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 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 and tested. If the second test also fails, additional considerations should be made to try and identify variables to alter in order to reach the minimum acceptance criteria and produce a successful batch (YCT) or additional *Artemia* purchased (requesting a different "Lot #" than what was previously used).

   2.2. Testing should be done before known reference food is completely depleted. However, if old food is not available for concurrent testing, new food can be tested (single concentration chronic bioassay) and is considered acceptable if the test acceptability criteria (survival and reproduction for *Ceriodaphnia dubia*, and survival and growth for *Americamysis bahia*) are met. The method (EPA -821-R-02-013) states that, "If the food is used for culturing (i.e. C. dubia), its suitability should be determined using a chronic test". However, the performance of cultures and/or control results in PT, SRT, or facility bioassays can substitute for the direct comparison tests, when necessary.

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

3. EQUIPMENT AND SUPPLIES

   3.1. Chronic *Ceriodaphnia dubia* and *Americamysis bahia* SRT 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 (only cylinder necessary)

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

3.5. Lab cultured *Ceriodaphnia dubia* (per SOP TA-04.01)

4. REAGENTS AND STANDARDS

4.1. Materials as noted in TA-7.05 for the YCT test and as noted in TA-07.15 for the *Artemia* test (excluding those steps necessary for SRT and effluent samples)

5. TEST PREPARATIONS

5.1. YCT

5.1.1. This test can be performed whenever a batch of YCT is prepared or purchased, regardless of whether the ingredients are from the same lot that was used to produce the previous batch. Testing should be done before known reference food is completely depleted. Supervisor should be notified of any problems.

5.1.2. Record test in *Ceriodaphnia dubia* Chronic SRT Bench Sheets

5.1.3. Follow the test method for SOP TA-07.05, *Ceriodaphnia dubia Chronic Toxicity Testing Method,* 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 YCT (the current batch of reference food), in addition to *Pseudokirchneriella subcapitata*, as food for the “Reference YCT” concentration.

5.1.3.3. Use the new batch of YCT (freshly prepared and untested), in addition to *Pseudokirchneriella 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 (no SRT toxicant water or effluent sample).

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. Supervisor should be notified of any problems.

5.2.2. Record test in *Americamysis bahia* Chronic Bioassay Bench Sheets.

5.2.3. Follow the test method for SOP TA-07.15, *Americamysis bahia Chronic Toxicity Testing Methods* for a **chronic screening test**.

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

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

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

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

5.2.3.5. Natural seawater (NSW) or Artificial Saltwater (ASW) is the only dilution water used for *Artemia* suitability (no SRT toxicant water or effluent sample).

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.).

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8. QUALITY CONTROL

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 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-07.17, *Using EPA Whole Effluent Toxicity (WET) Statistics for Acute and Chronic Bioassay Tests*

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

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

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

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

10.9. DEP Control Data for Chronic Tests

10.10. DEP Laboratory Quality Manual

10.11. DEP Laboratory Health and Safety Plan

10.12. MSDS Data Sheets

Appendix of Changes

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

08/14/2009 Replaced reference to SOP TA-07.08 with reference to TA-07.16

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07/21/2010 Removed reference to vinyl gloves in the Safety section comment and added a Caution statement

05/18/2011 Added to initial comment. 'The performance of cultures and/or control results in PT, SRT or facility bioassays can substitute for the direct comparison tests.' Corrected names of logbooks to match actual title

03/13/2013 Updated format

03/03/2014 Refined formatting

02/04/2015 Corrected numbering. Changed location of results.

03/04/2016 Updated links.

02/23/2018 Updated links. Added Hyperlinks for manuals and other SOPs.

02/12/2019 Updated links. Reworded Summary of Method for clarity.

02/12/2021 Reworded Summary of Methods, Test Preparation and Test Analysis for clarity. Edited formatting and updated links to Health and Safety Plan.

02/11/2022 Updated links and checked for grammar.

02/09/2023 Updated links and SOPs. Clarified only control water is required for suitability tests.

01/16/2024 Updated links and SOPs. Checked for grammar and word usage. Added section 3.5 to address *C. dubia* culturing method.

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