C. dubia QA evaluation study

Expert Science Panel Meeting

Tuesday April 12, 2022

Agenda

1. Opening Remarks and Review of the Agenda (5 min)

2. Perspective from Stakeholders (20 min)

3. Proposed Testing Plan (45 min)

4. Public Comments (10 min)

5. Schedule and Next Steps (5 min)

Stakeholders Perspectives

Public laboratories - Joshua Westfall (Los Angeles County Sanitation Districts)

Private laboratories – Peter Arth (Enthalpy)

Proposed Testing Plan

Agenda

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Background

CA State Water Board has adopted a statewide policy and plan to address aquatic toxicity

- Known as Toxicity Provisions

During public comment period, stakeholders shared some of their concerns regarding the *C. dubia* reproduction test

- E.g., Split samples tested by two labs can provide two different results

The current study was developed in response to these concerns to improve consistency and comparability amongst labs

Goals of the Study

1) Investigate differences in *C. dubia* test laboratory practices used by ELAP accredited laboratories

2) Evaluate intra-lab consistency and inter-lab comparability of *C. dubia* test results amongst accredited laboratories

3) Standardize select test laboratory practices to reduce intra- and inter-laboratory variability in *C. dubia* test responses

Approach

Task 1- Investigate differences in C. dubia test laboratory practices used by ELAP accredited laboratories

- **Develop a comprehensive database documenting historical data and lab techniques**

Task 2- Evaluate intra-lab consistency and inter-lab comparability of *C. dubia* test results amongst accredited laboratories

- **Conduct in depth data analyses to assess intra- and inter-lab variability and identify possible sources of variability**

Task 3- Standardize select test laboratory practices to reduce intra- and inter-laboratory variability in *C. dubia* test responses?

- **Perform intercomparison laboratory exercises using existing and optimized lab procedures**

Task 1 Update

COMPLETED

We now have one of the largest and comprehensive databases of its kind

- Control and reference toxicant test data including neonate production, water quality parameters
- Culture and brood board information
- Dilution water and food recipes
- *C. dubia* lab practices
- General lab information and operating procedures

Task 2 Update

COMPLETED

Exploratory analyses revealed

- No two labs in our database are conducting the test in exactly the same manner
- Relatively high intra- and inter-laboratory variability in control responses (mean, CV), reference toxicant responses (EC50) and some water quality parameters (mean, CV)

Statistical analyses were inconclusive

- Different methods used: random forest, generalized linear model, logistic regression
- No consistent or predominant lab technique identified from statistical analyses

Approach

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- Conduct in depth data analyses to assess intra- and inter-lab variability and identify possible sources of variability

Task 3- Standardize select test laboratory practices to reduce intra- and inter-laboratory variability in *C. dubia* test responses?

- **Perform intercomparison laboratory exercises using existing and optimized lab procedures**

Task 3 Planning

- SCCWRP was asked to draft a testing plan

- Two key questions were identified:

  Q1: Does standardizing lab practices improve consistency and comparability in *C. dubia* test results?

  Q2: Which lab practice should be standardized to reduce intra- and inter-lab variability?

Task 3 Planning

- SCCWRP was asked to draft a testing plan
- Two key questions and testable hypotheses were identified
- To address these questions, we have developed a two-phase testing approach
  - We are looking for Expert's feedback to ensure that the proposed study workflow is appropriate and will answer the study questions

Standardizing lab practices to reduce variability

One main challenge is defining what is acceptable within and between lab biological variability

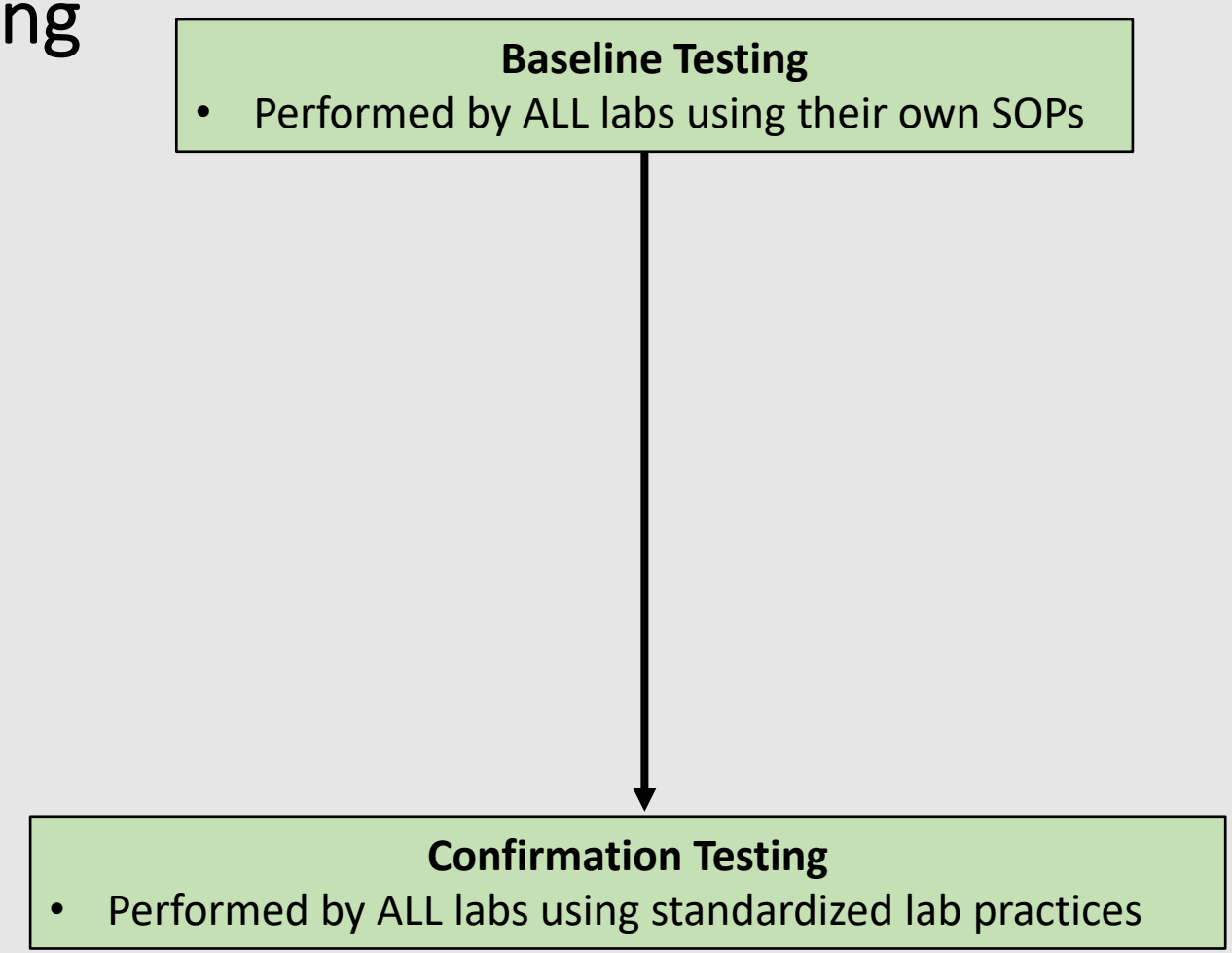
For this study, reduced variability and improved comparability could be defined in different ways

- Any reduction in CV compared to the current observations
- Use EPA 2001 data – CV ≤0.17 within and ≤0.28 among labs for IC25
- Ensure that mean, SD and/or CV of labs are as good as the referee lab

Testable Hypothesis 1

Standardizing lab practices does reduce intra- and inter-variability in test ‘control’ and ‘spiked’ samples (mean, SD, CV) among laboratories.

Proposed workflow for C. dubia testing



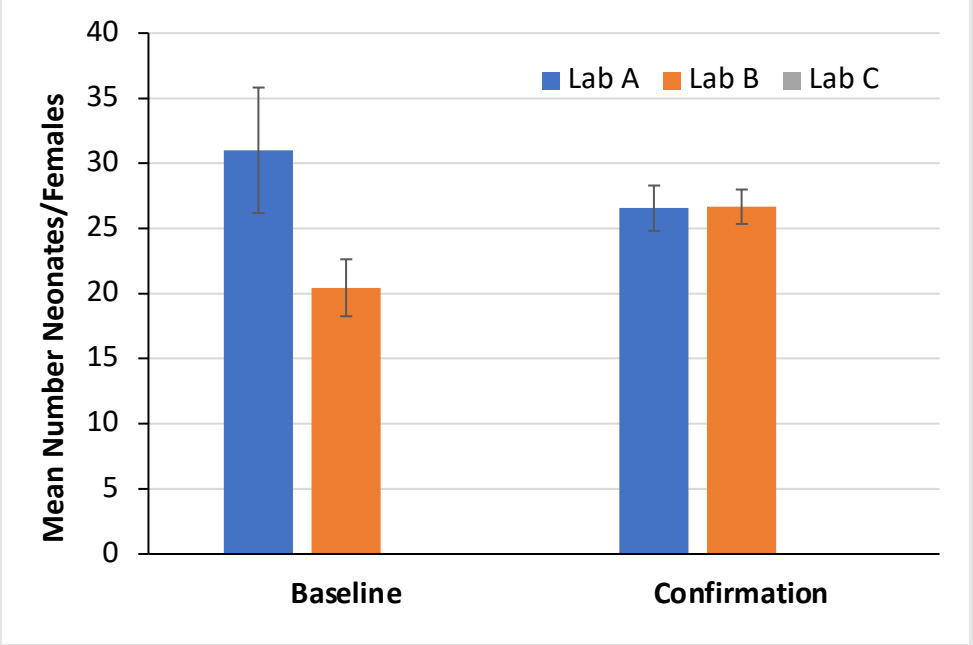
Conceptual Study Design to Address Q1

Blind sample analyses performed by all 16 labs

- Baseline testing using current SOPs vs confirmation testing using standardized lab practices
- 8-day tests with daily neonate production
- Additional data collection (e.g., specific age window, brood board health, ionic composition, etc..)
- Blind samples would include dilution waters, spiked sample
- 3-4 rounds of testing to calculate CV (with input from biostatisticians)

Note: Before baseline and confirmation testing occurs, coordination meeting will be scheduled with the labs to go over procedures, data reporting and submission…

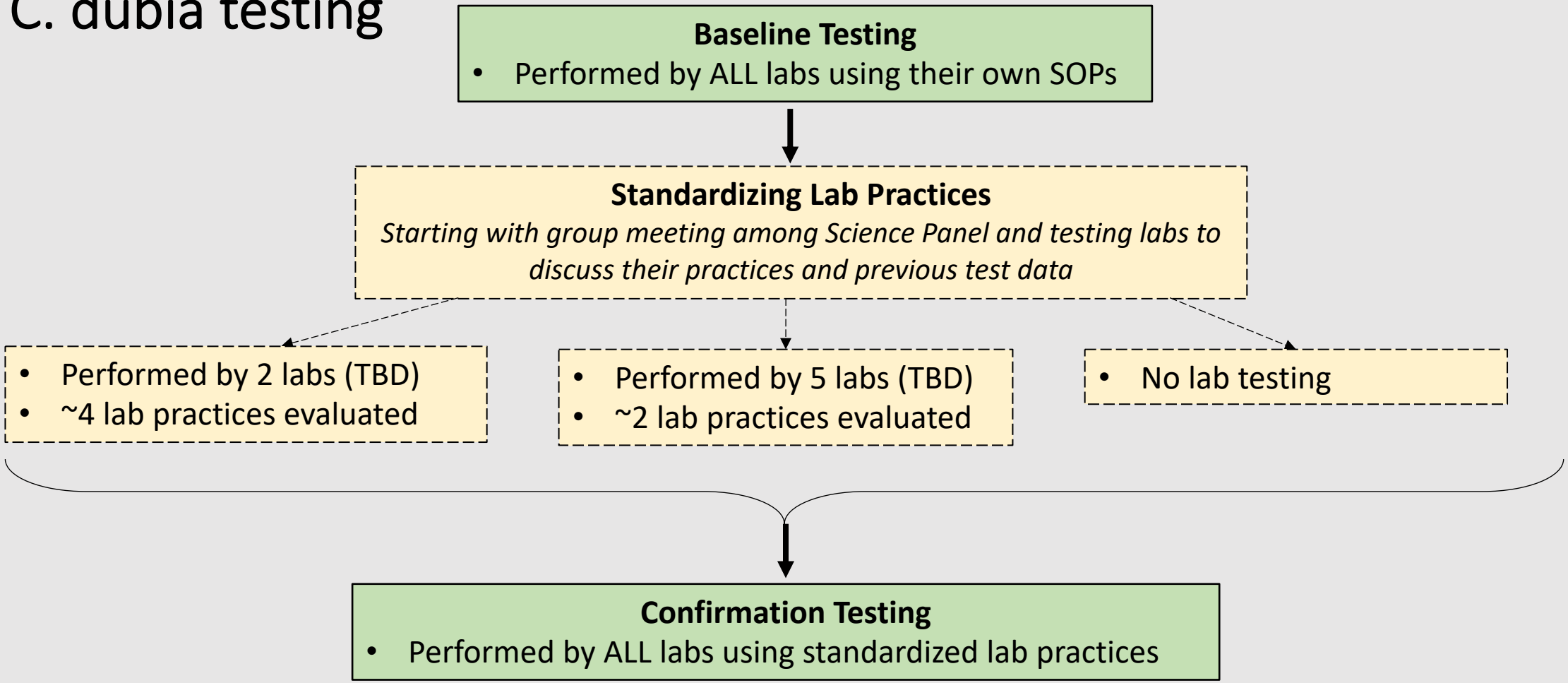
Example of key graphic (not REAL data)



Testable Hypothesis 2

“Lab practice x” does impact variability in test ‘control’ and ‘spiked’ sample responses (mean, SD, CV).

Proposed workflow for C. dubia testing



Conceptual Study Design to Address Q2

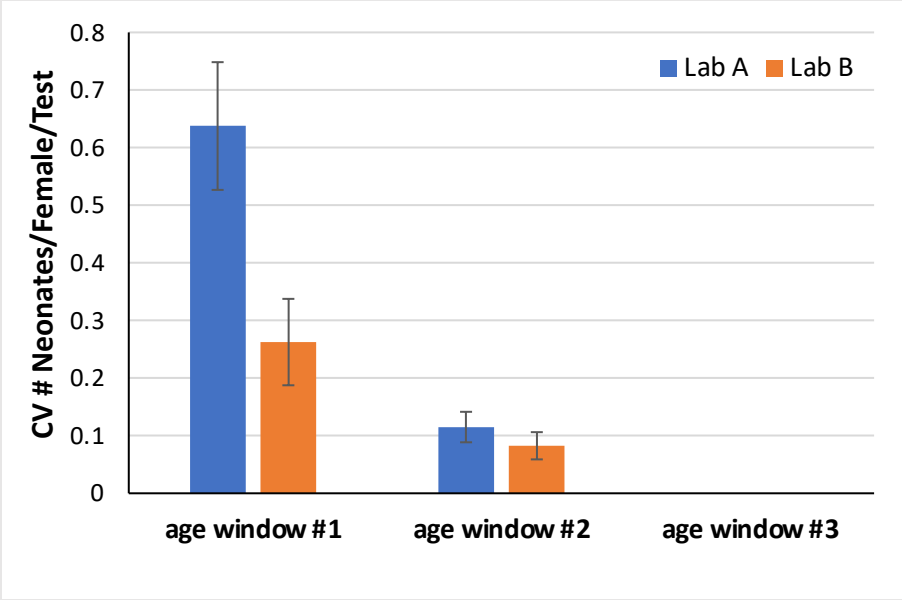
Multiple rounds of testing by 2 to 5 labs(selection criteria TBD).

Lab practices to optimize will be prioritized by the Science Panel with input from laboratories. These may include:

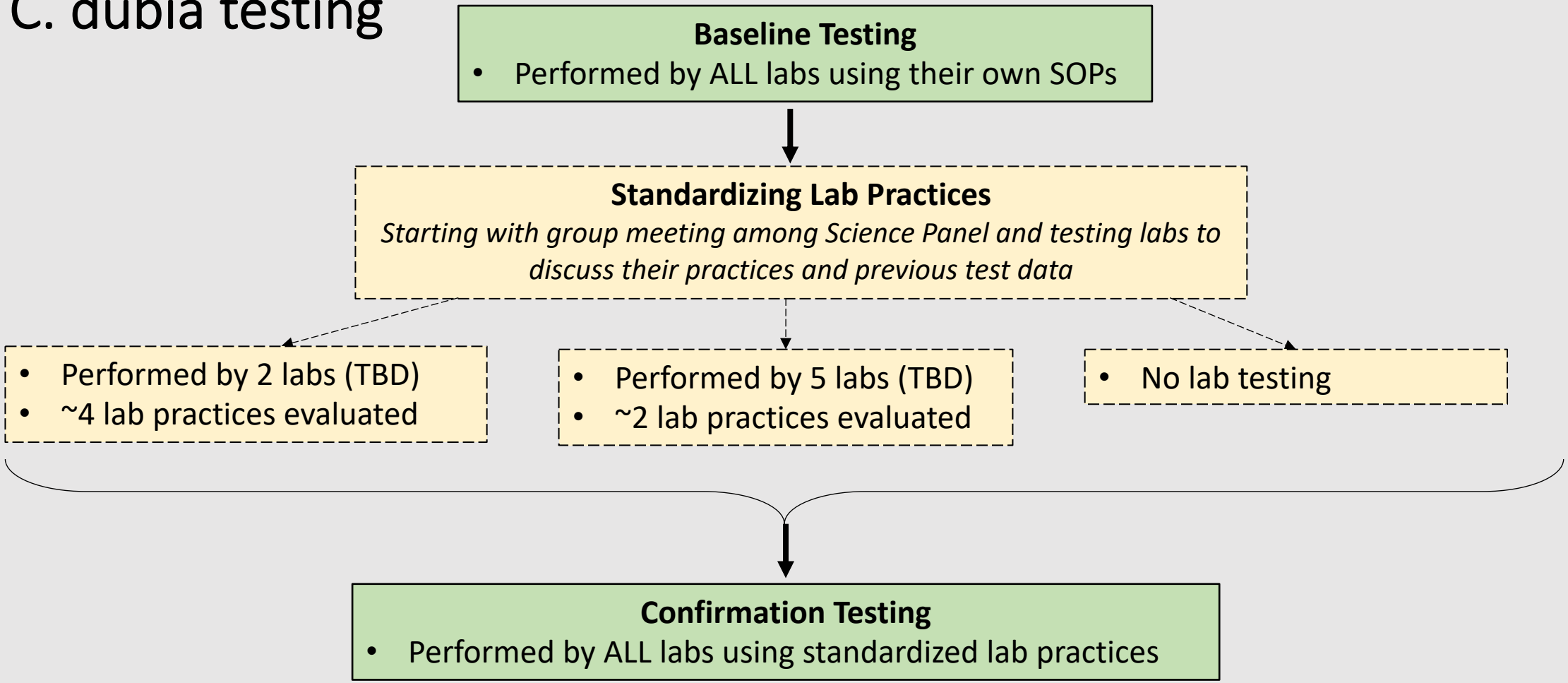
- Different age windows
- Different water recipes
- Different YCT food recipes

These will be 8-day tests with dilution waters, spiked samples (e.g., ref tox chemical)

Example of key graphic (not REAL data)



Proposed workflow for C. dubia testing



Schedule and Next Steps

Next Steps and Schedule

If study plan is approved, the following activities will be conducted:

- Produce written lab testing plan for review by SAC and ESP (by end April/early May)
  - Including description of all three phases, and suggested timeline to complete testing
- Initiate coordination and logistics for baseline testing (May/June 2022)
  - Develop study plan and QA plan, data sheets and submission, data management
  - Schedule group meeting with participating labs and science panel

We should aim to prepare and ship the first batch of split samples by early July