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 (usually Sundays), but not two consecutive calendar days including during a weekend or holiday period.

3. EQUIPMENT AND SUPPLIES

3.1. Volumetric pipettes of appropriate size

3.2. Plastic transfer pipettes

3.3. 0.2 mL Pipettor (fixed)

3.4. YCT, prepared per SOP TA-01.01

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

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

3.6.1. Toxicity Pipettes and Pipettors are calibrated per SOP TA-06.32

3.7. *Feeding Log and Culture Records for Ceriodaphnia dubia* logbooks

3.8. *Culture Log for Ceriodaphnia dubia* logbook

3.9. *Artemia nauplii Culture and Feeding* logbook

4. FEEDING METHODS

4.1. ***Ceriodaphnia dubia***

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

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4.1.1.1. *C. dubia* mass cultures are kept in a minimum of two vessels (4 1L beakers and/or 8" diameter Carolina dishes), in incubator #4 or #5 in room B246. Typically the Tox lab uses one 1L beaker per tray (A, B, C…) for the neonates and one Carolina dish for all tray adults. The details of the mass cultures are recorded in the *Feeding Log and Culture Records for Ceriodaphnia dubia* logbook and *Culture Log for Ceriodaphnia dubia* logbook. The vessels are labeled: *Ceriodaphnia dubia* Mass Culture *A*, *B*, etc., with the date the culture was started/renewed.

4.1.1.2. Cultures are started or renewed weekly.

4.1.2. Chronic-test culture: Feed 0.2 mL of a mixture of 50% YCT and 50% *Raphidocelis subcapitata* per 20 mL of culture medium daily during test duration (i.e. per 30-mL disposable plastic cup). Organisms are fed at initiation/renewal.

4.1.3. Acute-test culture: Feed 0.2 mL of a mixture of 50% YCT and 50% *Raphidocelis subcapitata* per 20 mL of culture medium on day 0 and day 2 of the test (i.e. per 30-mL disposable plastic cup). Organisms are fed two hours prior to test initiation/renewal and at renewal.

4.1.4. *C. dubia* in-house cultures are kept in Incubator #4 in room B246. Feed these cultures 0.2 mL of a mixture of 50% YCT and 50% *Raphidocelis subcapitata* per 20 mL of culture medium daily (i.e. per 30-mL disposable plastic cup), including Saturdays and holidays (see Section 2 - 2.1). When the *C. dubia* are transferred to a new tray, the food is added to the new organism cups prior to transferring in the *C. dubia*.

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 and at least two hours prior to test initiation.

4.2.2.1. *C. leedsi* and *P. promelas* should be > 1 day 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).

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4.2.3. During testing, feed fish or mysids 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 as possible to the error or 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 with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

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 Assurance Manual for sample disposal.

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

6.4. Read MSDS information for reagent handling and disposal.

7. REFERENCES

7.1. EPA 821-R-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, or Trout Chow) 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*

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

7.6. Laboratory Quality Assurance Manual

7.7. Laboratory Health and Safety Manual

7.8. MSDS Information Sheets

APPENDIX OF SIGNIFICANT 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.

10/06/2017 *Hyallela azteca* section removed

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