Ceriodaphnia dubia

Quality Assurance Study

Commission September 8, 2023

Background and Goals of the Study

- The *Ceriodaphnia dubia* reproduction test is the most common toxicity test in California for NPDES permit monitoring
 - Approved test method for 20 years
- Stakeholders have expressed concerns regarding consistency within and comparability among labs
 - Potentially challenging with the new Toxicity Provisions
- This study aims to assess laboratory performance and determine whether laboratory best practices can be recommended to improve consistency
 - This is not an evaluation of the test of significant toxicity (TST)

Ceriodaphnia dubia Toxicity Test



- Freshwater flea (~ 1 mm length)
- Mostly females, reproduce asexually
- Chronic toxicity test (up to 8 days)
- Test endpoints: survival and reproduction (i.e., number of neonates produced per female)
- Test acceptability criteria for controls
 - \circ ≥ 80% survival
 - Average of 15 neonates produced per surviving female
 - \circ ≥ 60% surviving females produced 3 broods

Expert Science Panel

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

Stakeholder Advisory Committee

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

Study Approach

- Document laboratory practices among CA-accredited laboratories
- Evaluate correlations between lab practices and test outcome using historical data from the labs
- Conduct a split-sample testing exercise with laboratories using their own lab techniques
- Host a workshop to agree on a list of standardized practices
- Conduct a second split-sample testing exercise with laboratories using the predetermined list of standardized lab techniques
- Develop test method guidance/recommendations to reduce variability and improve inter-laboratory comparability

Study Findings

- It's not the method, it's the labs
 - No two labs run the test exactly the same
- Several labs run the test with relatively low and consistent variability, with comparable responses to spiked toxicant samples
 - Based on lab intercalibrations, historical data
- Standardization helped but did not resolve the problem for labs with high variability
 - Based on first to second intercalibration study results
- Science Panel has made some recommendations that should help moving forward

Science Panel Recommendations Come in Three Categories

• Best practices – primary audience is the laboratories

• Accreditation – primary audience is the state

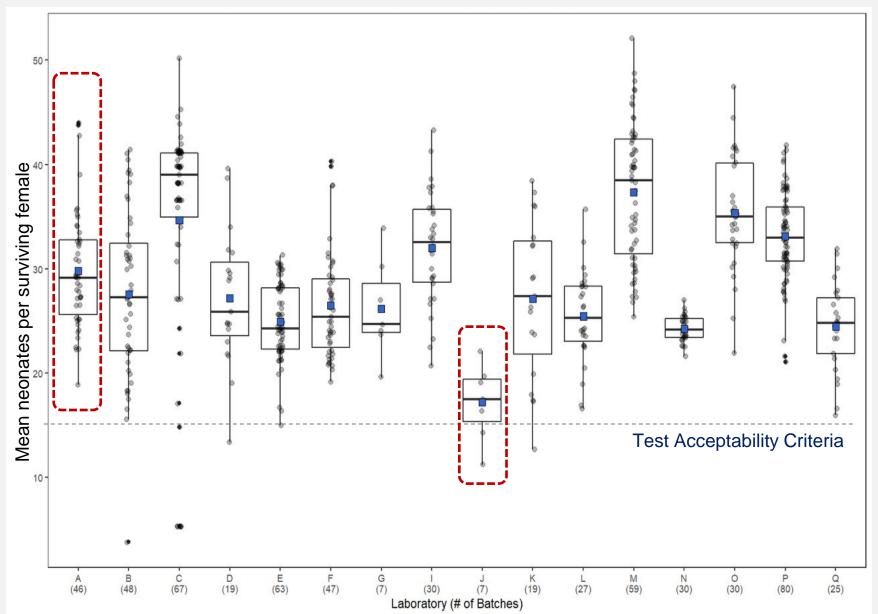
Training – primary audience are labs, state, and regulated

Best Practices Recommendations

- End test when 60% of surviving control females have produced 3 broods, within a 2-hr window of test initiation
- MUST
- Independently quantify food density and loading rate
- Use water source that meet requirements of the EPA manual
- Randomize test chambers
- Conduct detailed quantitative assessments of brood board health
- Document split broods on bench sheets
- SHOULD
- Renew test solutions daily within a 2-hour window
- Update lab documentation
- Store reagents appropriately

Variability in reproduction in lab controls

- Represents last 30 tests and up to 3 years
- Most labs show consistency; average coefficient variation ≤ 20
- Few of them have difficulties meeting test acceptability criteria



Accreditation Recommendations

- Increase number and/or frequency of proficiency sample testing for CAaccredited laboratories
 - Similar to the study intercalibration
- Collect and evaluate additional data associated with proficiency testing
- Optimize lab audits to ensure effective and consistent implementation of best practices

Variability could not be explained because all labs do things slightly differently

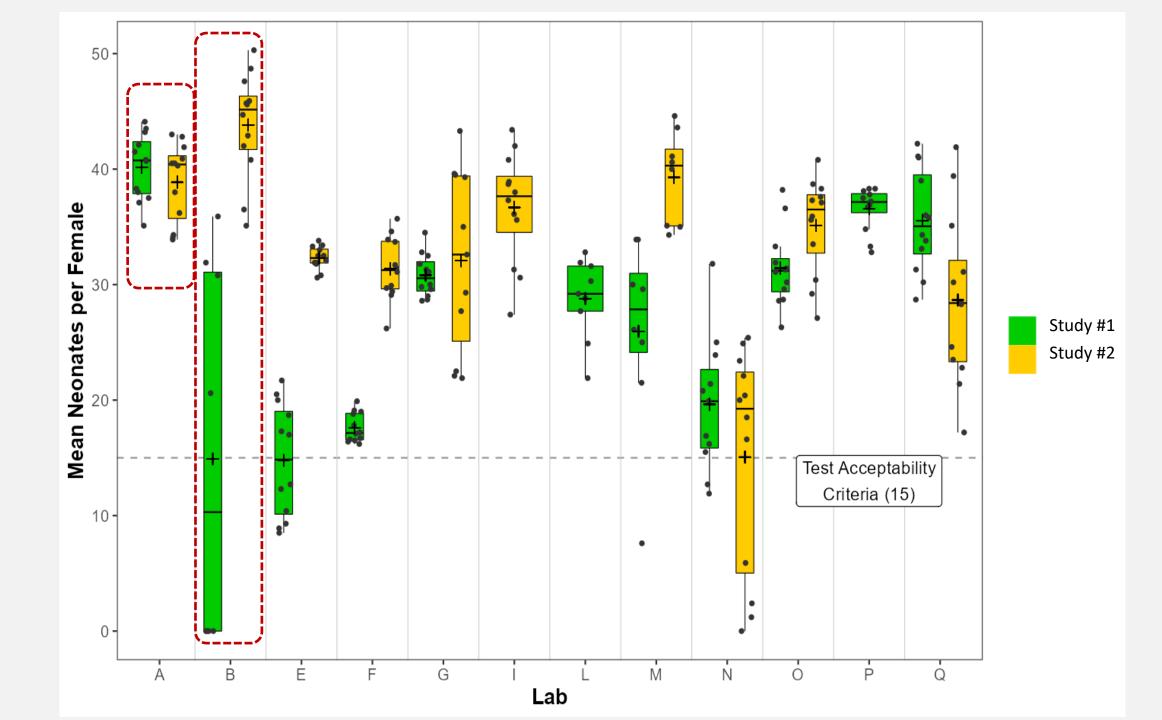
Lab	Dilution Water Recipe	Culture maintenance	Test Termination Trigger	Reference Chemical	Sample Volume (ml)	Light Source
Α	EPAMH + vitamins + Se	daily	≥60% with 3 broods	Sodium chloride	20	fluorescent
В	Modified EPAMH	daily	≥60% with 3 broods	Sodium chloride	15	fluorescent
С	EPA Hard + vitamins + Se	daily	≥60% with 3 broods	Copper chloride	15	fluorescent
D	EPAMH + Se	daily	≥60% with 3 broods	Copper chloride	15	fluorescent
E	EPAMH	daily	7 days	Copper chloride	30	fluorescent
F	80% DIW: 20% Perrier	daily	≥60% with 3 broods	Copper chloride	15	fluorescent
G	80% DIW: 20% Perrier	daily	≥80% with 3 broods	Copper (unknown type)	15	fluorescent
н	80% DIW: 20% Evian	daily	≥70% with 3 broods	Zinc sulfate	Not sent	fluorescent
I	Hoheisl +vitamins + Se	every other day	≥60% with 3 broods	Sodium chloride	15	fluorescent
J	EPAMH	daily	≥60% with 3 broods	Sodium chloride	15	fluorescent
к	L1650% + vitamins + Se	daily	≥60% with 3 broods	Sodium chloride	15	fluorescent
L	EPAMH + vitamins	daily	≥60% with 3 broods	Sodium chloride	15	fluorescent
М	Modified EPAMH + vitamins	every other day	≥60% with 3 broods	Sodium chloride	15	fluorescent
N	EPAMH + Se	every other day	≥60% with 3 broods	Sodium chloride	15	LED
0	EPAMH + vitamins + Se	every other day	≥60% with 3 broods	Sodium chloride	15	LED
Р	80% DIW: 20% Perrier	daily	≥60% with 3 broods	Copper (unknown type)	30	fluorescent
Q	80% DIW: 20% Perrier	daily	≥60% with 3 broods	Sodium chloride	15	fluorescent

Training Recommendations

• Implement auditors' training program.

• Implement training program with defined performance goals for all personnel involved in performing or reviewing *C. dubia* test.

 Provide guidance to regulated parties to evaluate toxicity test data and understand the results.



Study Constraints

- Number of laboratories and timing may not capture all sources of variation
- Quantified the improvement in variability cumulatively across all nine standardized best practices, but cannot quantify the variability for each best practice individually.
- Quantifying variability associated with dilution water of varying hardness.
- Quantified variability for concentration response for a single toxicant and a single species.

Next Steps

- Completed all testing activities, drafted a final report
- Held a joint meeting of the Science Panel and Stakeholder Committee on Wednesday
- Final Report due Sept 30
 - Raw data, too
- SWRCB Informational Item on Oct 17