Americamysis bahia Chronic Toxicity Testing Methods

1. SCOPE AND APPLICATION

1.1. This method is based on EPA-821-R-02-014 *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*, 3rd Edition, Test Method 1007.0. It describes the procedures used to estimate the chronic toxicity of whole effluent and receiving water (marine or salinity adjusted aqueous samples) on 7-day old *Americamysis bahia* (mysid shrimp) during a 7-day static renewal exposure. Refer to EPA-821-R-02-014 Section 14.1.

2. SUMMARY OF THE METHOD

2.1. Samples are received and prepared in the laboratory. Sample physical-chemistry (phys-chem) parameters are measured and samples are adjusted as needed. Sample and control waters are placed into test vessels. Test organisms are received, fed and held for observation prior to loading into test jars. Organisms are then loaded into test jars. Tests are incubated in laboratory incubators for seven days. While tests are in progress, survival/ mortality and phys-chem parameters are measured and recorded daily before and after sample renewal. The test endpoints are survival and growth (measured as dry weight). Results are entered into the Laboratory Information Management System (LIMS).

2.2. Test acceptability criteria: 80% or greater survival in the controls and an average dry weight of at least 0.20mg per surviving control organism, dried immediately after test termination.

2.3. For this test, it is presumed that the recommended salinity of 20‰-30‰ (±2‰ of the selected test salinity) has been met and all effluents and control water have been salinity adjusted as needed (Refer to SOP TA-07.13). The required test salinity is determined per the facility's NPDES permit, by consultation with FDEP Regional Operation Center (ROC) Personnel, or by SOP TA-07.13. Filter sample through a 60 µm screen to remove debris and/or indigenous organisms if needed. Also, adjust pH (TA-08.06) and adjust dissolved oxygen concentration; dechlorinate (TA-09.18), etc.

2.4. Interferences: Refer to EPA-821-R-02-014 Section 14.3. Includes toxic substances introduced by contaminants in dilution water or on hands, glassware, sample hardware, testing equipment, dissolved oxygen <4.0 mg/L, pH <6.0 or >9.0 SU, salinity < 5 or > 35 parts per thousand, ammonia, chlorine, debris, and indigenous organisms that may attack or be confused with the test organisms.

3. EQUIPMENT AND SUPPLIES

3.1. Eight autoclaved, 500-mL glass bioassay jars for each test concentration, larger containers can be used.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.1.1. A test with 5 test concentrations and a control, using 8 replicates (required minimum) per concentration would require 48 jars. Test solution volume is a minimum of 250 mL per replicate.

3.2. Autoclaved 250mL, 500mL and 2000mL graduated cylinders for making test solutions.

3.3. Autoclaved 4000L Erlenmeyer flask for mixing test dilutions.

3.4. Autoclaved 6000mL Erlenmeyer flask to hold effluent sample

3.5. Serological pipettes—1ml and 10 mL, graduated

3.6. Pipetter for serological pipettes

3.7. Magnetic stir bars and stirrer

3.8. Large plastic Carolina dish to hold mysids for acclimation and loading into test jars.

3.9. 60µm mesh to filter sample

3.10. <200µm mesh screen used to transfer test organisms.

Note: Droppers and glass tubing with fire polished edges, 4 mm ID, can be for transferring test organisms.

3.11. Effluent samples to be tested (recommended to be warmed to 26 ± 1 °C)

3.12. Meters to measure:

3.12.1. pH calibrated per SOP TA-06.23

3.12.2. Dissolved oxygen (DO) calibrated per SOP TA-06.04

3.12.3. Temperature using digital thermometer calibrated per SOP TA-06.13

3.12.4. Conductivity/salinity calibrated per SOP TA-06.36

3.13. Hach® kits to measure:

3.13.1. Total residual chlorine (TRC) per SOP TA-06.22

3.13.2. Alkalinity per SOP TA-06.08

3.13.3. Hardness per SOP TA-06.08; usually not measured when conductivity is > 5,000 µmhos/cm.

3.13.4. Ammonia per SOP TA-06.35 or SOP TA-06.33

3.14. Thermo Scientific Incubator (incubator #1, 5, or 7).

3.14.1. Incubator should maintain test temperature at 25 ± 1 °C and a photoperiod of 16h light: 8h dark. Light intensity should be 50-100 ft. candles (recommended). Sample temperatures in jars must not deviate more than 3 °C for the duration of the test.

3.14.2. Other incubators or a water bath can be used to regulate test temperature.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

3.15. 30 ml cups contained in parameter tray

3.15.1. Two vials per test concentration. One is for the "old" solution parameters; the other is for the "new" solution parameters. Rinse cups daily.

3.16. Waste bucket

3.17. *Artemia* nauplii (<24 hours old, enhanced with Selcon™).

3.17.1. Used to feed test organisms at the rate of 150 nauplii per mysid per day; half at renewal and half at the end of the day. Refer to SOP TA-01.03 to culture *Artemia*.

3.18. Drying oven. Capable of sustaining 105 °C for eight hours or 60 °C for 24 hours. Refer to SOP TA-06.21.

3.19. Weigh boats (prepared per SOP TA-06.21).

3.20. "S" weights

3.20.1. Two weights that span the weight range of the empty weigh boats, and then two weights that span the weights of the weigh boats plus the dried mysids.

3.21. Mettler Toledo Balance XA105DU (calibrate per SOP PCR-2.2), to weigh weigh-boats and dried mysids.

3.22. *Americamysis bahia* test organisms: age 7 days, 40 per test concentration, 5 per replicate for a total of 240 organisms.

3.22.1. Order from supplier. Arrange shipping so as not to exceed required organism age and to allow time for organism acclimation as needed. Refer to SOP TA-04.20.

3.23. *Chronic Bioassay Bench Sheets Americamysis bahia*.

3.24. *Daily Physical-Chemistry Calibration Log*

4. REAGENTS AND STANDARDS

4.1. Artificial Sea Water (ASW) prepared with laboratory grade artificial sea salts (SOP TA-03.02); or, Natural Sea Water (NSW).

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1. See SOP FS 1000 for sample collection and preservation.

5.1.1. For a valid test: a) the sample should be held at 0-6 °C prior to test initiation, and b) sample holding time should not exceed 36-hrs from time of collection (last sample time when 4 grab samples are collected over a 24-hour period, or the end of the 24-hour collection period for a composite sample) to test initiation.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

5.1.2. A series of three samples (designated as samples 1, 2, and 3) are collected and used upon receipt. Sample 1 is used for 2 days, sample 2 is used for 2 days, and sample 3 is used for 3 days.

5.1.2.1. The remainder of each sample is stored in the walk-in cooler for solution renewal the following day or days.

5.2. See SOP TA-06.14 for bioassay sample log-in procedure.

6. TEST INITIATION

6.1. Place clean test jars onto laboratory cart. Label each jar to identify the replicate (A–H), test concentration, and facility sampled.

6.1.1. Place tape across front of jar, beneath jar #, and use waterproof marker to identify. Eight jars for each set of control replicates needed for each sample tested, plus eight jars per concentration for each sample tested.

6.2. Calibrate meters and record readings in *Daily Physical-Chemistry Calibration Log* daily during test. Check meters at end of day and record values.

6.3. If necessary, filter effluent sample through 60µm mesh into 6000mL Erlenmeyer flask to remove debris and indigenous organisms.

6.4. Fill a disposable, 30 ml cup with sample and take initial pH measurement.

6.4.1. If pH is outside acceptable range of 6.0–9.0, then an additional test with a pH-adjusted aliquot will be run. The test with the original sample need now only be a screening test, regardless of whether the pH-adjusted aliquot is used for a screening or definitive test. Refer to SOP TA-08.06 for sample pH adjustment.

6.5. In same plastic cup with sample, take initial DO measurement.

6.5.1. If DO is <4.0 mg/L (low DO) or > 9.0 mg/L (super saturated DO), aerate entire sample to ~ 90% saturation prior to test initiation. Samples with a potential DO problem generally show a downward trend in DO during the first few hours of the test. Monitor the DO closely. If DO begins to fall, set up gentle aeration at <100 bubbles/min to ALL test solutions for duration of test using micropipettes and the aeration manifold.

6.6. In same plastic cup with sample, measure the conductivity, temperature, and salinity (by direct measurement or conversion) of the sample.

6.6.1. If conductivity is less than 2,000 µmhos/cm (corrected to 25 °C) or the salinity is less than 1 ppt, freshwater organisms probably should be used rather than *Americamysis bahia*. Reconfirm the NPDES permit test requirements and/or contact appropriate District personnel.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.6.2. The following web site: http://www.fivecreeks.org/monitor/sal.html can be used for salinity conversions from conductivity and temperature measurements.

6.7. Measure total residual chlorine (TRC) in sample per SOP TA-06.22. Check field data sheet supplied by sampler for field TRC and atypical field measurements.

6.7.1. If TRC >0.1 mg/L and non-chlorine toxicity is to be measured, dechlorinate a portion of the sample using sodium thiosulfate per SOP TA-09.18. Test dechlorinated replicates of all chosen test concentrations concurrently with additional chlorinated (100% sample ONLY) replicates.

6.8. Measure alkalinity, hardness (if appropriate), and total ammonia of sample per SOP TA-06.08 and TA-06.17 or TA-06.33.

6.9. Using a graduated cylinder, pour 300 to 500mL of diluent into each control replicate jar.

6.10. Pour 300 to 500mL of 100% samples into each of the 100% sample concentration jars.

6.11. Measure the desired volumes of diluent and sample into a 6000-mL Erlenmeyer flask using two graduated cylinders. Shake/swirl flask by hand to mix or place flask on stir table and stir until homogenized. Measure 2000 mL of the mixture back into a 2,000-mL cylinder. Transfer 250 mL from the 2000 mL cylinder to a 250 mL cylinder. Empty the 250 mL cylinder into test jar A. Repeat the transfer until 250 mL of solution has been placed into containers B through H for that particular concentration. Rinse the cylinders with deionized water between concentrations. Repeat for each test concentration.

6.11.1. For each sample tested, use eight replicates per concentration in a dilution series based on the Facility's NPDES permit, instruction from the appropriate FDEP ROC or, if applicable, previous knowledge of toxicity level. Usually a minimum of 5 concentrations plus the control are used.

6.11.2. Once concentrations are poured, immediately record temperatures to prevent loss or gain of sample temperatures as best as possible

6.11.3. Example of sample volumes (0.5 dilution) below for eight 250 mL replicates of each concentration in a mysid *chronic* toxicity test:

Concentration	Diluent	Sample	Total Volume
Control	2000 mL	0 mL	2000 mL
6.25%	2000 mL	2000 mL of 12.5%	4000 mL
12.5%	2000 mL	2000 mL of 25%	4000 mL
25%	2000 mL	2000 mL of 50%	4000 mL
50%	2000 mL	2000 mL of 100%	4000 mL
l00%	0 mL	2000 mL of 100%	2000 mL

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

6.12. Perform QA standard reference toxicant (SRT) tests monthly, using potassium chloride (refer to SOP TA-09.20) or other toxicants for the SRT, unless the chronic tests are not performed on a regular basis (at least one per month). SRTs must be performed concurrently with the facility test. (toxicants and concentrations can vary with the needs of the lab)

6.12.1. Example of KCl concentrations and toxicant volumes for *chronic* SRT test. SRTs are performed at 20‰ salinity.

Concentration	Diluent	Toxicant	Total Volume
Control	2000 mL	0 mL	2000 mL
0.2 g/L	3200 mL	800 mL of 2 g/L KCl	4000 mL
0.4 g/L	2400 mL	1600 mL of 2 g/L KCl	4000 mL
0.6 g/L	1600 mL	2400 mL of 2 g/L KCl	4000 mL
0.8 g/L	800 mL	3200 mL of 1 g/L KCL	4000 mL
1.6 g/L	800 mL	3200 mL of 2 g/L KCl	4000 mL

6.13. Pour aliquots from all "A" replicates into the appropriate 30 mL cups in the parameter tray. Measure pH, D.O., temperature, conductivity, and salinity of the solutions from the aliquots. Record all readings in appropriate columns on *Chronic Bioassay Bench Sheet*. Record any comments pertaining to the condition or appearance of the sample. On the following seven days, rotate through the replicates and take parameters before and after renewing the sample.

6.13.1. Conductivity measurements (µmhos-cm) and salinity measurements (ppth) are reported to 3 significant figures. pH, DO, and temperature measurements should be read to one decimal place. Take measurements from lowest to highest concentration.

6.13.2. Day 0 "A" replicates; Day 1 "B" replicates, etc. If a replicate is not available (removed from rotation due to 100% mortality), measure aliquot from the next alphabetically sequential replicate of the same concentration.

6.14. Test initiation (o-hour and 0-Day):

6.14.1. Using a <200µm mesh screen, load **one** (20% of test organisms per replicate) mysid into each test chamber. Repeat the loading process until **five** mysids have been loaded into each replicate. Verify count over a light table. Record counts in appropriate columns on the *Chronic Bioassay Bench Sheet*.

6.14.2. (5 mysids per replicate) x (8 replicates per test concentration) = 40 mysids per concentration tested.

6.14.3. A person other than the loader should verify the number of mysids in each replicate.

6.15. Prepare *Artemia nauplii* and feed at rate per step 3.17.1. Feed after initial loading and after daily renewal. Do not feed on test day 7 (see step 6.3). Feed without contacting test liquid or jar with the pipet, by gently dispensing the *Artemia* along

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

the inner wall of the jar to minimize disturbance to the mysids and prevent loss or cross contamination.

6.15.1. See Step 3.17.1 for feeding instructions. Adjust amount fed as necessary to compensate for mortality and/or dissolved oxygen issues.

6.16. Arrange the chambers in the incubator using the randomizer on the whiteboard in the Toxicology lab and cover with clear Plexiglas.

Note: Covers prevent accidental contamination and minimize evaporation.

6.17. Measure alkalinity and hardness if appropriate (SOP TA-06.08), total residual chlorine (SOP TA-06.22) and total ammonia (SOP TA-06.35 or TA-06.33) of sample, salinity adjusted sample (as needed) and control water. Record each in the corresponding log book along with control water parameters in *Dilution Water Logbook.*

Note: Hardness is not usually measured if conductivity is greater than 5,000 µmhos/cm.

7. INTERIM READINGS AND RENEWALS

7.1. At 24 (± 2-3 hours) hour intervals, pour aliquots of the appropriate replicates into 30 mL cups. Count the mysids in each replicate. Measure pH, temperature, DO, conductivity and salinity in the aliquots before renewing the test solutions and record the readings in appropriate columns in the *Chronic Bioassay Bench Sheet*. Clean test jars by siphoning, the "old" test solutions to remove debris, dead *Artemia*, and any dead test organisms. Using a tube with a very fine mesh capping one end, carefully siphon all but 10%-15% of the solution from each jar into a waste bucket. Try to keep the mysids within the jar. Replace the contents of each jar with respective fresh concentrations of the sample. Count the mysids in each replicate. Record the number of live, dead, or missing mysids on test bench sheets. Pour aliquots of renewed solutions into 30ml cups. Measure pH, temperature, DO, conductivity, and salinity of the aliquots and record the readings in appropriate columns in the *Chronic Bioassay Bench Sheet.*

7.1.1. Generally, Day 0 aliquots are taken from the "A" replicates; Day 1 aliquots are taken from the "B" replicates; Day 2 from "C", etc. However, before renewal and after renewal aliquots should be from the same replicate for each concentration; EXCEPT, if all organisms are dead in a replicate, the after-renewal aliquot is taken from a replicate with living organisms (if applicable for a concentration).

Note: The remaining amount of solution after siphoning should measure a half-inch to an inch from the bottom of the test jar. All counting is done over a light table. Mysids are recorded as live if they move when lightly prodded or if any external or internal organs of the organism are still active.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

Note: If a mysid becomes stuck on the siphoning mesh, immediately return the mysid to the jar. To return the mysid, dip siphoning hose tip with net attached back into correct jar and allow mysid to swim off.

7.2. Pour aliquots from the appropriate replicate after renewal into the corresponding 30 mL cup. Measure pH, temperature, DO, conductivity, and salinity after renewing the test solutions and record readings in the correct columns in *Chronic Bioassay Bench Sheet*.

7.3. Feed mysids with *Artemia nauplii* concentrate daily after the renewals. Prepare *Artemia* and adjust volume per step 5.11. Return jars to incubator and cover to reduce evaporation.

7.3.1. On weekends, the *Artemia* are fed at one time with consideration given to potential DO problems.

8. TEST TERMINATION

8.1. Make weigh boats two days before test termination. The day of termination, weigh the empty weigh boats. Refer to SOP TA-06.21

8.2. On day 7 (t = 168 hrs ±6 hrs), count and record number of surviving and dead mysids. Remove and discard dead mysids. Pour aliquots and measure parameters of the solutions. Record all counts and readings along with test termination date and time in appropriate columns in *Chronic Bioassay Bench Sheet*. Record any comments pertaining to the appearance or condition of the mysids.

Note: Counting is done over a light table. Physical-chemical readings are taken from the aliquot to minimize disturbance to the mysids.

8.3. Pour the contents of Control replicate “A” onto a fine mesh net held over the waste bucket. Immediately euthanize the mysids by immersing them in ice water in the Carolina dish. Keep mysids immersed for two minutes to ensure they are fully euthanized.

8.4. When the mysids are dead, gently rinse them with DI water. Use forceps and place each mysid into the respective weigh boat. Return weigh boat to the Carolina dish. Repeat Steps 7.3 and 7.4 for each replicate.

8.5. Cover the dish (containing weigh boats with mysids) with foil. Dry the weigh boats containing the mysids for 8 hours in the sediment-drying oven after test termination. Store the Carolina dish in the desiccator in a vacuum after they are dried.

8.5.1. Dry for 8 hours (minimum of six hours) at 105 °C.

8.5.2. Mysids can also be dried for 24 hours at 60 °C.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

8.6. Remove the dish after cooling and weigh the weigh-boats containing the mysids (per SOP TA-06.21). Record the weights in the *Chronic Bioassay Bench Sheet*.

9. STATISTICAL ANALYSIS

9.1. Complete the weight page in the *Chronic Bioassay Bench Sheets Americamysis bahia*.

9.2. Enter test data into EPA statistical programs to obtain LC_{50}, IC_{25}, NOEC, and LOEC per SOP TA-07.16

9.2.1. Refer to data analysis in EPA-821-R-02-014. TSK, Dunnetts, and ICP programs can be used to analyze data. *TOXCALC®, CETIS®* or the *EPA WET Spreadsheet* statistical programs can also be used to calculate endpoints.

9.3. Enter test results into LIMS after two-person data verification. Refer to SOP BG-01.

10. DATA ARCHIVAL

10.1. Log sample receipt, temperature and condition into *Bioassay Sample Log-in* book per SOP TA-06.14, including LIMS numbers from Chemistry

10.2. FDEP Laboratory Information Management System (LIMS)

10.3. Record information in appropriate logbooks (*Chronic Bioassay Bench Sheets* and *Daily Physical-Chemistry Calibration Log Book*).

10.4. Fill in information requested at top of bench sheets as completely as possible prior to test initiation. Include full name of facility, address, contact person, date and time collected, field TRC, LIMS sample and job numbers, test chamber ID, test chamber size, test solution volume, food type, and food batch numbers. Access scanned Field Sheets via the Receiving folder, \\fldep1\DEAR\Labs\Lab3\Receiving to obtain sampling information.

11. QUALITY CONTROL

11.1. Refer to FDEP Laboratory Quality manual for corrective action and handling out-of-control data. Refer to EPA-821-R-02-014 Test Method 1007.0 for test conditions and acceptability criteria.

11.2. Sample should be held at 0-6ºC prior to test initiation and between renewals.

11.3. Sample holding time should not exceed 36 hours from time of collection until initial use of sample.

11.4. Incubator maintained at the selected test temperature of 26±1ºC.

11.5. Test temperature must not deviate more than 3ºC in the test jars for the duration of the test.

11.6. If a problem occurs during sample processing or testing, the following steps are performed: 1) lab supervisor is notified, 2) client is notified (client makes decisions

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

for additional testing, 3) Program Administrator is notified, and 4) a nonconformance report (NCR) describing the problem and the corrective action is created in LIMS.

11.7. Perform QA standard reference toxicant (SRT) tests monthly or concurrently as needed. (See step 6.12).

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

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

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

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

11.9.3. 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.).

12. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

12.2. Refer to the DEP Laboratory Quality Manual for sample disposal

12.3. Refer to the Laboratory Health and Safety Plan.

12.4. Read MSDS information for reagent handling and disposal.

13. REFERENCES

13.1. DEP Laboratory Quality Manual.

13.2. DEP Laboratory Health and Safety Plan.

13.3. EPA 821-R-02-014 *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 3rd Ed.* Test Method 1007.0. October 2002.

13.4. DEP SOP FS 1000, General Sampling (Document download Link).

13.5. DEP SOP TA-01.03, *Artemia Nauplii Culture*

13.6. DEP SOP TA-03.02, *Preparation of Artificial Seawater (ASW)*

13.7. DEP SOP TA-04.20, *Receiving and Maintaining Purchased Test Organisms*

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

13.8. DEP SOP TA-06.04, *YSI Model 58 Dissolved Oxygen Meter Calibration and Measurement*

13.9. DEP SOP TA-06.08, *Measuring Total Alkalinity and Hardness*

13.10. DEP SOP TA-06.13, *Thermometer Accuracy Check and Use*

13.11. DEP SOP TA-06.14, *Bioassay Sample Log-In Procedure*

13.12. DEP SOP TA-06.21, *Weighing Chronic Tests*

13.13. DEP SOP TA-06.22, *Measuring Total Residual Chlorine using the Hach Pocket Colorimeter Test Kit*

13.14. DEP SOP TA-06.23, *Calibration and Use of Accumet Model AR10 pH Meter*

13.15. DEP SOP TA-06.25, *Calibration and Use of Mettler Toledo SevenMulti for Measuring pH*

13.16. DEP SOP TA-06.33, *Measuring Ammonia using HACH Ammonia Nitrogen Test Kits for Freshwater and Saltwater Samples.*

13.17. DEP SOP PCR-2.2, *Calibration, Use and Maintenance of Mettler Toledo XA105DU Balance*

13.18. DEP SOP TA-07.07, *Statistical Analysis of Acute Toxicity Test Data for LC50*

13.19. DEP SOP TA-09.01, *Sodium Chloride and Potassium Chloride Reference Toxicant Stock Solutions*

13.20. DEP SOP TA-09.18, *Dechlorination Procedure using Sodium Thiosulfate*

13.21. DEP SOP TA-08.06, *pH adjustment of Samples: Algal Assay and Toxicity Bioassay*

13.22. DEP SOP TA-07.13, *Salinity Adjustment of Samples: Toxicity Bioassay*

13.23. DEP SOP TA-07.16, Using *TOXCALC* for *Statistical Analysis of Chronic Bioassays*

APPENDIX OF CHANGES:

12/20/2007 Added *Scope and Application*; *Summary of Method,* and *Appendix of Changes.* Moved reference text from title to Scope and Application. Moved Scope and Summary text from comment section to Scope and Summary Sections. Step 8 under *Test Initiation* changed copper to cadmium; changed copper concentrations to cadmium concentrations in Step 8 comment.

11/10/2008 Added references. Put SOP references in Materials initial comment; Materials #22 comment; Materials #24 comment; and, Methods #8. Removed reference to *Toxstat®* program. Added reference to TOXCALC. Deleted reference to SOP #TA-06.06.

11/03/2009 Deleted “Vinyl gloves can be substituted for individuals allergic to latex.” from Safety comment.” Changed “must” to “should” in Test Termination Step 4 comment. Updated Biology Quality Manual reference. Deleted TOXCALC® from

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.

	Statistical Analysis Step 2 and added "*TOXCALC® or CETIS®* statistical programs can also be used to calculate endpoints" to Step 2 comment.
02/19/2010	Corrected concentrations in comment for Step 8 of Test Initiation.
02/07/2011	Updated reference to Biology Quality Manual – now Bureau Quality Manual. Added SOP TA-06.13 to References.
03/17/2011	Updated references to Bureau Quality Manual. Changed 105º C to 100º C throughout the SOP.
02/21/2012	Updated references to Bureau Quality Manual. Updated log-book names.
01/10/2014	Reformatted SOP.
02/13/2015	Formatted for 508 compliance.
03/03/2016	Updated links. Added XA105DU balance. Updated names of and moved the FDEP Laboratory Quality Manual and Laboratory Health and Safety Plan links to the reference section. In the reference section, listed SOP identifier before the SOP title. Italicized SOP titles. Formatted for Toxicity SOP consistency.
03/14/2016	Accepted format edits.
03/08/2017	Corrected Step references and updated links.
04/17/2017	Clarified enhanced artemia use.
02/28/2018	Added hyperlinks for SOPs and manuals.
02/21/2019	Updated referenced SOPs and SRT concentrations.

The OFFICIAL COPY is the on-line version. All other copies are considered unofficial and uncontrolled.