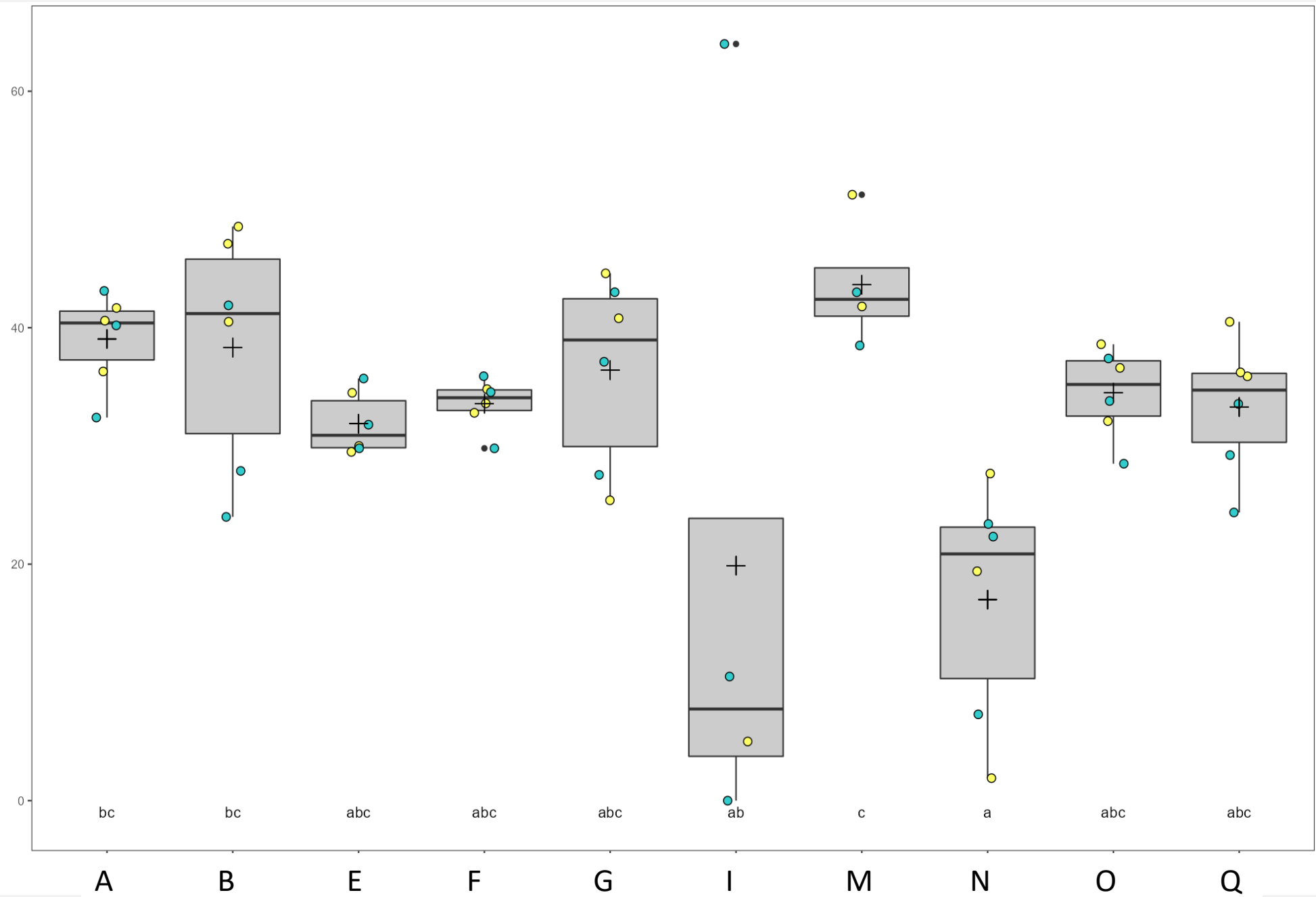
| Lab ID | Lab controls | #1- EPA MH | #2A- Perrier | #2B-F- SCCWRP dose-response | #3- Lab dose-response |
|-----------------|--------------|------------|--------------|-----------------------------|-----------------------|
| A | 12 | 3 | 3 | 3 | 3 |
| B | 12 | 3 | 3 | 3 | 3 |
| E | 12 | 3 | 3 | 3 | 3 |
| F | 12 | 3 | 3 | 3 | 3 |
| G | 12 | 3 | 3 | 3 | 2 |
| I | 12 | 3 | 3 | 3 | 3 |
| L | - | - | - | - | - |
| M | 8 | 2 | 2 | 2 | 2 |
| N | 12 | 3 | 3 | 3 | 3 |
| O | 12 | 3 | 3 | 3 | 3 |
| P | - | - | - | - | - |
| Q | 12 | 3 | 3 | 3 | 3 |
| All Labs | 116 | 29 | 29 | 29 | 28 |

Second Intercalibration – Data Analyses

- Descriptive statistics (mean, range, CV and SD for neonate production, mortality, water quality measures)
- Comparisons of IC values, CV, PMSD within and among labs
- Direct comparisons of toxicity endpoints and point estimates between baseline and second ILS
- Multivariate analyses to identify standardized test parameters that may reduce variability

Second ILS - Neonate production

Mean neonates per surviving female



- Sample 1 EPA MH
- Sample 2A Perrier
- outlier

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Science Panel Recommendations

- The Panel has been meeting every two weeks to review all the data generated in this project
 - Historical data analyses
 - Lab interviews
 - Site visits information
 - Baseline ILS data
 - *Awaiting second ILS data*

Science Panel Recommendations

- The Panel is meeting every two weeks
- Objectives are:
 - To review all the data generated in this project
 - Formulate their recommendations
 - Produce final report and supporting documents

Science Panel Documentation Plan

- Executive summary with bulleted list of recommendations
- Technical guidance manual providing **scientific** rationale and discussion for the recommendations
- Appendices (**in journal format with methods, results and discussion**)
 - Historical data analyses
 - Two intercalibration exercises

Science Panel Recommendations

- Based on data available to date, they are developing four types of recommendations
 - Accreditation
 - Best practices
 - Training
 - Possible future studies

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Next Steps

| Activity | Timeline |
|--|---|
| Science Panel public meeting | July 5 |
| List of recommendations | SAC feedback by July 10 |
| Second ILS data analyses and interpretation | By end of July (meeting date TBD) |
| Draft executive summary | By Aug 31 SAC feedback by Sept 15 |
| Draft technical guidance manual and appendices | Draft by Aug 31 SAC feedback by Sept 15 |
| Final guidance document and appendices | Sept 30 to SWRCB |