Americamysis (formerly Mysidopsis) bahia Acute Toxicity Testing Methods

DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF LABORATORIES BIOLOGY SECTION
TALLAHASSEE FLORIDA

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1. SCOPE AND APPLICATION

1.1. This method is based on EPA 821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edition, Test Method 2007.0. It is used to estimate the acute toxicity of whole effluent and receiving waters to the mysid shrimp, *Americamysis bahia* (formerly known as *Mysidopsis bahia*), using 1 to 5-day old organisms in a 48-hour static exposure or in a 96-hour static renewal exposure. Tests conducted by this procedure are BSY-AS-MMY and BSY-AD-MMY.

2. SUMMARY OF METHOD

2.1. Samples are received and prepared in the laboratory. Sample physical-chemical (phys-chem) parameters are measured. Samples are adjusted as needed to meet permit or method test requirements. Sample is filtered through a 60µm screen to remove debris and/or indigenous organisms. Sample dilutions and controls are placed into test chambers. Test organisms are collected, fed, and held for observation prior to loading into test chambers. Tests are incubated in laboratory incubators during the duration of the test. Tests are checked at ~24-hour intervals for mortality. Survival/mortality is recorded daily. Physical-chemical parameters are measured and recorded daily. The test endpoint is mortality of the organisms. Statistical endpoints are obtained from the EPA WET Statistics Excel spreadsheet per SOP TA-07.17. Results are entered in the Laboratory Information Management System (LIMS) per SOP TA-06.42. 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. For this test, it is presumed that the salinity requirement of 5 to 32 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.

2.3. Interferences: refer to EPA-821-R-02-012 Section 9. Includes toxic substances introduced by contaminants in dilution water or on hands, glassware, sample hardware, and testing equipment. Dissolved oxygen <4.0 mg/L or >9.0 mg/L; pH <6.0 SU or >9.0 SU; ammonia, chlorine, debris, indigenous organisms that may attack or be confused with the test organisms.

2.4. Test acceptability criteria: 90% or greater survival in the controls.

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2.5. If the test acceptability criteria is not met, the test is invalidated and reported with "No Result" and a comment explaining why (no qualifier).

2.6. Method modification allows for a reduction of organisms per chamber from 10 to 5 and an increase in chambers per concentration from 2 to 4.

3. EQUIPMENT AND SUPPLIES

3.1. *A. bahia* test organisms (1–5 days old, 24-hour range) acclimated to test salinity.

3.1.1. Test organisms are obtained from vendors. Refer to SOP TA-04.20.

3.2. 500mL Test chambers, autoclaved

3.2.1. Test chambers can be air dried or dried in a drying oven for eight hours at 105 °C in place of autoclaving.

3.3. 10 mL Volumetric pipets with Pipettor/bulb

3.4. 250 ml, 500 ml, and 1 L Graduated cylinders (as needed)

3.4.1. For measuring amounts of toxicant/sample or diluent needed for each concentration. One set for toxicant/sample and one set for diluent.

3.5. 1 L, 2 L, and 4 L Erlenmeyer flasks (as needed)

3.5.1. For mixing test dilutions. One set for toxicant/sample and one set for diluent.

3.6. 8" Carolina dish or plastic bowl

3.6.1. Transfer mysids from vendor bag to Carolina dish/bowl for easier loading into test chambers. The dish/bowl is used to hold purchased organisms for feeding and examination prior to test initiation.

3.7. 5cm by 5cm spectra® mesh screen having openings ≤ 200 µm

3.7.1. Used to transfer mysids, without damage, from Carolina dish to test chambers.

3.8. Meters to measure physical-chemical parameters:

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

3.8.2. Dissolved Oxygen (DO) (Calibrate per SOP TA-06.44.)

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

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

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

3.9.1. Total Residual Chlorine (TRC) (Measure per SOP TA-06.22.)

3.9.2. Alkalinity (Measure per SOP TA-06.08.)

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3.9.3. Hardness (Measure per SOP TA-06.08.)

3.9.4. Ammonia (Measure per SOP TA-06.35)

3.10. Thermo-Scientific Incubator (Precision Model 818) with temperature and lighting control.

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

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

3.11. 30 mL disposable plastic cups w/ parameter tray

3.11.1. Parameters are measured on aliquots of sample taken from test chambers. This is done to reduce disturbance of the test organisms, and to minimize risk of possible contamination.

3.12. Live *Artemia* nauplii (enhanced with Selcon™ fatty acid concentrate)

3.12.1. Selcon is an Omega-3 Highly Unsaturated Fatty Acid (HUFA) concentrate containing Vitamin B12 and Vitamin C.

3.12.2. Prepare per SOP TA-01.03.

3.13. Disposable pipettes

3.13.1. Used for feeding. A 0.2mL fixed volume pipette can be used to feed organisms after Artemia has been counted per SOP TA-01.03.

3.14. Computer with access to the Laboratory Information Management System (LIMS).

3.14.1. Refer to SOP TA-06.42 for recording parameters.

3.15. *Bioassay Sample log-in* logbook.

3.16. *Acute Bioassay Bench Sheets* logbook.

4. REAGENTS AND STANDARDS

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

4.2. ASW or NSW (refer to SOP TA-03.02) diluted to the salinity of the sample is used as the control. In a test where salts are added to the sample to accommodate the salinity requirements of the test organisms, ASW or NSW diluted to the original sample salinity and then adjusted with salts to the test salinity is used as a 'salt' control. (This is in addition to the control). Salt adjustments are made per SOP TA-07.13. A minimum of 1 hour, preferably 2 hours, should pass prior to loading organisms in salted solutions.

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

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5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

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

5.3. See SOP TA-06.14 for Bioassay Sample Log-in Procedure.

6. TEST INITIATION

6.1. Place clean test chambers onto a laboratory cart. Label each chamber to identify the replicate (A, B, C, etc.), test concentration, and facility. Place tape across front of chamber, beneath jar #, and use waterproof marker to identify.

6.1.1. For receiving water samples, use a minimum of four chambers with 20 organisms per concentration including controls. Alternative method, minimum of two chambers with 20 organisms per concentration including controls for effluent samples.

6.1.2. Minimum test chamber size is 250 mL and minimum test volume is 200 mL. We generally use 500 mL chambers and 250 mL of sample loaded with 5 organisms per chamber. We reduce test volumes and number of test chambers when we have inadequate sample for "regular" testing.

6.2. Calibrate meters and record calibration readings. Calibrate meters per respective SOPs. Record calibration readings in LIMS logbooks. Meter calibrations are verified daily when tests are in progress, otherwise calibrate as needed.

6.3. Fill a 30 mL disposable plastic cup with sample and take an initial pH measurement per SOP TA-06.43. Record value the Bioassay Sample log-in logbook and LIMS TOX_pH.

6.3.1. If the pH is outside the acceptable range of 6.0–9.0 S.U., then an additional test with a pH-adjusted subsample is run. Adjust pH of sample per SOP TA-08.06. Record pH adjustment and/or measurement in the appropriate bench sheet. Regardless of whether the pH adjusted sample is used for a screening or definitive test, the test with the non-adjusted sample is then analyzed as a screening test. The control for this additional screen is dilution water plus the same amount of sodium thiosulfate added to dechlorinate the sample.

6.4. In the same plastic cup containing the sample, take an initial DO measurement per SOP TA-06.44. Record value in *Bioassay Sample Log-in* logbook and LIMS TOX_DO logbook.

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6.4.1. If the DO is ≤ 4.0 mg/L or > 9.0 mg/L, aerate the entire sample at the rate of ~100 bubbles per minute to as near saturation as possible prior to test initiation. Record aeration (initial and final DO, aeration rate, and duration) in the appropriate bench sheet. 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 the duration of test using micropipettes and the aeration manifold. No action is required if DO increases after test initiation.

6.5. In the same sample cup, measure the conductivity, salinity, and temperature of the sample. Conductivity readings can be rounded to the nearest 5 µmhos-cm or 0.1 mmhos-cm. Record value in the *Bioassay Sample Log-in* Logbook and LIMS TOX_COND_SAL.

6.5.1. Read the salinity directly from the Fisherbrand Traceable Bench Conductivity Meter. If the conductivity is below 2,000 µmhos/cm or 1 ppth, then *A. bahia* is probably not the appropriate test organism for this effluent sample. Re-check the facility NPDES permit requirements. Occasionally, saltwater species are used in addition to freshwater species at the request of district personnel.

6.5.2. If the salinity is <6 ppth, and no ion imbalance is suspected, increase sample salinity up to 6 ppth with sea salts. If the salinity < 6 ppth and ion imbalance is suspected add an additional 6 ppth to the original apparent salinity of the sample. If the salinity is > 6, then no adjustment is necessary. Salinity adjustments are made per SOP TA-07.13. Salinity can be 7 ppth, if necessary. If permit or district personnel requests a specific salinity, the sample, control, and salt control can be run at the requested salinity. When salt adjustments are made to the sample, an additional salt control must be prepared by diluting NSW or ASW to the sample's original salinity and adding artificial sea salts to match the salinity of the salt-adjusted sample. This salt-control is tested with the control and the salt-adjusted sample.

6.5.3. If the salinity is between 6 and 20 ppth, then add well water to the Carolina dish holding the mysids to begin their acclimation to a lower salinity. The mysids are cultured and held in 20 ppth ASW from the vendor unless otherwise requested. Fish are tested at the original salinity of the sample or per permit requests.

6.5.4. 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. The mysids are cultured and held in

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20¬ ppth ASW from the vendor unless otherwise requested. Fish are tested at the original salinity of the sample or per permit requests.

6.5.5. If the sample is salinity adjusted, then an additional test with an equivalently adjusted well water (control) aliquot will be run in addition to the other concentrations. The same number of replicates are used for this salinity adjusted control. The test will be added to the logbook and called 'Salt Control'. At test termination, the salt control should be analyzed to determine if the salinity adjustment of the effluent may have adversely affected the results of the bioassay.

6.5.6. The EPA document recommends that mysids should not be subjected to a change of more than 3 ppth salinity within a 12-hour period. If the salinity is not known until the time of sample receipt, then acclimate mysids as closely as possible to the EPA rate. Mysids can be acclimated from 20 ppth by ± 5 ppth initially, then after two hours adjust by ± 3 ppth every two hours until the test salinity is matched. If the test salinity is known before testing, request that the organism supplier starts acclimation prior to shipping the organisms to the laboratory.

6.5.6.1. Proficiency tests (PTs) are usually run at 25 ppth. Request organisms ahead of time acclimated to 25 ppth.

6.6. Measure total residual chlorine (TRC) in the sample per SOP TA-06.22. Record the result in the LIMS TOX_CHLORINE logbook and on the bioassay bench sheets. Check the field data sheet supplied by sampler for field TRC and atypical field measurements. 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 method per SOP TA-09.18. Set-up 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.

6.7. **Screening Test:** Measure 250 mL of NSW or ASW of the appropriate salinity into each of the control replicate chambers. Measure 250-mL aliquots of the 100% sample into the 100% test concentration chambers.

6.7.1. The sample volumes below are an example for four 250 mL replicates for an acute screening toxicity test: (Diluent is synonymous with control water.)

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Concentration	Diluent	Sample	Total Volume
Control	1000 mL	0 mL	1000 mL
100%	0 mL	1000 mL	1000 mL

6.8. **Definitive Test (0.5 dilution):** Measure and pour 250 mL of control water into each of the control replicate chambers. Measure and pour 250 mL aliquots of the 100% sample into each of the 100% sample concentration chambers.

6.8.1. The sample volumes below are an example for four 250 mL-replicates of each concentration for an acute definitive toxicity test: Diluent is synonymous with control water. Adjust volumes and replicates as needed; ensure that minimum volumes and minimum number of replicate requirements are met.

Concentration	Diluent	Sample	Total Volume
Control	1000 ml	0 mL	1000 mL
6.25%	500 mL	500 mL of 12.5% sample	1000mL
12.50%	500 mL	500 mL of 25% sample	1000 mL
25%	500 mL	500 mL of 50% sample	1000 mL
50%	500 mL	500 mL of 100% sample	1000 mL
l00%	0 mL	500 mL	1000 mL

6.8.2. **For the 50% Concentration:** Measure and pour 1000 mL of diluent and 1000 mL of 100% sample in the 4000-mL flask. Swirl to mix. Then, using the 250-mL graduated cylinder measure and dispense 250 mL of the solution into each of the four replicate chambers for the 50% concentration.

6.8.3. **For the 25% Concentration:** Add 1000 mL of the diluent to the 50% concentration remaining in the flask (should be 1000 mL), mix, and dispense 250 mL to each of the four 25% concentration chambers.

6.8.4. **For the 12.5% Concentration:** Add 1000 mL of the diluent to the 25% concentration remaining in the flask (should be 1000 mL), mix, and dispense 250 mL to each of the four 12.5% concentration chambers.

6.8.5. **For the 6.25% Concentration:** Add 1000 mL of the diluent to the 12.5% concentration remaining in the flask (should be 1000 mL), mix, and dispense 250 mL to each of the four 6.25% concentration chambers.

6.9. Perform QA standard reference toxicant (SRT) tests monthly, or concurrently if bioassays are performed less frequently. The SRT must be done within 30 days of the start of a facility bioassay. FDEP Laboratory uses Potassium chloride as the standard toxicant. Other toxicants can be used.

6.9.1. An example of sample/toxicant and diluent volumes for four 250 mL replicates of each concentration for an acute definitive potassium

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chloride SRT test, using the toxicant made per SOP TA-09.01. Concentrations and toxicants can be modified as needed. Diluent is synonymous with control water. Volumes rounded.

Concentration	Diluent	SRT Toxicant	Total Volume
Control	1000 mL	0 mL of 1 g/L	1000 mL
0.2 g/L	800 mL	200 mL of 1 g/L	1000 mL
0.4 g/L	600 mL	400 mL of 1 g/L	1000 mL
0.5 g/L	500 mL	500 mL of 1 g/L	1000 mL
0.6 g/L	400 mL	600 mL of 1 g/L	1000 mL
0.8 g/L	200 mL	800 mL of 1 g/L	1000 mL

6.10. Pour aliquots from the A replicates into disposable plastic cups held within a parameter tray. Measure pH, temperature, DO, salinity, and conductivity from the subsamples. Record all readings. Record any comments pertaining to the condition or appearance of the sample.

6.10.1. Conductivity readings can be rounded to the nearest 0.1 mmhos/cm. Readings for pH, DO, and temperature shall be read to one decimal place. Take measurements from lowest to highest concentration.

6.10.2. Record all readings in the appropriate columns on *Acute Bioassay Bench Sheet*.

6.11. Use the screen to load 20% of test organisms per replicate - into each test chamber. Repeat the loading until the desired number of mysids has been loaded into each chamber. Load 2 organisms at a time for 10 organisms per replicate and 1 organism at a time for 5 organisms per replicate.

6.12. Verify the count over a light table. A person other than the loader should verify the number of organisms per replicate. Record counts and physical-chemical parameter readings in appropriate columns in *Acute Bioassay Bench Sheet*.

6.13. Feed test organisms (in test chambers). Feed 0.2 mL concentrated (~100 *Artemia* nauplii per mysid) each day. To avoid potential DO problems, feed half the amount twice daily, checking to determine appropriate amount of *Artemia* to add (based on Artemia remaining in test chamber).

6.13.1. *A. bahia* are required to be fed twice daily regardless of a renewal, with the termination day being the exception. First feeding should be performed two hours prior to cleaning.

6.14. Place chambers together in an incubator using the randomizers in the lab and cover with clear Plexiglas. Covers prevent accidental contamination and minimize evaporation.

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6.15. Measure alkalinity and total ammonia of sample following test initiation. Measurements made per SOP TA-06.08 and TA-06.35, respectively.

7. INTERIM READINGS

7.1. On day 1 (t = 24 hour and any other interim readings if test duration is > 48 hour), count and record the number of surviving mysids. This may occur at any time or during the cleaning period, two hours after the morning feeding. Use a transfer pipet to remove any dead test organisms and to remove dead *Artemia* nauplii. Pour aliquots (see 6.1.2 comment) into the parameter tray and measure pH, temperature, DO, and conductivity daily. Record readings. When done, feed mysids, return test chambers to incubator, and cover.

7.1.1. All counting is done over a light table. Mysids are recorded as live if they move when lightly prodded or appendage movement is seen. Record counts and physical-chemical readings in appropriate columns in Acute Bioassay Bench Sheet.

7.1.2. Although not a requirement, we generally follow: (four replicate concentrations) Aliquots are taken from B replicates at 24 hours, C replicates at 48 hours (for 48 hour termination or “old” and “new” solutions for renewals), D replicates at 72 hours and A replicates at 96 hours. If the test only has two replicates, aliquots are taken from B replicates at 24 hours, A replicates at 48 hours (for 48-hour termination or “old” and “new” solutions for renewals), B replicates at 72 hours and A replicates at 96 hours. If a replicate is not available (all dead in the replicate the previous day), the next sequential replicate is used.

7.2. If test duration is >48 hours, repeat step 7.1 at 72 hours.

8. TESTS WITH DURATION >48 HOURS

8.1. On test day 2 (t = 48 hour), add 0.2 mL of concentrated *Artemia* nauplii at least 2 hours prior to test renewal.

8.2. Collect aliquots of "old" solution for parameters; then, use a screened siphon to carefully remove all but ~ 50 mL of "old" test solution. Remove as much debris as possible during this process. Count and record number of surviving organisms; remove dead organisms. Record counts in appropriate columns on *Acute Bioassay Bench Sheets*.

8.3. Use the 500-mL cylinder to carefully pour "new" test solutions into the appropriate test chambers.

8.3.1. New test solutions are prepared from sample received on test-day 0 that has been stored at 0-6°C. Generally, the sample is from an unopened container. Double-check DO level and aerate if necessary. If chlorine or

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ammonia were not detected in the original sample, it is generally not necessary to check for these components or measure alkalinity in the sample used for test renewal.

8.3.2. The "new" sample should be prepared during the two-hour feeding period. All sample manipulations (screening, pH adjustment, aeration, etc.) should be completed prior to use of the sample. Generally, two people work together performing the test renewals. One person removes the "old" solution; the other person adds "new" solution to the test chambers.

8.4. Collect aliquots of "new" solution (from test chambers) into clean parameter vials. Measure and record DO, pH, temperature, and conductivity in each of the vials containing "new" solution.

8.5. Verify that the number of surviving organisms per replicate is the same as the survival counts prior to renewal. Record the after-renewal counts on the bench sheet.

8.6. Perform Step 7.1 at 24-hour intervals until test termination. Feed only 0.2 mL concentrated *Artemia* nauplii immediately after renewal. Resume regular feeding at 72 hours.

9. TEST TERMINATION

9.1. On termination day (t = 48 hour (±~2 hours) or t = 96 hour (±~4 hour) or whatever the test duration is (acute bioassay test duration generally does not exceed 96 hours), count the number of surviving mysids. Measure parameters of the solutions in the chambers or in aliquots from the chambers poured into vials in the parameter tray. Record all counts and parameter readings. Record any comments pertaining to the appearance or condition of the mysids. Record test termination date and time.

9.1.1. Test may be terminated prior to the intended duration if 100% mortality has occurred in the test concentrations AND control mortality is ≤ 10%. Test can also be terminated in the event of a control failure.

9.1.2. Counting is done over a light table. Physical-chemical readings (pH, DO, and conductivity) are taken in the appropriate replicate chamber for each concentration per Step 7.1.2.

9.1.3. Record counts, readings, date, and time in appropriate columns in Acute Bioassay Bench Sheet.

9.2. Pour the contents of all chambers into a bucket of ice water to euthanize fish. Place glassware to be washed on appropriate cart.

9.3. Pour the bucket into the sink and flush drain with water. Refer to Bureau Quality Manual for waste disposal procedures.

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9.4. Enter test results into statistical program to obtain LC50, per SOP TA-07.17 for definitive tests.

9.5. LC50s for screening tests are reported in LIMS as "100 L" if mortality is < 50% in the 100% sample test solution; "100 K" if mortality in the 100% sample test solution is > 50%; and, "100" if mortality in the 100% sample test solution is exactly 50%. The 100% percent test solution does not have to be 100% sample but does have to comply with the percent effluent designated in the facility's NPDES permit.

9.6. Enter test results into LIMS.

9.6.1. Test results are verified by a second person prior to data being uploaded in LIMS.

9.6.2. 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. Record information in appropriate logbooks, Acute Bioassay Bench Sheet, and FDEP Laboratory Information Management System (LIMS).

10.4. *Bioassay Sample Log-In* logbook. Record per SOP TA-06.14.

10.5. Enter the LC50 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.

10.6. Log sample receipt, temperature, and condition into *Acute Bioassay Bench Sheets* 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,

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test chamber size, test solution volume, and food type, and food batch numbers. Access scanned Field Sheets via \\floridadep\data\DEAR\Labs\Receiving to obtain sampling information.

11. QUALITY CONTROL

11.1. Refer to DEP Laboratory Quality Manual for corrective action and handling out-of-control data. Refer to EPA 821-R-02-012 Test Method 2007.0 for test conditions and acceptability criteria.

11.2. Perform QA standard reference toxicant (SRT) tests monthly. If an SRT fails the test acceptability criteria or control chart limits, it must be repeated as soon as possible to get a passing test within the month timeframe. If two or more consecutive tests do not fall within control chart limits, the SRT must be repeated immediately and corrective action must be taken. If two or more tests fall outside of the control chart limits in 20 samples, the SRT must be repeated immediately and corrective action must be taken. When the cause of the outlier can be documented, the outlier should be removed from the future calculations of the control chart.

11.3. Samples must be held at 0-6 degrees C prior to test initiation and between renewals (exception - hand delivered sample day of collection - sample should be cooling relative to collection temperature). Sample holding time should not exceed 36 hours from time of collection until initial use of sample.

11.4. Tests and cultures shall be maintained at 25 ± 1°C (26 ± 1°C for *A. bahia*, where applicable). Test temperature shall not deviate by more than 3°C (maximum minus minimum) during the test period. Verify continuous temperature of equipment using a NIST- traceable thermometer. Apply correction factors as needed for accuracy.

11.4.1. Temperature Excursion Response:

11.4.1.1. If the test solution temperature deviation (maximum minus minimum) exceeds 3°C and no toxicity is observed, document the deviation and add the comment, “Temperature of test solutions deviated from method specifications.”

11.4.1.2. If the incubator monitoring temperature deviation (maximum minus minimum) exceeds 3°C and no toxicity is observed, document the deviation and add the comment, “Continuous temperature monitoring in surrogate test vessels deviated from method specifications.”

11.4.1.3. If the test solution temperature deviation (maximum minus minimum) exceeds 3°C and toxicity is observed, document the deviation, qualify results as estimated (“J”), and add the comment, “Estimated value. Deviation of test solution

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temperatures from method specifications may be contributing to toxicity."

11.4.1.4. If the temperature deviation (maximum minus minimum) exceeds 3°C and the test does not meet method acceptability criteria, report results as "No Result" and notify the client that the test should be repeated.

11.5. If controls fail to meet minimum acceptability criteria, the test will be invalidated, and the analyses are 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 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.

11.6. 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.

11.7. 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.

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

11.9. If a problem occurs during the sample processing or testing, the following steps are performed: 1) Lab supervisor is notified. 2) Client is notified (client makes decision for additional testing). 3) Section administrator is notified. 4) A non-conformance report (NCR) is submitted describing the problem and the corrective action.

11.9.1. To create an NCR: open the LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops for

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sample collection and preservation, and the Laboratory Quality Assurance Manual for corrective action and handling out-of-control data.

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 Health and Safety Plan.

12.3. Refer to the DEP Laboratory Quality Assurance Manual for sample disposal.

12.4. Read MSDS information for reagent handling and disposal.

13. REFERENCES

13.1. DEP Laboratory Quality Assurance Manual

13.2. DEP Laboratory Health and Safety Manual

13.3. MSDS Information Sheets

13.4. EPA 821-R-02-012, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition, Test Method 2007.0.

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-06.44, *YSI Model 58 Dissolved Oxygen Meter Calibration and 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-06.35, *Measuring Total Ammonia using HACH Ammonia Nitrogen Pocket Colorimeter II Test Kit*

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

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

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

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

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

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

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

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

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

APPENDIX OF SIGNIFICANT CHANGES

02/11/2015 Formatted for 508 compliance.

03/23/2024 Added information about what to do if temperatures exceed the ±3˚C limit. Added SOP TA-06.42. Updated Ph and DO Meter SOPs. There is no SOP TA-06.33. No longer makes hypersaline brine so removed. Added proper way to euthanize fish.

04/17/2026 Section 11: Quality Control, was expanded to further address test/culture temperature requirements (section 11.4) and control chart failures (section 11.2) to address the 2026 audit deficiencies.

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