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 twice daily Monday through Friday, and once each day on weekends and holidays. At the discretion of the toxicity lab coordinator, maintenance of laboratory cultured organisms (*Ceriodaphnia dubia* and *Hyalella azteca*) 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 & 10 mL Volumetric pipettes

3.2. 2.5 mL Plastic transfer pipettes

3.3. 0.2 mL Pipetter (fixed)

3.4. Tropical fish food flakes

3.5. YCT, prepared per SOP TA-01.01

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

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

NOTE: Toxicity Pipettes and Pipetters 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*" with the date the culture was started (or renewed/changed weekly).

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 (i.e. per vial).

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4.1.2.1. *C. dubia* chronic cultures are kept in Incubator #4 in room B246. These cultures are fed daily, including Saturday and Sunday (see Section 2 - 2.1). On Mondays, Wednesdays, and Fridays, the chronic cultures are fed after they are transferred.

4.2. ***Hyalella azteca*** (See SOP TA-04.13)

4.2.1. Mass culture: Feed approximately 1 crumbled tablespoon full per glass culture tank every day.

4.2.1.1. Cultures are maintained on rack A3 in B246.

4.2.2. Organisms collected for test: Feed a pinch of crumbled flake food plus 5 mL of YCT per day.

4.2.2.1. Organisms collected for test are kept in a Carolina® dish.

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

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

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

4.3.2.1. *C. leedsi* and *P. promelas* should be ≥ 1day old before they are fed. *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.

4.3.2.2. 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).

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.

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.).

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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-04.13, Maintenance of *Hyalella azteca* Cultures.

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

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

7.8. 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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