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. This SOP describes the procedures used to estimate the chronic toxicity of whole effluent or receiving water (marine or salinity adjusted aqueous samples) to the mysid shrimp, *Americamysis bahia*, during a seven-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. Samples and controls 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 or waterbath for seven days. Daily during tests, test vessels are checked, survival/mortality is recorded, and phys-chem parameters are measured and recorded before and after sample renewal. The test endpoints are survival, growth (measured as dry weight) and fecundity (if required - measured as the percentage of females with eggs in the oviduct and/or brood pouch of the organisms). 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 ≥ 0.20 mg (minimum) per surviving control organism. If fecundity is used; 50% (minimum) of control females must have eggs.

2.3. For this test, it is presumed that the EPA Test Method 1007.0 **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). **NOTE:** The **required** test salinity should be determined per the facility's NPDES permit or by consultation with FDEP Regional Operation Center (ROC) Personnel. The source of the required test salinity must be recorded in the comments section on page one (1) of the bioassay benchsheets. It is also presumed that the sample has been manipulated as necessary to remove debris (filter through a 2-4 mm screen), remove indigenous organisms (filter through a 60 µm screen), adjust pH (TA-08.06), adjust dissolved oxygen concentration, dechlorinate (TA-09.18), etc.

2.4. Interferences: Refer to EPA-821-R-02-014 Section 14.3. Include 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 SU 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.

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3. EQUIPMENT AND SUPPLIES

3.1. Eight autoclaved, 400-mL glass beakers or eight 250-mL plastic disposable cups for each test concentration

3.1.1. A test with 5 test concentrations and a control, using 8 replicates (required minimum) per concentration would require 48 beakers or cups. Test solution volume is a minimum (recommended) of 150 mL per replicate. The 400-ml beakers and the 250 ml (~ 8 oz.) cups are recommended by the method. Larger contains can be used.

3.2. Autoclaved graduated cylinders: 100, 250, 500, and 2,000 mL for making test solutions. One set for toxicant and one set for diluent.

3.3. Autoclaved Erlenmeyer flasks, 2-L, 4-L and 6-L (for mixing test dilutions).

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

3.5. Pipetter for serological pipettes

3.6. Magnetic stir bars and stirrer

3.7. Large plastic Carolina dish (to hold mysids for acclimation and loading into test vessels.)

3.8. Droppers and glass tubing with fire polished edges, 4 mm ID, for transferring test organisms.

Note: Nytex™ screen having opening < 200µm can also be used to transfer test organisms.

3.9. Sample warmed to 26 ± 1 °C (recommended).

3.10. Meters to measure:

3.10.1. pH (calibrate per SOP TA-06.23 or TA-06.25)

3.10.2. dissolved oxygen (DO) (calibrate per SOP TA-06.04)

3.10.3. temperature (digital thermometer preferred, other type can be used SOP TA-06.13)

3.10.4. conductivity/salinity (calibrate per SOP TA-06.25)

3.11. Hach® kits to measure:

3.11.1. total residual chlorine (TRC) (SOP TA-06.22)

3.11.2. alkalinity (SOP TA-06.08)

3.11.3. hardness (SOP TA-06.08; usually not measured when conductivity is > 5,000 µmhos/cm).

3.12. Denver Instruments® Model 225 pH/ion meter with ammonia probe (calibrate per SOP TA-06.17.) (Ammonia test strips and/or a HACH ammonia kit can be used to measure total ammonia.)

3.13. Thermo Scientific Incubator. (Incubator #1, 3, or 5).

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3.13.1. Incubator should maintain test temperature (26 ± 1 °C recommended) and a photoperiod of 16h light: 8h dark. Light intensity should be 50-100 ft. candles (recommended). Test temperature must not deviate (maximum minus minimum) more than 3 °C in the test beakers/cups for the duration of the test.

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

3.14. Digital thermometer (calibrate SOP TA-06.13)

3.15. 30 ml cups contained in parameter tray

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

3.16. Waste bucket

3.17. *Artemia* nauplii (<24 hours old).

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

3.18. Drying oven. Capable of sustaining 100 °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-2.1), to weigh weigh-boats and dried mysids.

3.22. *Americamysis bahia* test organisms: age 7 days, 40 per test concentration/5 per replicate.

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. Effluent to be tested

3.24. Control water.

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

4. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

4.1.1 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 hr from time of collection (last sample time when 4 grab samples are collected over a 24 hour period; the end of the 24 hour collection period for a composite sample; the time of the grab for a

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single grab sample) to test initiation. Usually samples for this test are 24-hour composites or single grab samples.

4.2 Refer to SOP TA-06.14 for bioassay sample log-in procedure

5. TEST INITIATION

5.1. Place clean test beakers or cups onto laboratory cart. Label each beaker to identify the replicate (A–H), test concentration, and facility sampled.

5.1.1. Place tape across front of beaker/cup, beneath beaker/cup #, and use waterproof marker to identify. Eight beakers or cups for each set of control replicates needed for each sample tested, plus eight per concentration for each sample tested.

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

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

5.3.1. 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.

5.4. In same plastic cup with sample, take initial D.O. measurement.

5.4.1. 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 the DO begins to fall, set up gentle aeration (<100 bubbles/min) to ALL test solutions for duration of test using micropipettes and the aeration manifold.

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

5.5.1. 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*. Review NPDES permit test requirements and/or contact appropriate District personnel.

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

5.6. Measure total residual chlorine (TRC) in the sample. Check field data sheet supplied by sampler for field TRC and atypical field measurements.

5.6.1. Measure TRC per SOP TA-06.22. If TRC >0.1 mg/L and non-chlorine toxicity is to be measured, dechlorinate a portion of the sample by the sodium

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thiosulfate method per SOP TA-09.18 and test dechlorinated replicates of all chosen test concentrations concurrently with additional chlorinated (100% sample ONLY) replicates.

5.7. Measure the desired volumes of diluent and sample into a 6,000-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 beaker/cup 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.

5.7.1. 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 District off or if applicable, previous knowledge of toxicity level. Usually a minimum of 5 concentrations plus the control are used.

5.7.2. 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

5.8. Perform QA standard reference toxicant (SRT) tests monthly, using cadmium chloride (refer to SOP TA-09.20) 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.

5.8.1. Example of cadmium concentrations and sample volumes for *chronic* SRT test. SRTs are performed at 20‰ salinity.

Concentration	Diluent	Sample	Total Volume
Control	2000 mL	0 mL	2000 mL
2.5 µg/L	2000 mL	2000 mL of 5 µg/L	2000 mL
5.0 µg/L	2000 mL	2000 mL of 10 µg/L	2000 mL
10 µg/L	2000 mL	2000 mL of 20 µg/L	2000 mL
20 µg/L	2000 mL	2000 mL of 40 µg/L	2000 mL
40 µg/L	0 mL	4000 mL of 40 µg/L	4000 mL

5.9. Pour aliquots from all "A" replicates into the appropriate 30 mL cups in the parameter tray. Measure pH, D.O., temperature and conductivity (salinity by conductivity/temperature conversion) of the solutions from the aliquots. Record all

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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 all of the replicates and take parameters before and after renewing the sample.

5.9.1. Conductivity measurements (mmhos-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.

5.9.2. 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).

5.10. Using a wide bore pipette (or a Nitex® screen), load **one** (20% of test organisms per replicate) mysid into each test beaker. 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*.

5.10.1. (5 mysids (required) per replicate) x (8 replicates (required minimum) per test concentration) = 40 mysids (required minimum) per concentration tested.

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

5.11. Prepare *Artemia nauplii* and feed at rate per step 5.11.1. 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 beaker/cup with the pipet, by gently dispensing the artemia along the inner wall of the bealer/cup to minimize disturbance to the mysids and prevent loss or cross contamination.

5.11.1. Feed test organisms at the rate of 150 nauplii per mysid (~0.1 ml concentrated artemia); half at renewal and half 6–8 hours later (if practicable). Refer to SOP TA-01.03. Adjust amount fed as necessary to compensate for mortality and/or dissolved oxygen issues.

5.12. Randomly arrange and place beakers/cups in incubator (or waterbath) and cover with clear Plexiglas.

Note: Covers prevent accidental contamination and minimize evaporation. If water bath is used, ensure that a temperature recorder is installed and that the light timer is set and turned on.

5.13. Measure alkalinity and hardness (if appropriate) (SOP TA-06.08), total residual chlorine (SOP TA-06.22) and total ammonia (SOP TA-06.17)of sample, salinity adjusted sample (as needed) and control water. Record each in the corresponding log book. Also record control water parameters in *Dilution Water LogBook.*

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

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6. INTERIM READINGS AND RENEWALS

6.1. At 24 (± 2-3 hours) hour intervals, pour aliquots of the appropriate replicates into 30 mL cups and 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 the appropriate columns in the *Chronic Bioassay Bench Sheet*. Clean, by siphoning, the "old" test solutions to remove debris, dead *Artemia*, and any dead test organisms. Carefully siphon all but 10%-15% of the solution from each beaker/cup into the waste bucket (or pour all but 10%-15% of the solution in each beaker/cup through a fine mesh net into the waste bucket). Try to keep the mysids within the beaker/cup. Replace the contents of each beaker/cup with respective fresh concentrations of the sample. Count the mysids in each replicate. Record the number of live, dead, or missing mysids on the bench sheets. Record physical-chemical readings in appropriate columns in *Chronic Bioassay Bench Sheet.*

Note: The remaining water after pouring should measure a half-inch to an inch from the bottom of the beaker. All counting is done over a light table. Mysids are recorded as live if they move when lightly prodded.

Note: Siphoning should be the first choice of test solution removal due to sensitivity of *Menidia* to handling.

Note: The net should retain any mysids poured from the beaker. If a mysid falls on the net, immediately use the loading pipette to return the mysid to the beaker. To return the mysid to the beaker, pipette water from the beaker about an inch into the loading pipette. Place the tip of the pipette near the mysid, taking care not to crush the mysid, squeeze a little of the fluid from the pipette onto the mysid. Pipette the mysid into the loading pipette, and release the mysid beneath the surface of the liquid remaining in the appropriate replicate beaker.

6.2. Pour aliquots from the appropriate replicate after renewal into the appropriate 30 mL cup. Measure pH, temperature, DO, conductivity (salinity by conductivity/temperature conversion) after renewing the test solutions and record readings in the appropriate columns in *Chronic Bioassay Bench Sheet*.

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

6.3. Feed mysids with *Artemia nauplii* concentrate daily after the renewals. Return beakers to incubator and cover to reduce evaporation.

6.3.1. Organisms are fed at the rate of 150 24-hour old *Artemia nauplii* per mysid per replicate, half after test solution renewal and half at the end of the day (if practicable). Weekends, the *Artemia* are fed at one time with consideration given to potential DO problems.

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7. TEST TERMINATION

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

7.2. 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.

7.3. Pour the contents of Control replicate "A" onto a fine mesh net held over the waste bucket. If fecundity is not used as an endpoint, immediately euthanize the mysids by immersing them in ice water in the Carolina dish. Go to Step 7.4. If fecundity is used as an endpoint, place the mysids in a petri dish, anesthetize (see 7.3.2) and examine under the dissecting microscope. Determine and record the number of males, females, gravid females, and immature organisms. Go to Step 7.4.

7.3.1. If greater than 50% of the mysids are gravid females, fecundity can be used as one of the test endpoints.

7.3.2. Two or three drops of 90% ethanol added to 15-20 mL of water in a 30 mL cup (containing the mysids) will be sufficient to anesthetize the mysids to allow examination without turning the mysids opaque.

7.4. When the mysids are dead, gently rinse them with DI water. Use forceps and place each mysid into the respective weigh boat. Place the weigh boat in a 4" or 8" Carolina dish. Repeat Step 7.3 and Step 7.4 for each replicate.

7.4.1. Replicate "A" mysids of the Control are placed into the weigh boat for Control replicate "A". The drying / weighing sequence is generally: 1. Carolina dish with mysids into desiccator overnight. 2. Next day, Carolina dish with mysids into drying oven for 8-hours. Carolina dish with mysids returned to desiccator overnight. 3. Mysids weighed.

7.5. Cover with the dish (containing weigh boats with mysids) with foil. Place the dish in the desiccator. Dry the weigh boats containing the mysids for eight hours in the sediment-drying oven the day after test termination. Store the Carolina dish in the desiccator in a vacuum before and after they are dried.

7.5.1. Dry for eight (8) hours (minimum of six hours) at 100 °C.

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

7.6. 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*.

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8. STATISTICAL ANALYSIS

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

8.2. Enter test data into EPA statistical programs to obtain LC_{50}, IC_{25}, NOEC, and LOEC per SOP TA-07.07 and TA-07.16

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

8.3. Enter test results into LIMS after two person data verification.

9. DATA ARCHIVAL

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

9.2. FDEP Laboratory Information Management System (LIMS)

9.3. Fill in information requested at top of benchsheets 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.

10. QUALITY CONTROL

10.1. 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.

10.2. If a problem occurs during sample processing, a nonconformance report (NCR) describing the problem and the corrective action is created in LIMS.

10.3. Perform QA standard reference toxicant (SRT) tests monthly or concurrently as needed.

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

10.5. 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.

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

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

10.5.3. 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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11. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

11.2. Refer to the FDEP Laboratory Quality Manual for sample disposal

11.3. Refer to the Laboratory Health and Safety Plan.

11.4. Read MSDS information for reagent handling and disposal.

12. REFERENCES

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

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

12.3. 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.

12.4. DEP SOP FS 1000, *General Sampling*

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

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

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

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

12.9. DEP SOP TA-06.05, *Temperature Calibration and Measurement using YSI Model 58 Dissolved-Oxygen Meter*

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

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

12.12. DEP SOP TA-06.17, *Measuring Total Ammonia*

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

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

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

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

12.17. DEP SOP TA-06.25, *Calibration and Use of Mettler Toledo SevenMulti for Measuring Conductivity, Salinity and TDS*

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12.18. DEP SOP PCR-2.2, *Calibration, Use and Maintenance ofMettler Toledo XA105DU Balance*

12.19. DEP SOP TA-07.07, *Statistical Analysis of Acute ToxicityTest Data for LC50*

12.20. DEP SOP TA-09.19, *Preparation and Testing of Cadmium Chloride and Copper Chloride Reference Toxicant Solutions*

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

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

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

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

12.25. 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 *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.

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