Americamysis bahia Chronic Toxicity Testing Methods

DEPARTMENT OF ENVIRONMENTAL PROTECTION
LABORATORY BIOLOGY SECTION
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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 is used to estimate the chronic toxicity of saltwater whole effluent and receiving waters on *Americamysis bahia* (mysid shrimp) using 7-day old organisms in a 7-day static renewal exposure. Refer to EPA-821-R-02-014 Section 14.1. Tests conducted by this procedure are BSY-CD-MMY and BSY-CS-MMY.

2. SUMMARY OF 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 is filtered through a 60 µm screen to remove debris and/or indigenous organisms. Sample dilutions and control waters are placed into test chambers. Test organisms are received, fed and held for observation prior to loading into test chambers. Organisms are then loaded into test chambers. 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). Statistical endpoints are obtained from the EPA WET Statistics Excel spreadsheet per SOP TA-07.17. Results are entered into the Laboratory Information Management System (LIMS). If the reported result is less than the top concentration tested (i.e. <100%), add “Upper and lower confidence intervals are, ##.#% and ##.#%, respectively.” to the comment field when entering into LIMS.

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

2.3. For this test, it is presumed that the recommended salinity of 20 ppth (parts per thousand) to 30 ppth (± 2 ppth 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, as needed.

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 ppth, 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 500 mL autoclaved glass bioassay chambers (glass jars) for each test concentration, larger containers can be used.

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

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

3.3. 4 L autoclaved Erlenmeyer flask for mixing test dilutions.

3.4. 6 L autoclaved Erlenmeyer flask to hold effluent sample.

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

3.6. Pipettor 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 chambers.

3.9. 60 µm mesh to filter sample.

3.10. < 200 µm 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. Meters to measure physical-chemical parameters:

3.11.1. pH (Calibrate per SOP TA-06.43.).

3.11.2. Dissolved Oxygen (DO) (Calibrate per SOP TA-06.39).

3.11.3. Digital Thermometer (Calibrate per SOP TA-06.13).

3.11.4. Conductivity/Salinity (Calibrate per SOP TA-06.36).

3.12. Hach® kits to measure physical-chemical parameters:

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

3.12.2. Alkalinity and Hardness (Measure per SOP TA-06.08).

3.12.2.1. Hardness usually not measured when conductivity is > 5,000 µmhos/cm.

3.12.3. Ammonia (Measure per SOP TA-06.35 or SOP TA-06.33).

3.13. Thermo-Scientific Incubator (Precision Model 818) (#1, #5 or #7) with temperature and lighting control.

3.13.1. Refer to QC section for incubator requirements for valid testing.

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

3.14. 30 mL disposable plastic cups contained in parameter tray.

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

3.15. Waste bucket.

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

3.16.1. 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. Prepare in advanced per SOP TA-01.03.

3.16.2. Used for feeding *A. bahia* per SOP TA-02.00.

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

3.18. Weigh boats (Prepared per SOP TA-06.21).

3.19. Class 1 weights.

3.19.1. 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.

3.20. Mettler Toledo Balance XA105DU (calibrate per SOP BB-36), to weigh weigh-boats and dried mysids.

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

3.21.1. 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.

3.22. *Chronic Bioassay Benchsheets Americamysis bahia*.

3.23. Computer with access to LIMS.

3.24. *Bioassay Sample Log-In* logbook.

3.25. *Organism Log-In Benchsheets* logbook.

4. REAGENTS AND STANDARDS

4.1. Natural seawater (NSW) or Artificial Saltwater (ASW)

4.1.1. ASW is prepared in advance per SOP TA-03.02.

4.1.2. Used as diluent to make up test concentration and for control water.

4.1.3. Warmed to 26 +/- 1° C.

4.1.4. Refer to SOP TA-07.13 for salinity adjusting samples.

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

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 hours 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.

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(s).

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

6. TEST INITIATION

6.1. Place clean test chambers onto laboratory cart. Label each chamber to identify the replicate (A–H), test concentration, and facility sample.

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

6.2. Calibrate meters and record readings in LIMS logbooks daily during test. Check meters at end of day and record values.

6.3. Filter effluent sample through 60 µm mesh into 6 L Erlenmeyer flask to remove debris and indigenous organisms.

6.4. Fill a 30 mL disposable cup with sample and take initial pH measurement per SOP TA-06.43. Record value in the *Bioassay Sample Log-in* logbook and LIMS under TOX_pH (report in accordance with SOP TA-06.42).

6.4.1. If pH is outside acceptable range of 6.0-9.0 SU, then an additional test with a pH-adjusted subsample will be run. Adjust pH of sample per SOP TA-08.06. Record pH adjustment and/or measurement in the appropriate benchsheet. The test with the non-adjusted sample is then analyzed as a screening test, regardless of whether the pH adjusted aliquot is used for a screening or definitive test.

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6.5. In same disposable plastic cup with sample, take initial DO measurement per SOP TA-06.39. Record value in *Bioassay Sample Log-in* logbook and LIMS TOX_DO logbook.

6.5.1. If the D.O. is ≤ 4.0 mg/L or ≥ 9.0 mg/L, aerate the entire sample at a rate of ~100 bubbles per minute to as near saturation as possible prior to test initiation. Record aeration (initial and final DO, as well as aeration rate and duration) in the appropriate benchsheet. 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 (<100 bubbles per minute) to ALL test solutions for the duration of test using micropipettes and the aeration manifold. No action is required if DO increases after test initiation.

6.6. In the same plastic cup with sample, measure the conductivity, temperature, and salinity (by direct measurement or conversion) of the sample. Record value in the *Bioassay Sample Log-in* Logbook and LIMS TOX_COND_SAL.

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

6.6.2. If the salinity is greater than 6 ppth, no salinity adjustment is required. If the salinity is between 1 and 6 ppth and no ion imbalance is suspected, no salinity adjustment is required. If the salinity is between 1 and 6 ppt and an ion imbalance is suspected, salinity adjustment to 7 ppth is required. If the permit requires a specific salinity, or the district personnel request a specific salinity, the saltwater tests can be performed at that salinity. Salinity adjustments are made per SOP TA-07.13.

6.6.2.1. If the salinity is between 6 and 20 ppth, then add well water to the Carolina dish containing the mysids to begin their acclimation to a lower salinity. If the salinity is above 20 ppth, add a higher salinity of ASW or hypersaline brine to the Carolina dish containing the mysids to begin their acclimation to a higher salinity. Fish are tested at the original salinity of the sample.

6.6.3. The following web site:https://www.hamzasreef.com/Contents/Calculators/SalinityConversion.php 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. Record the result in the LIMS TOX_CHLORINE logbook and on the bioassay benchsheets Check field data sheet supplied by sampler for field TRC and atypical field measurements.

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6.7.1. If TRC ≥0.1 mg/L, and non-chlorine toxicity is to be measured, dechlorinate a subsample (volume needed to initiate the test) of the sample using sodium thiosulfate per SOP TA-09.18. Setup a concurrent screening test using the 100% sample that has not been dechlorinated and a control. Record chlorine result, amount of sodium thiosulfate used and final chlorine result after dechlorination in the logbook and in the designated section in LIMS.

6.8. Measure alkalinity, hardness (if appropriate), and total ammonia of sample per SOP TA-06.08 and TA-06.35, or TA-06.33.

6.9. Using a graduated cylinder, pour 250 to 500 mL of diluent into each control replicate chamber.

6.10. Pour 250 to 500 mL of 100% samples into each of the 100% sample concentration chambers.

6.11. To make concentrations for a definitive test, measure the desired volumes of diluent and sample into a 6 L 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 2000 mL cylinder. Transfer 250 mL from the 2000 mL cylinder to a 250 mL cylinder. Empty the 250 mL cylinder into test chamber A. Repeat the transfer until 250 mL of solution has been placed into containers B through H for each 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 below of sample and diluent volumes needed 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

6.12. Perform QA standard reference toxicant (SRT) tests monthly (or concurrent with bioassay if bioassays performed less frequently; a concurrent SRT with one bioassay can be used as the SRT for a series of tests as long as the time interval does not exceed 30 days prior to the start of a bioassay test), using potassium chloride for the

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SRT. Toxicant can be changed as needed. SRTs are currently performed concurrently with the facility test.

6.12.1. Example below of diluent and toxicant volumes for chronic SRT test using the toxicant made per SOP TA-09.01. SRTs are performed at 20% salinity.

Concentration	Diluent	Toxicant	Total Volume
Control	2000 mL	0 mL	2000 mL
0.2 g/L	3500mL	500 mL of 1.6 g/L KCl	4000 mL
0.4 g/L	3000 mL	1000 mL of 1.6 g/L KCl	4000 mL
0.6 g/L	2500 mL	1500 mL of 1.6 g/L KCl	4000 mL
0.8 g/L	2000 mL	2000 mL of 1.6 g/L KCl	4000 mL
1.6 g/L	0 mL	2000 mL of 1.6 g/L KCl	2000 mL

6.13. Pour aliquots from all A replicates into the appropriate 30 mL disposable plastic cups in the parameter tray. Measure pH, DO, temperature, conductivity, and salinity of the solutions from the aliquots. Record all readings in appropriate columns on *Chronic Bioassay Benchsheets* logbook. 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 loading (0-Hour and 0-Day):

6.14.1. Using a < 200 µm 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 Benchsheets* logbook.

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 steps 3.16.13.16.2. Feed after initial loading and after daily renewal. Do not feed on test day 7 (see step 7.3). Feed without contacting test liquid or chamber with the pipet, by gently dispensing the

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Artemia along the inner wall of the chamber to minimize disturbance to the mysids and prevent loss or cross contamination.

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

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 logbook along with control water parameters in LIMS logbooks.

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 disposable plastic cups. 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 Benchsheets* logbook. Mysids must be fed 2 hours before renewal. Count the mysids in each replicate. Pipette out any dead test organisms. Using a tube with a very fine mesh capping one end, carefully siphon the "old" test solutions to remove debris and dead *Artemia*. Siphon all but 10%-15% of the solution from each chamber into a waste bucket. Try to keep the mysids within the chamber. Replace the contents of each chamber 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 disposable plastic cups. Measure pH, temperature, DO, conductivity, and salinity of the aliquots and record the readings in appropriate columns in the *Chronic Bioassay Benchsheets* logbook.

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 chamber. 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.

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

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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 Benchsheets* logbook.

7.3. Feed mysids with *Artemia* nauplii concentrate daily after the renewals. Prepare *Artemia* and adjust volume per TA-01.03. Return chambers to incubator and cover to reduce evaporation.

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 ±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 Benchsheets* logbook. Record any comments pertaining to the appearance or condition of the mysids.

Note: Counting is done over a light table. Phys-chem 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 8.3 and 8.4 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 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.

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 Benchsheets* logbook.

9. STATISTICAL ANALYSIS

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

9.2. Enter test data into EPA statistical programs to obtain LC_{50}, IC_{25}, NOEC, and LOEC per SOP TA-07.17.

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9.2.1. Refer to data analysis in EPA-821-R-02-014. TSK, Dunnetts, and ICP programs can be used to analyze data. *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.

9.3.1. If the reported result is less than the top concentration tested (i.e. <100%), add "Upper and lower confidence intervals are, ##.#% and ##.#%, respectively." to the comment field when entering into LIMS.

10. DATA ARCHIVAL

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

10.2. 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.

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

10.2.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

10.2.3. 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.).

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

10.4. *Organism Log-In Benchsheets* logbook. Record per SOP TA-04.20.

10.5. FDEP Laboratory Information Management System (LIMS).

10.6. Record information in appropriate logbooks (*Chronic Bioassay Benchsheets*).

10.7. Log sample receipt, temperature, and condition into *Chronic Bioassay Benchsheets* logbook. 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 \\floridadep\data\dear_scans\Labs\BizhubScans\Receiving to obtain sampling information.

10.8. Enter the LC50, EC50, NOEC, LOEC, and/or IC20 and IC25 results from the *EPA WET Excel spreadsheet* into the appropriate logbook, appropriate computer file and LIMS results editor. A second person must verify data prior to uploading into LIMS.

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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 0-6°C 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 ± 1°C.

11.5. Test temperature must not deviate more than 3°C in the test chambers for the duration of the test.

11.5.1. If the test vessel temperatures for a facility test exceeds ±3˚C, but results indicate no toxicity, add the comment, “Temperature of test solutions deviated from test specifications.” to the reported test result.

11.5.2. If the incubator checkpoint temperatures exceed ±3˚C, but results indicate no toxicity, add the comment, “Continuous temperature monitoring in surrogate test vessels deviated from test specifications.” to the reported test result.

11.5.3. If room temp checkpoints are out, but there is no toxicity and the incubator and test vessel temps were fine, do not qualify or add a comment.

11.5.4. If results do indicate toxicity in a sample, the degree of departure and objectives of the test, as well as, best professional judgement and experience of the lab staff should be considered when reporting the data.

11.5.4.1. If after review, the temperature deviation is suspected of contributing to the toxicity of a test, report as “No Result”.

11.5.4.2. If after review, the temperature deviation is not suspected of contributing to toxicity, report with the comment, “Temperature of test solutions deviated from test specifications. Deviation not suspected of contributing to toxicity.”

11.6. If controls fail to meet minimum acceptability criteria, the test will be invalidated, and the analyses rerun as soon as possible/the schedule allows for SRT tests. Facility tests are rerun at the discretion of the district toxicity coordinator. If the test organism sensitivity falls outside the acceptable range (+/- 2 standard deviations of the mean) during the standard reference toxicant (SRT) tests, the SRT test will be repeated until an acceptable test result is achieved and the cause of the aberration is determined. Test results will be reported as usual (not invalidated) if there is a successful (within the acceptable range) SRT for that test organism within 30 days. Note that based on the nature of the statistics, it can be expected for one in 20 points will fall outside the acceptable range (+/- 2 standard deviations of the mean). If the

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SRT shows that the test species are overly sensitive, but the facility test results in a >100% effluent value, the result should still be reported with no qualifier.

11.7. The subsection manager is responsible for assessing and approving corrective actions. Samples which exceed holding time criteria (36 hours) by no more than eight (8) hours will be analyzed, the results will be qualified, the client will be notified, and a non-conformance report will be prepared. Samples which exceed holding time by more than eight (8) hours shall not be analyzed. The client will be notified and given the option of cancelling the job or rescheduling.

11.8. Analysis of samples which do not meet preservation requirements (temperature ≤ 6 ºC for shipped samples (overnight) and temperature decreasing (from field measurement) in hand delivered samples) is determined on a case by case basis. Generally, samples that are to be used for compliance purposes will not be analyzed; samples that are for information only will be processed. All data from samples not meeting temperature requirements will be qualified, non-conformance reports generated, and the client notified.

11.9. Incubator light intensity maintained at ~50-100 ft-candles for a photoperiod of 16 hours light to 8 hours dark.

11.10. 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 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.

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

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.

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13.4. MSDS

13.5. DEP SOP FS 1000, *General Sampling*

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

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

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

13.9. DEP SOP TA-06.39, *Thermo Scientific Orion Star A213 Benchtop RDO/DO Meter Calibration and DO Measurement*

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

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

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

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

13.14. DEP SOP TA-06.22,*Measuring Total Residual Chlorine using the Hach® Pocket Colorimeter™ II Test Kit*

13.15. DEP SOP TA-06.36, *Calibration and Use of the Fisherbrand™ Traceable™ Bench Conductivity Meter for Conductivity and Salinity*

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

13.17. DEP SOP TA-06.42, *LIMS Entry for Laboratory Parameters*

13.18. DEP SOP TA-06.43, *Calibration and Use of Fisherbrand™ Accumet™ AB315 pH/mV Meter*

13.19. DEP SOP BB-036, *Calibration, Use, and Maintenance of Mettler Toledo XA105DU Balance*

13.20. DEP SOP TA-09.01, *Potassium Chloride Reference Toxicant Stock Solutions*

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

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

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

13.24. DEP SOP TA-07.17, *Using EPA Whole Effluent Toxicity (WET) Statistics for Acute and Chronic Bioassay Tests*

13.25. DEP SOP TA-06.35, *Measuring Total Ammonia using the HACH® Ammonia Nitrogen Pocket Colorimeter™ II Test Kit*

13.26. DEP SOP TA-02.00, *Feeding Test-Organism Cultures*

13.27. DEP SOP BG-01, *Data-Entry Verification*

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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 compliances.

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.

02/13/2020 Updated and added hyperlinks. Updated pH (TA-06.37), conductivity and salinity (TA-06.36), DO (TA-06.39) and ammonia (TA-06.35) meters used. Updated SRT toxicant concentration used. Sentence added to 6.12. Grammar fixes.

02/12/2021 Updated to universal language.

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02/11/2022 Updated links. Added table of contents. Added missing SOPs.

02/09/2023 Updated pH meter SOP. Changed language in reference to “cups” and “Jars”. Added LIMS entry instructions SOP. Changed all font to Calibri (Body).

01/18/2024 Updated links and references. Edited for grammar, clarity, and continuity.

03/12/2024 Added information about what to do if temperatures exceed the ±3˚C limit.

04/19/2024 Added section 9.3.1 and added the same information to 2.1.

11/07/2024 Added sections 11.6, 11.7, and 11.8.

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