Feeding Test-Organism Cultures

1. SCOPE AND APPLICATION

1.1. These methods are based on EPA 821-R-02-012 "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" and practical experience maintaining cultures in the laboratory. They are used for organisms cultured in the laboratory and organisms purchased from vendors.

2. SUMMARY OF METHOD

2.1. Unless otherwise noted, organisms are fed the appropriate quantity and type of food once daily Monday through Friday, as well as on weekends and holidays. At the discretion of the toxicity lab coordinator, maintenance of laboratory cultured organisms (*Ceriodaphnia dubia*) may be suspended for one calendar day, but not two consecutive calendar days during a weekend or holiday period.

3. EQUIPMENT AND SUPPLIES

3.1. 1 mL and 10 mL Volumetric pipettes

3.2. 2.5 mL Plastic transfer pipettes

3.3. 0.2 mL Pipetter (fixed)

3.4. YCT, prepared per SOP TA-01.01

3.5. *Pseudokirchneriella subcapitata*, prepared per SOP TA-01.04

3.6. *Artemia nauplii*, prepared per SOP TA-01.03 (less than 24 hr old nauplii are fed to test organisms)

NOTE: Toxicity Pipettes and Pipettors are calibrated per SOP TA-06.32

4. FEEDING METHODS

4.1. ***Ceriodaphnia dubia***

4.1.1. Mass Culture: Feed 2 mL of YCT and 2 mL of *Pseudokirchneriella subcapitata* per liter of culture using a disposable transfer pipette. A new pipette is used for each feeding.

4.1.1.1. *C. dubia* mass cultures are kept in a minimum of two vessels (4 L beaker(s) and/or 8" diameter Carolina dish(es)), on tray B3 in room B246. The vessels are labeled "*Ceriodaphnia dubia*" "Mass Culture *A"*, *B*, etc., with the date the culture was started (or renewed/changed weekly) and MHWCD batch.

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4.1.2. Chronic-test culture: Feed 0.2 mL of a mixture of 50% YCT and 50% *Pseudokirchneriella subcapitata* per 20 mL of culture medium daily during test duration (i.e. per vial).

4.1.3. Acute-test culture: Feed 0.2 mL of a mixture of 50% YCT and 50% *Pseudokirchneriella subcapitata* per 20 mL of culture medium on day 0 and day 2 of the test. Organisms are fed two hours prior to test initiation and renewal.

4.1.4. *C. dubia* chronic cultures are kept in Incubator #4 in room B246. Feed these cultures 0.2 mL of a mixture of 50% YCT and 50% *Pseudokirchneriella subcapitata* per 20 mL of culture medium daily, including Saturday and Sunday (see Section 2 - 2.1). On Mondays, Wednesdays, and Fridays, the food is added to the new organism vials prior to transferring the *C. dubia* into the new vials

4.2. ***Cyprinella leedsi, Pimephales promelas, Menidia beryllina, Americamysis bahia***

4.2.1. Place purchased organisms in 4 L plastic Carolina® dishes (1 per culture).

4.2.2. Feed 1-3 mL *Artemia nauplii* per Carolina® dish, once per day.

4.2.2.1. *C. leedsi* and *P. promelas* should be > 1day old; *M. beryllina* should be > 5 days old and *A. bahia* should be 1 day old before they are fed artemia. Organisms are generally received at appropriate ages to be fed upon arrival.

4.2.2.2. Check the bottom of each dish for accumulation of *Artemia nauplii* and adjust the amount used accordingly. Remove and discard excess/dead artemia from the culture dishes (daily).

4.2.3. During testing, feed fish 0.2 mL of artemia 2 hours prior to renewal and after renewal. Artemia is prepared and counted per SOP TA-01.03

5. DATA ARCHIVAL

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

5.2. Record feedings in the appropriate *Culture Records* logbook.

5.3. 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.

5.3.1. The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

5.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

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5.3.3. 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.).

6. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

6.2. Refer to Laboratory Quality Manual for sample disposal

6.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

6.4. Read MSDS information for reagent handling and disposal.

7. REFERENCES

7.1. EPA 821-R-02-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.

7.2. DEP SOP TA-01.01, YCT (Yeast, Cereal Leaves, Flake-Fish Food) Preparation.

7.3. DEP SOP TA-01.03*, Artemia Nauplii* Culture.

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

7.5. DEP SOP TA-06.32, Calibration/Verification of Mechanical and Disposable Pipettes in the Bioassay-AGP Lab

7.6. Laboratory Quality Manual http://www.dep.state.fl.us/labs/library/lab_sops.htm

7.7. Laboratory Health and Safety Manual and Hazardous Waste Management https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

APPENDIX OF CHANGES

01/22/2008 Added Scope and application, Summary of Method and Appendix of Changes. Deleted *D. pulex* chow from Materials. Added "4 mL of YCT and 4 mL of *Pseudokirchneriella subcapitata*" to *D. pulex* Step 1.

09/30/2013 Major revision. Removed feeding instructions for newly hatched fry and adults that were cultured in-house that are now being purchased.

02/09/2015 Formatted for 508 compliance.

09/11/2015 Updated references.

10/07/2016 Updated references.

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10/06/2017 *Hyallela azteca* section removed

08/29/2018 Updated Font. Revised the frequency for feeding organisms.

07/02/2019 Removed unused SOP from references08/26/2020 Added test feeding instructions

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