Production of *Ceriodaphnia dubia* for Chronic and Acute Toxicity Testing

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 821-R-02-012 Short-term 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 organisms are available for bioassay testing. Tests conducted by this procedure are BSY-CS-FCD, BSY-CD-FCD, BSY-AS-FCD and BSY-AD-FCD.

2. SUMMARY OF METHOD

2.1. Cultured organisms are fed and transferred on a regular basis to 30-ml plastic disposable cups containing fresh media. The day prior to setting up bioassay tests, *C. dubia* adults are transferred to cups (one per cup) as "adults for neonates". The transfer can be performed after noon (or earlier as needed) and is checked before the end of the day and on the morning of the test to ensure that neonates are < 24 hours old and that the age range is less than 8 hours when the organisms are loaded into test replicates. Reproduction, mortality, and health of the organisms is monitored and recorded in the *Culture Records for Ceriodaphnia dubia (water flea) Adults for Neonates* logbook.

2.2. Interferences:

2.2.1. Contaminated culture vessels or contaminated culture media and poor-quality food can be detrimental to the survival and reproduction of cultured *C. dubia*.

3. EQUIPMENT AND SUPPLIES

3.1. 30-mL plastic disposable cups

3.2. Pasteur pipet (2 mm bore) or wide-bore plastic transfer pipet

3.3. *C. dubia* culture tray(s) (120 places "Adult for Neonates")

3.4. *C. dubia* organism collection tray

3.5. Clear plastic culture tray cover

3.6. *Culture Records for Ceriodaphnia dubia (water flea) Adults for Neonates* logbook

3.7. Magnifying glass

3.8. Black permanent marker

3.9. Light table

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4. REAGENTS AND STANDARDS

4.1. 20% DMW or MHWCD, per SOPs TA-03.01 or TA-03.15

4.2. *Pseudokirchneriella subcapitata*, per SOP TA-01.04 formerly *Selenastrum capricornutum*

4.3. YCT per SOP TA-01.01

5. PREPARATION

5.1. On the day prior to test initiation, usually Monday, set up the *C. dubia* culture tray labeled "Adults for Neonates" with adults that have produced ≥ 1 brood from the *C. dubia* Culture A, Culture B Culture C and Culture D (when in use) trays which are in Incubator # 4 (B246). Feed per SOP TA-02.00. Additional cultures may be available depending upon the test schedule. Refer to the general *C. dubia* chronic culture methods in SOP TA-04.01.

5.1.1. For chronic toxicity tests, neonates < 24 hours old and born within an eight-hour period are required. For acute tests, neonates < 24 hours old are needed. Laboratory experience will greatly aid in determining the quantity of "Adults for Neonates" cultures (cups) needed. We set up 90 – 120 cultures cups for any tests we are running.

5.2. After setting up the "Adults for Neonates" tray(s) check (cups containing adults) as needed to confirm reproduction in accordance with the needs of the tests. Record any vials containing neonates and the number of neonates in each vial.

5.2.1. To collect C. dubia for chronic tests the day prior to initiation, analysts will do hourly counts and record when they see any cups containing neonates.

5.2.1.1. If there are less than 8 neonates in a cup, use a black permanent marker to mark the rim of each cup.

5.2.1.2. If there are more than 8 neonates in a cup, mark the side of the cup with the position of the cup (Ex. A13) and time of count.

5.2.1.3. Adults from vials having ≥ 8 neonates, should be transferred to new vials containing relevant media and food, then returned to appropriate "Adults for Neonates" tray. The cup still containing the neonates are place on the organism collection tray for use in chronic testing.

5.2.1.4. Eight to twelve (8-12) cups containing ≥ 8 neonates are needed for a single chronic definitive test.

5.2.1.5. If reproduction is less than needed by the end of the workday, the bioassay lab supervisor should be notified, and steps are taken to ensure the < 24-hour old / ≤ 8-hour age limit for neonates for chronic tests.

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5.2.1.5.1. Steps can include but are not limited to returning to the lab after hours and checking reproduction progress; or, resetting the cultures the day of the test and monitoring as in Section 5.2. For acute tests, adults can be collected from the Ceriodaphnia dubia "Mass" cultures (SOP TA-04.01), placed in a culture dish (beaker or Carolina dish) with appropriate media and food and monitored for reproduction.

5.2.2. To collect C. dubia for acute tests, the day of initiation all Adults for Neonates cups will be counted, and the numbers will be recorded. Once all vials have been counted, the top four from each tray will be put to the side for consideration to use in the tests.

5.2.2.1. Fifteen (15) vials minimum containing ≥ 8 neonates would be needed for an acute definitive test.

5.2.3. The organism batch number is the date (mm/dd/yy) that the tests will be initiated.

5.3. Use appropriate age neonates for bioassay tests.

5.4. Maintain the "Adults for Neonates" culture until Thursday of the test initiation week in case additional tests are scheduled for non-normal initiation days (other than Tuesday) per SOP TA-04.01.

5.5. Record batch information in the *Culture Records for Ceriodaphnia dubia Adults for Neonates* logbook.

6. DATA ARCHIVAL

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

6.2. Record the culture set up, transfers, batch numbers and number of neonates in the *Culture Records for Ceriodaphnia dubia Adults for Neonates* logbook per SOP TA-04.01.

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

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

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

6.4. 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.).

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7. QUALITY CONTROL

7.1. Refer to DEP Laboratory Quality Manual for corrective action and handling of out-of-control data.

7.2. Autoclaved glassware, clean-rinsed cups, suitable media, and food is used to minimize the potential for contamination of the cultures.

7.3. Culture records are maintained and checked for performance. Appropriate staff are notified in the event of culture problems.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

8.2. Excess/unneeded organisms are humanely euthanized, and waste is disposed per (Refer to FDEP Laboratory Quality Manual for media disposal.

8.3. Refer to the FDEP Laboratory Health and Safety Plan and Hazardous Waste Management.

8.4. Read MSDS information sheets for reagent handling and disposal.

9. REFERENCES

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

9.2. DEP Laboratory Quality Manual

9.3. DEP Laboratory Health and Safety Plan

9.4. DEP SOP TA-01.04, *Pseudokirchneriella subcapitata culture for Ceriodaphnia dubia Food*

9.5. DEP SOP TA-03.01, *Preparation of 20% Dilute Mineral Water (DMW)*

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

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

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

9.9. DEP SOP TA-04.01, *Maintenance of Ceriodaphnia dubia Mass and Chronic-Bioassay Cultures*

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APPENDIX OF CHANGES

06/06/2013 Combined and Reformatted TA-05.01-3.14 and TA-05.02-1.13. SOP TA-05.01 was removed from the LIMS tracking system. The new SOP was named TA-05.02-2.0.

02/11/2015 Formatted for 508 compliance.

06/13/2016 Updated links.

06/30/2017 Updated links.

05/30/2018 Updated links.

05/29/2019 Updated steps for clarity and elaboration

05/12/2020 Updated links. Updated equipment and section on the laboratory quality manual in the Quality Control section. Updated Data Archival section.

04/14/2021 Updated to current links.

05/24/2022 Updated links, amended and expanded preparation section, and checked for grammar.

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