Maintenance of *Ceriodaphnia dubia* Mass and Chronic-Bioassay Cultures

1. SCOPE AND APPLICATION

1.1. This method is based on EPA-821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Ed. October 2002 and the test organism culturing experience of laboratory personnel. It is used to ensure that *Ceriodaphnia dubia* (*C. dubia*) bioassay test organism cultures are maintained to provide suitable test organisms. Tests affected by this SOP are BSY-AS-FCD, BSY-AD-FCD, BSY-CS-FCD and BSY-CD-FCD.

2. SUMMARY OF METHOD

2.1. Culture vessels (disposable plastic cups and beakers) are prepared by soaking or washing. Cultured organisms are fed and transferred on a regular basis to prepared vessels containing fresh media. Reproduction, mortality, and health of the organisms are monitored and recorded in the *Feeding Log and Culture Record for C. dubia* logbook.

2.2. Interferences:

2.2.1. Improperly prepared culture vessels, culture media, and food can be detrimental to the survival and reproduction of cultured *C. dubia*.

3. EQUIPMENT AND SUPPLIES

3.1. Mass Culture

3.1.1. *C. dubia* used for “Mass Culture” (neonates from Monday counts)

3.1.2. 2 L beakers (2) and/or large, 20 cm, Carolina® dish

3.1.2.1. Other suitable culture containers may also be used if they meet the material guidelines found in EPA 821-R-01-012. Substitute the appropriate container name in place of “beaker” as needed.

3.1.3. Labeling tape and waterproof black marker

3.2. Chronic Cultures

3.2.1. *C. dubia* chronic culture ‘A’, *C. dubia* chronic culture ‘B’, *C. dubia* chronic culture ‘C’ and *C. dubia* mass cultures ‘A’ and ‘B’. Additional cultures as needed.

3.2.2. Ninety 30-mL disposable plastic cups

3.2.2.1. Preferably, presoak new 30-mL disposable plastic cups with deionized (DI) water for at least 12 hours before use; or triple rinse with DI water (spray) from the Toxicity lab DI hose.

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3.2.3. (3) 30-place Plexiglas trays

3.2.3.1. Label the first tray "*C. dubia* chronic culture A", the second tray "*C. dubia* chronic culture B" and the third "*C. dubia* chronic culture C."

3.2.3.1.1. Label along the front of the tray. The left rear corner contains 30-mL disposable plastic cup #5. The right rear corner is the 30^{th} slot and it contains the reserve 30-mL disposable plastic cup, labeled "Res." The 30-mL disposable plastic cups across the front of the tray from left to right are #1, #6, #11, #16, #21, and #26.

3.2.4. On Mondays when a *C. dubia* test will be set up that week, an additional ninety 30-mL disposable plastic cups and 3 additional 30-place Plexiglass trays are labeled "Adults for Neonates Culture A", "Adults for Neonates Culture B", and "Adults for Neonates Culture C".

3.2.4.1. One 60-place tray can substitute for two of the 30-place trays. Label as if it were two 30-place trays. A piece of tape separates the tray into two 30-cup sections.

3.2.5. 250 mL or 500 ml Graduated cylinders or Erlenmeyer flasks

3.2.6. Dropper bulb (use with Pasteur pipet)

3.2.7. Pasteur pipet or large-bore plastic transfer pipet

3.2.8. Disposable plastic transfer pipet

3.2.9. Light table

4. REAGENTS AND STANDARDS

4.1. MHWCD (Moderately Hard Water for *C. dubia*). Prepared per SOP TA-03.15.

4.2. YCT (Yeast, Cereal Leaves, Trout Chow) (food for *C. dubia*). Prepared per SOP TA-01.01.

4.3. *Pseudokirchneriella subcapitata (P. sub* formerly *Selenastrum capricornutum* -algae for *C. dubia*). Prepared per SOP TA-01.04.

4.4. Deionized water

5. MASS CULTURE PREPARATION

5.1. Label two clean 2 L beakers "Mass Culture A" and "Mass Culture B". Write the current date on the label and the current MHWCD batch #.

5.2. After the chronic cultures have been transferred on Mondays, empty remaining *C. dubia* into the beakers labeled "Mass Culture".

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5.2.1. Neonates from culture tray “C” can be split so that half are transferred to “Mass Culture A” and the other half to “Mass Culture B”.

5.3. Add MHWCD to the new “Mass Culture” beakers to bring the volume to approximately 1.8 liters.

5.4. Take the old “Mass Culture” beakers to the sink and empty them. Rinse with water and place the dirty beakers on the “to be cleaned” glassware cart.

5.5. Place the new “Mass Culture” beakers on Culture rack B4.

5.6. Feed cultures per SOP TA-02.00.

5.7. Record feeding, cleaning, parameters and media replenishment in the *Feeding Log and Culture Records for Ceriodaphnia dubia* logbook.

5.7.1. Parameters for the “Mass Culture” beakers are typically done on Fridays along with the DI water parameters but can be done any day of the week. DO, pH, temperature, and conductivity are taken and recorded.

6. CHRONIC BIOASSAY CULTURE PREPARATION

6.1. Empty the DI water from the disposable plastic cups soaking in the Plexiglas trays labeled “*C. dubia* chronic culture ‘A’, ‘B’ and ‘C’”. On **Mondays**, empty the DI water from the disposable plastic cups soaking in the Plexiglas trays labeled “Adults for Neonates”. If disposable plastic cups were not soaked the night before, rinse disposable plastic cups with DI water three times.

6.2. Use a cylinder or flask to pour 20 (± 2) mL of MHWCD into each of the disposable plastic cups from Step 6.1.

6.3. Place the trays containing the *C. dubia* chronic ‘A’, ‘B’ and ‘C’ bioassay cultures on the light table.

6.4. On **Mondays**, count the neonates and record their number in the *Culture Records for Ceriodaphnia dubia* logbook. Also, record any dead or missing adults. Using a Pasteur pipet with a dropper bulb or a plastic transfer pipet, transfer one neonate to the corresponding new culture disposable plastic cup (1-29). Transfer 15 neonates from the old reserve disposable plastic cup to the new one. Any dead or missing adults should be replaced using adults from the reserve disposable plastic cup. At the same time, transfer the adult from the old culture to the corresponding disposable plastic cup on the “Adults for Neonates” tray to produce <24-hour old neonates for use in Tuesday test set up.

6.4.1. If a culture disposable plastic cup does not contain at least eight healthy neonates, a neonate is transferred from a disposable plastic cup containing eight or more healthy neonates. The letter “X” is noted in the culture logbook to account for this replacement. More than one disposable plastic cup can be

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loaded from a disposable plastic cup containing more than eight healthy neonates.

6.4.2. While transferring, do not crush the *C. dubia* by capturing them against the side or bottom of the cup. When releasing the *C. dubia* into the new disposable plastic cup, release them under the surface of the liquid by gently squeezing the dropper bulb or transfer pipet.

6.4.3. The neonate transferred to the culture disposable plastic cups on Monday matures into the adult that is transferred on Wednesdays and Fridays, and is replaced the following Monday.

6.4.4. If *C. dubia* tests are not being run, the adults that would be transferred to the "Adults for Neonates" tray should be transferred to a glass Carolina dish instead. Label the dish "Adults for Neonates", the date, and the MHWCD batch #.

6.5. Every weekday, repeat Steps 6.1.-6.4. Transfer all adults in the old reserve disposable plastic cup to the new reserve disposable plastic cup. On **Wednesdays** and **Fridays**, count neonates similar to Monday.

6.6. After all transfers are complete for the day, feed the newly transferred *C. dubia* (refer to SOP TA-02.00). Food can be added to the culture disposable plastic cups after the MHWCD is poured and prior to the transfers. Place the trays in Incubator #4 (maintained at ~25 ºC).

6.7. Empty remainder of young neonates and adults from the old culture disposable plastic cups into the culture room sink.

6.8. Rinse the inside of each empty disposable plastic cup with a strong stream of DI water, then fill the emptied disposable plastic cups with DI water and allow to soak until the next transfer.

6.8.1. If a disposable plastic cup has a film build-up that the DI water cannot dislodge, discard the disposable plastic cup and replace it with a new one.

6.9. Record daily feeding in the *Feeding Log and Culture Record for C. dubia* logbook. (Feeding on Culture transfer days are also recorded in the *Culture Records for Ceriodaphnia dubia* logbook).

7. CULTURE CRASH PROTOCOL

7.1. In the event of a culture crash, a new tray may be reestablished via two methods.

7.1.1. If there is at least one healthy tray you may use *C. dubia* in its mass culture beaker to set up a new tray.

7.1.2. If none of the trays seem healthy, or the lab wants to bring in fresher cultures, an order must be placed for new *C. dubia.* Refer to SOP TA-04.20, steps 6.1-6.14.

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8. DATA ARCHIVAL

8.1. Use a black, waterproof pen for all bench sheets and logbooks.

8.2. Record transfers in the *Culture Records for Ceriodaphnia dubia* logbook. Record culture maintenance and feedings in the *Feeding Log and Culture Records for Ceriodaphnia dubia* logbook.

8.3. To correct raw data entries, place a single line through the incorrect or illegible entry. Write the corrected entry near the error, initial and date the correction. Do not overwrite entries.

8.3.1. The corrected entry is written as near as possible to the error or in the comment box on the appropriate bench sheet.

8.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

8.4. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

9. QUALITY CONTROL

9.1. Preferably, new disposable plastic cups are soaked with DI water for at least 12 hours before use; else, disposable plastic cups are rinsed three times with DI water (from the Toxicity lab DI water hose). The rinse is to remove any potential water-soluble contaminants from the inner surface of the disposable plastic cups.

9.2. To keep the cultures healthy, mass cultures are generally cleaned and replenished every Monday unless cleaning is needed sooner (the water develops a foul odor, excessive algae growth, the cultures crash, etc.).

9.3. Chronic-bioassay cultures are transferred every Monday, Wednesday, and Friday. Transfer days can be adjusted to account for holidays and based on knowledge of when samples will be received, to ensures that the organisms will be an appropriate age for the test(s) being performed.

9.4. Culture media and food are prepared per their respective SOPs using sterile glassware and aseptic techniques to prevent contamination.

9.5. Every Monday and Friday *C. dubia* culture disposable plastic cups are replaced with fresh disposable plastic cups.

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10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Proper laboratory protection—including lab hood or ventilation system, lab coat, gloves, and safety glasses—is required when working with chemicals and unprocessed samples.

10.2. Refer to the DEP Laboratory Quality Manual.

10.3. Refer to the Laboratory Health and Safety Plan.

10.4. Read MSDS information for reagent handling and disposal.

11. REFERENCES

11.1. EPA 821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Ed. October 2002

11.2. DEP SOP TA-03.15, *Preparation of Synthetic Moderately Hard Freshwater*

11.3. DEP SOP TA-01.01, *YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) Preparation*

11.4. DEP SOP TA-01.04, *Pseudokirchneriella subcapitata* Culture for *Ceriodaphnia dubia* Food

11.5. DEP SOP TA-02.00, *Feeding Test-Organism Cultures*

11.6. DEP SOP TA-04.20, Receiving and Maintaining Purchased Test Organisms

11.7. FDEP Laboratory Quality Manual

11.8. FDEP Laboratory Health and Safety Plan

11.9. MSDS Information Sheets

APPENDIX OF CHANGES

09/25/2008 Added Scope and Application, Summary of the Method, Interferences, References and Appendix of Changes.

06/27/2013 Reformatted SOP

06/05/2014 Updated procedure with ‘C’ culture included

01/29/2015 Formatted for 508 compliance

06/08/2016 Updated links

05/29/2018 Updated procedure with “Mass Culture” parameter information and “Adults for Neonates” Carolina dish information. Update logbook names.

05/29/2019 Updated logbook names

05/13/2020 Updated wording for 7.3.1 and 7.3.2. Added links.

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04/13/2021 Updated for universal language and elaborated on 6.4.1 for more clarity.

05/24/2023 Updated for universal language, grammar, and accuracy.

05/09/2024 Updated links and references, adding missing ones to the text and removing unused ones. Edited for grammar, word choice, and readability.

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