YCT (Yeast, Cereal Leaves, Flake Fish Food or Trout Chow) and *Artemia* Suitability Tests

1. SCOPE AND APPLICATION

1.1. This SOP is based on EPA 821-R-02-013 and EPA 821-R-02-014. It is used to determine suitability of YCT (yeast, cereal leaves, and flake fish food or trout chow) and *Artemia*, for use in feeding *Ceriodaphnia dubia* and *Americamysis bahia*, respectively, in cultures and tests.

2. SUMMARY OF METHOD

2.1. A new batch of food is compared to a known reference batch of food by running a side-by-side 7-day chronic survival and reproduction screening test with *Ceriodaphnia dubia* (for YCT) or a 7-day chronic survival and growth screening test with *Americamysis bahia* (for *Artemia*) using the known reference food and the new food. Both tests utilize only control water (no SRT toxicant water or effluent sample). If minimum test acceptability requirements are not met, the new food is discarded, and another batch is prepared or ordered and tested. If the second test also fails, additional changes should be made.

2.2. Testing should preferably be done before known reference food is depleted. However, new food can be tested alone and is considered acceptable if the test acceptability criteria are met.

2.3. New batches of YCT and *Artemia* can be used if determined to be suitable for *Ceriodaphnia dubia* and *Americamysis bahia* respectively.

3. EQUIPMENT AND SUPPLIES

3.1. YCT Suitability Bench Sheets and FDEP Biology Laboratory-Toxicity Section *Artemia* Suitability Bench Sheets.

3.2. Known reference YCT and new YCT (per SOP TA-01.01)

3.3. Known reference *Artemia* and new *Artemia* (per SOP TA-01.03)

3.4. Materials as noted in TA-07.05 for the YCT test and as noted in TA-07.15 for the *Artemia* test

3.4.1. One 2 L Autoclaved, graduated cylinder for pouring control water

3.4.2. Screen test conditions (TA-07.05 and TA-07.15)

3.5. Lab cultured *Ceriodaphnia dubia* (per SOP TA-04.20)

4. REAGENTS AND STANDARDS

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4.1. See TA-7.05 for the YCT test and TA-07.15 for the *Artemia* test

5. TEST PREPARATIONS

5.1. YCT

5.1.1. This test should be performed whenever a batch of YCT is prepared or purchased and before reference food is depleted. Notify supervisor of any problems.

5.1.2. Record test in Chronic YCT Suitability QC Bench Sheets *Ceriodaphnia dubia* Logbook.

5.1.3. Follow the test method for SOP TA-07.05 for a chronic screening test.

5.1.3.1. Label one concentration “Reference YCT” and one concentration “New YCT”. Use the same synthetic moderately hard water for *Ceriodaphnia dubia* (MHWCD) for the two concentrations.

5.1.3.2. Use current batch of YCT plus *Raphidocelis subcapitata* as food for the “Reference YCT” concentration.

5.1.3.3. Use new batch of YCT plus *Raphidocelis subcapitata* as food for the “New YCT” concentration.

5.1.3.4. Moderately Hard Freshwater (MHWCD) is the only dilution water used for YCT suitability.

5.2. *Artemia*

5.2.1. This test should be performed whenever a new lot of cysts is purchased and before the last lot is depleted. *Artemia* is cultured (hatched) per TA-01.03. Notify supervisor of any problems.

5.2.2. Record test in Chronic *Artemia* Suitability QC Bench Sheets *Americamysis bahia* Logbook.

5.2.3. Follow the test method for SOP TA-07.15 for a chronic screening test.

5.2.3.1. Label the old cysts lot concentration “Reference *Artemia*” and the new cysts lot concentration “New *Artemia*”.

5.2.3.2. Use the same batch of artificial seawater (ASW) for the two concentrations.

5.2.3.3. Use current lot of *Artemia* as food for the “Reference *Artemia*” concentration.

5.2.3.4. Use the newly purchased lot of *Artemia* as food for the “New *Artemia*" concentration.

5.2.3.5. ASW is the only dilution water used for *Artemia* suitability.

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6. TEST ANALYSIS

6.1. YCT

6.1.1. Terminate each concentration when 60% of the surviving females have a 3rd brood of neonates.

6.1.2. The new YCT is deemed suitable for use if there is an average of ≥15 neonates per surviving organism and there is survival of ≥80%.

6.1.2.1. If the new YCT doesn't meet the requirements above, the batch should be discarded, and a new batch prepared and tested per this SOP.

6.2. *Artemia*

6.2.1. Terminate the test per SOP TA-07.15.

6.2.2. The new *Artemia* is deemed suitable for use if there is an average dry weight of ≥0.20 mg per surviving organism and there is survival of ≥80%.

6.2.3. If the new *Artemia* doesn't meet the requirements above, the lot should be returned to the supplier and a new lot purchased and tested per this SOP.

7. DATA ARCHIVAL

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

7.2. Record both the YCT and the *Artemia* results in control data for chronic tests and charts. \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\Bioassay Charts and Tables

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

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

7.3.2. 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.

7.3.3. 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.).

8. QUALITY CONTROL

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8.1. Test data should be carefully examined to ensure that poor results aren't attributable to problems within the test.

8.2. Refer to the FDEP Laboratory Quality Assurance Manual for corrective action and handling out-of-control data.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

9.2. Refer to the FDEP Laboratory Health and Safety Plan.

9.3. Read MSDS information for reagent handling and disposal.

10. REFERENCES

10.1. EPA 821-R-02-013, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*

10.2. EPA 821-R-02-014, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*

10.3. DEP SOP TA-07.05, *Ceriodaphnia dubia Chronic Toxicity Testing Method*

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

10.5. DEP SOP TA-01.03, *Artemia nauplii Culture*

10.6. DEP SOP TA-04.20, *Receiving and Maintaining Purchased Test organisms*

10.7. DEP SOP TA-07.15, *Americamysis bahia Chronic Toxicity Testing Methods*

10.8. DEP Laboratory Quality Assurance Manual

10.9. DEP Health and Safety Manual

10.10. MSDS Data Sheets

APPENDIX OF SIGNIFICANT CHANGES

07/24/2008 Added Scope and Application, Summary of the Method, References and Appendix of Changes.

01/16/2024 Added section 3.5 to address *C. dubia* culturing method.

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