

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

April 13, 2023

# Stakeholder Advisory Committee

- Katie Fong (SWRCB)
- Amelia Whitson (EPA Region IX)
- Veronica Cuevas (RWQCB4)
- Mitch Mysliwec (Larry Walker Assoc/CASA)
- Jian Peng (Orange County Public Works/CASQA)
- Sarah Lopez (Central Coast Water Quality Preservation Inc)
- Josh Westfall (Los Angeles County Sanitation Districts)
- Peter Arth (Enthalpy Laboratories)
- Annelisa Moe (Heal the Bay)
- Steve Boggs (California ELAP)

# Agenda

1. Opening remarks and review of agenda (5 min)
- 2. Approval of minutes from the last Stakeholder Committee meeting (5 min)**
3. Draft Workplan for the second intercalibration (25 min)
4. CASA proposal for additional samples (15 min)
5. Laboratory training (15 min)
6. Questions from the public (15 min)
7. Next steps (5 min)

# At your last meeting

## Update on Task 2

- Summary of the lab visits and roundtable workshop
- Review of the list of standardized parameters for the second ILS

## Preparation for Task 3 - second ILS

- Study question – ***Does standardizing lab practices improve consistency and comparability in *C. dubia* test results?***
- Study design – Recommendation to use same as baseline ILS

# Overall progress to date

- Task 1- Identify potential sources of intra- and inter-variability
  - Compile and analyze historical data
  - Conduct baseline intercalibration

COMPLETED

- Task 2- Lab training
  - Lab visits by Science Panel members
  - Roundtable workshop with laboratories

COMPLETED

- Task 3- Evaluate efficacy of test conditions and QA refinements
  - Conduct second intercalibration **TOPIC OF TODAY'S MEETING**
  - Develop test guidance to improve test comparability and consistency

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# Draft Workplan for second ILS

Workplan and QAPP remain largely the same

11 out of the 12 labs that participated in baseline ILS

Main changes are

- List of standardized parameters
- Additional documentation requested

The Science Panel reviewed and made minor changes

# Standardized parameters

- Renew or terminate test boards daily at 24 hrs **within a 2-hr window (i.e., +/- 1 hour)**
- Quantify initial food density **and document feeding method to estimate density in test chambers.**
- Holding times of YCT  $\leq 7$  days after thawing and algae  $\leq 21$  days. **Use same batch for an entire testing round.**
- Evaluate brood boards for 2 weeks prior testing. **Organisms should be out of mass culture.**
- **Store reagents in desiccator at least one week prior to testing.**



# Standardized parameters

- Use 6-10 days old adults to start the test.
- Use < 24 hr old neonates produced within an 8-hr window. Record brood number used.
- Run test for 8 days.
- Use randomization by blocking of known parentage AND randomization of cups on the board.
- Document split broods on bench sheets at the time of observation is required.

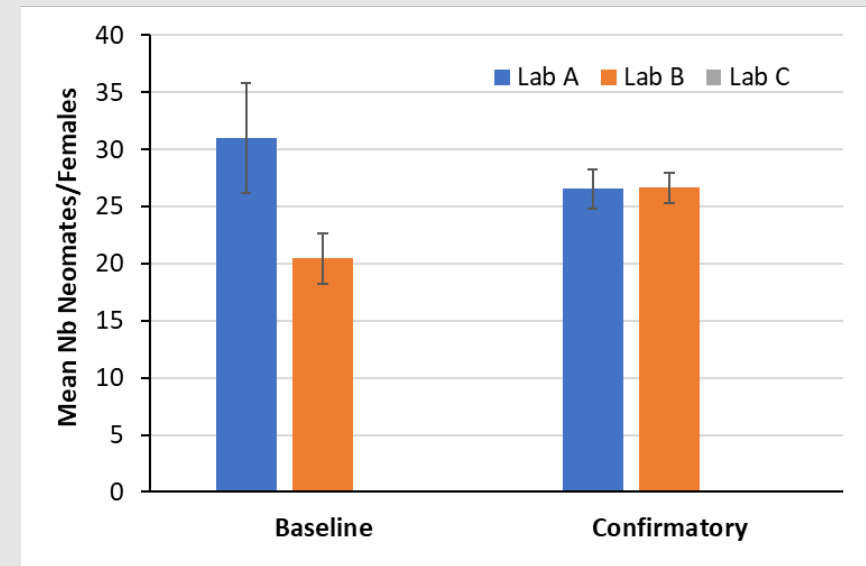
# New documentation requested

- Day/time when labs would have ended the test
- Detailed observations used to determine split broods
- Vendors for all reagents
- Food creation date and feeding method

# Proposed data analyses

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

*Example of key graphic (not REAL data)*



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# CASA proposal for additional samples

- CASA requested that samples of varying hardness (soft and hard) be added to the second ILS
  - Provided a written proposal submitted to the Science Panel and Stakeholder Committee
- Science Panel discussed at length and raised the following concerns
  - Hypothesis is different than the current hypothesis for the second ILS
  - Existing study design is not compatible
  - Not all laboratories have the testing capacity
  - Ultimately agreed not to endorse these additional samples in the second ILS

# CASA proposal for additional samples

- Science Panel recommendations:
  - CASA Proposal could be tested separately from the ILS
  - Existing studies may provide the additional information desired
  - The testing manual should be used for guidance (e.g., hardness controls)
  - Dischargers with known soft or hard water should work closely with their selected labs prior to sending samples

# Test samples for second ILS

- Sample 1: EPA moderately hard dilution water recipe to be tested at full strength (i.e., 100%).
- Sample 2A: Perrier dilution water recipe #2 to be tested at full strength.
- Samples 2B, 2C, 2D, 2E, 2F: 5 dilutions of sodium chloride in Perrier water, provided prepared at SCCWRP (i.e., no additional sample dilution allowed)
- Sample 3: NaCl as a solid with instructions to prepare 5 dilutions using the lab's own dilution water.

# Schedule for second ILS

April 21: First batch of split samples prepared by SCCWRP

April 24: Cubitainers shipped to the labs

April 25: First batch of *C. dubia* tests performed

May 12: Second batch of split samples prepared

May 15: Cubitainers shipped to the labs

May 16: Second batch of *C. dubia* tests performed

June 2: Third batch of split samples prepared

June 5: Cubitainers shipped to the labs

June 6: Third batch of *C. dubia* tests performed

***Data submission required after each testing round; no later than 10 days after test termination.***



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# Laboratory training

Two 1.5-hr sessions with participating laboratories and the Science Panel

- Wed 4/19: Review of guidance documents
- Thur 4/20: Review of data submission process

# Laboratory documentation training

- How to document split brood
- Brood board health criteria including photos and description
- Randomization of test cups
  
- Detailed instructions for data submission
- Water treatment checklist
- Template bench sheets

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# Schedule and Next Steps

Activity	Output	Timeframe
<del>Lab visits and roundtable workshop</del>	<del>Create list of recommended standardized parameters</del>	<del>March 2023</del>
<b>Second ILS workplan and QAPP</b>	<b>Approved documentation with testing details</b>	<b>By April 17</b>
Laboratory training	Informational item (guidance documents) for participating laboratories	By April 20
Second Intercalibration	Data quantifying improvement for among lab variability	Mid-April thru mid-June
Intercalibration data analysis	Refined list of recommendations	By July 15
Draft Guidance Manual	Final product for review	By end of August
Final Guidance Manual and supporting documentation	Final product for Labs and SWRCB	End of September

- Recording and minutes available on SCCWRP Cerio project webpage <https://www.sccwrp.org/about/research-areas/additional-research-areas/ceriodaphnia-toxicity-testing-quality-assurance/>