YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) and Artemia Suitability Tests for NELAC D.2.8(i)

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 and *Artemia*, for use in feeding, respectively, *Ceriodaphnia dubia* and *Americamysis bahia* in cultures and in tests.

2. SUMMARY OF METHOD

   2.1. A new batch of food is compared to a known "good" batch of food by running a side by side 7-day chronic survival and reproduction screening test with *Ceriodaphnia dubia* and a 7-day chronic survival and growth screening test with *Americamysis bahia* using the known "good" food and the new food. 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, the current organism supplier lab is contacted and "good" food is purchased.

   2.2. If old food is not available for concurrent testing, "new" food can be tested (single concentration-chronic bioassay) and will be considered to be acceptable if the test acceptability criteria (survival and reproduction for *Ceriodaphnia dubia* and survival and growth for *Americamysis bahia*) are met. The performance of cultures and/or control results in PT, SRT or facility bioassays can substitute for the direct comparison tests.

   2.3. YCT and *Artemia* are used to feed species other than the aforementioned; food that is determined to be suitable for *Ceriodaphnia dubia* and *Americamysis bahia* is presumed suitable for the other species (utilizing the same food).

3. EQUIPMENT AND SUPPLIES

   3.1. Chronic *Ceriodaphnia dubia* and *Americamysis bahia* SRT Bench Sheets

   3.2. Known good YCT and new YCT

   3.3. Known good *Artemia* and new *Artemia*

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

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

5. TEST PREPARATIONS

   5.1. YCT

      5.1.1. This test can be performed whenever a batch of YCT chow is prepared or purchased, regardless of whether or not the ingredients are from the same lot as was used to produce the previous batch. Testing should be done before known good food is completely depleted. Supervisor should be notified of any problems.

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

5.1.3. Follow the test methods for SOP TA-07.05, *Ceriodaphnia dubia Chronic Toxicity Testing Methods* for a chronic screening test.

5.1.3.1. Label one concentration "YCT" and one concentration "New YCT." Use the same synthetic moderately hard water (MHWCD) for the two concentrations. (MHWCD is our name for MHW for *Ceriodaphnia dubia*)

5.1.3.2. Use YCT (the current batch of "good" food), in addition to *Pseudokirchneriella subcapitata*, as food for the "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.

5.2. *Artemia*

5.2.1. This test should be performed whenever a new lot of cysts is purchased and prior to the last use of the lot that is currently being utilized. *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 methods for SOP TA-07.15, *Americamysis bahia Chronic Toxicity Testing Methods* for a chronic screening test.

5.2.3.1. Label one concentration "*Artemia*" and one concentration "New *Artemia*." Use the same batch of artificial seawater (ASW) for the two concentrations.

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

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

6. TEST ANALYSIS

6.1. YCT

6.1.1. Terminate the test when the endpoint of the 3rd brood of neonates in 60% of surviving females is reached, in either concentration.

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 $\geq 80\%$.

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.

6.1.3. Run a "T-test" or the Dunnetts procedure (refer to SOP TA-07.16) to provide a statistical comparison of the "YCT" and the "New YCT."

6.1.3.1. If there is no difference, or if the "New YCT" is statistically better than the "old," the "New YCT" is acceptable.

6.1.3.2. If there is a "negative" significant difference (i.e., the "New YCT" produces significantly fewer neonates per organism than does "YCT"), but the requirements in STEP 6.1.1.1. are met, the "New YCT" can be used with the

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following caution. The "chronic" *C. dubia* culture (SOP TA-04.01) should be monitored to ensure that survival and reproduction do not fall below minimums listed in STEP 6.1.1.1.

6.2. *Artemia*

6.2.1. Terminate the test per SOP TA-07.15.

6.2.1.1. The new lot of *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%.

6.2.1.2. If the new lot of *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.

6.2.2. Run a "T-test" or the Dunnetts procedure (refer to SOP TA-07.16) to provide a statistical comparison of the good *Artemia* and the new lot of *Artemia.*

6.2.2.1. If there is no difference, or if the new *Artemia* lot is statistically better than the current *Artemia*, the new lot is acceptable. Record suitability result in *Culture Records for Artemia* logbook.

6.2.2.2. If there is a "negative" significant difference (i.e., the new lot produces significantly less dry weight per organism than does the current lot, or that there is significantly less survival of the new lot compared to the old lot), but the requirements in 6.2.1.1. are met, the lot can be used.

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 X:\Labs\Lab5\Biology\Bench Common\ToxCommon\growth and reproduction 2016 control data for chronic tests and charts.

7.3. 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.

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

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

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

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.

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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 Methods.

10.4. DEP SOP TA-7.16. Using ToxCalc for Statistical Analysis of Chronic Bioassays.

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

10.6. DEP SOP TA-01.03. *Artemia* Nauplii Culture

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

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

10.9. DEP Laboratory Health and Safety Plan, https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

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
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.
02/17/2017	Updated links.

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