Menidia beryllina Chronic Toxicity Testing Methods

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Menidia beryllina 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 1006.0. It describes the procedures used to estimate the chronic toxicity of saltwater whole effluent and receiving waters on 7 to 11-day old *Menidia beryllina* (inland silverside) during a 7-day, static renewal exposure. Tests conducted by this procedure are BSY-CS-MFS and BSY-CD-MFS.

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 to meet permit or method test requirements. Sample is filtered through a 60µm screen to remove debris and/or indigenous organisms. Sample dilutions and control water 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. Phys/chem parameters are measured and recorded daily. Survival/mortality is recorded daily. The test endpoints are survival and growth (measured as dry weight) (IC25) of the organisms. 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 in LIMS.

2.2. Test acceptability criteria: 80% or greater survival in the controls and an average dry weight of ≥ 0.50 mg per surviving control organism, dried immediately after test termination.

2.3. For this test, it is presumed that the salinity requirement of 5 ppth (parts per thousand) to 32 ppth (± 2 ppth of the selected test salinity) has been met and all effluents and control water have had their 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.

2.4. Interferences: Refer to EPA-821-R-02-014 Section 13.3. Includes toxic substances introduced by contaminants in dilution water or on hands, glassware, sample hardware and testing equipment, dissolved oxygen < 4.0 mg/L, pH < 6.0 or > 9.0 SU; salinity < 5 or > 35 parts per thousand; ammonia, chlorine, debris, and indigenous organisms that may attack or be confused with the test organisms.

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3. EQUIPMENT AND SUPPLIES

3.1. 1 L Test Chambers, 4 per test concentration

3.1.1. Test chambers can be air dried, dried in a drying oven, or autoclaved.

3.1.2. A test with 5 test concentrations and a control, using 4 replicates per concentration (required minimum) would require 24 chambers. Tests are run using 1 L glass chambers and 500 mL test solution/replicate.

3.2. 100, 250, 500 and 200 mL Autoclaved graduated cylinders for making test solutions.

3.3. 6 L Erlenmeyer flasks (two), autoclaved, for mixing test dilutions and to hold effluent sample.

3.4. 1 mL and 10 mL Serological pipettes—graduated

3.5. Pipettor for serological pipettes

3.6. Magnetic stir bars and stirrer

3.7. Large plastic Carolina dish to hold fish for acclimation and loading into test chambers

3.8. 60 µm mesh to filter sample

3.9. 4 mm ID glass tubing with fire polished edges (or any pipette with at least a 4 mm ID), and droppers, for transferring organisms

3.9.1. To avoid potential injury to the *M. beryllina* test organisms, nets or screens should not be used for transfers.

3.10. Meters to measure:

3.10.1. pH (Calibrate per SOP TA-06.43)

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

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

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

3.11. Hach® kits to measure:

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

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

3.11.2.1. Hardness usually not measured when conductivity is > 5000 µmhos/cm

3.11.3. Ammonia (Measure per SOP TA-06.35)

3.12. Thermo Scientific Incubator (Precision Model 818) (#1 or #5) with temperature and lighting control

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3.12.1. Incubators should maintain selected test temperature (25 ± 1°C recommended) and a photo period of 16-hours light to 8-hours dark. Light intensity should be 50-100 ft candles. Test temperature must not deviate more than ±3°C in the test chambers for the duration of the test.

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

3.12.3. Other incubators or the water bath can be used to regulate test dilution and toxicant waters temperature.

3.13. 30 mL disposable plastic cups contained in parameter tray

3.13.1. Two cups per test concentration. One is for the “old” solution parameters; the other is for the “new” solution parameters. Rinse cups daily.

3.14. Waste bucket

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

3.15.1. Prepare in advance per SOP TA-01.03.

3.15.2. Used for feeding *M. beryllina* per SOP TA-02.00

3.16. Drying oven capable of sustaining 60°C for at least 24 hours. Refer to SOP TA-06.21.

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

3.18. Laboratory Class 1 weights

3.18.1. 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 fish (0.01 g and 0.05 g are used).

3.19. Mettler Toledo balance XA105DU (calibrate per SOP BB-36), to weigh weigh-boats and dried fish.

3.20. *Menidia beryllina* test organisms: 7–11 days old, 40 per test concentration, 10 per replicate for a total of 240 organisms.

3.20.1. Test organisms are obtained from the vendor per SOP TA-04.20.

3.20.2. Organisms are acclimated to test salinity. Increase or decrease slowly, no more than 3 ppth per hour.

3.21. *Chronic Bioassay Bench Sheet*

3.22. *Bioassay Sample Log-in* logbook

3.23. *Organism Log-in Bench Sheets* logbook

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

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4. REAGENTS AND STANDARDS

4.1. Artificial Sea Water (ASW) prepared with laboratory grade artificial sea salts (SOP TA-03.02).

4.2. Effluent samples to be tested (recommended to be warmed to 25 ± 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 to test initiation (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).

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

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

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

6. TEST INITIATION

6.1. Place 24 clean 1 L test chambers onto a laboratory cart. Label each chamber to identify the replicate (A–D), test concentration, and facility sampled.

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

6.2. Calibrate meters and record readings in LIMS daily during test. Also, check meters at end of day and record values.

6.3. Filter effluent sample through 60µm mesh into 6 L Erlenmeyer flask and heat to 25±1°C.

6.4. Fish will arrive with the mail carrier (usually around 10:00 am) and must be collected from Receiving. Unpackage fish and record all necessary information in the Organisms Log-in Logbook. Collect some of the water the fish arrive in and use to take phys/chem parameter readings. Pour the contents of the fish bag into an 8’ Carolina dish and observe fish, noting and removing dead fish or fish acting erratically. The dish will be set aside for observation for at least two hours. During

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this time, the dish will remain under aeration, and the fish are fed Artemia nauplii (per SOP TA-01.03).

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

6.5.1. If pH is outside acceptable range of 6.0–9.0 SU, then an additional test with a pH-adjusted aliquot will be run. Adjust pH of sample per SOP TA-08.06. Record pH adjustment and /or measurement in the appropriate bench sheet. 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.

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

6.6.1. If the DO is < 4.0 mg/L or > 9.0 mg/L, aerate 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, 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 DO begins to fall, set up gentle aeration (< 100 bubbles per minute) to ALL test solutions for duration of test using micropipettes and the aeration manifold. No action is required if DO increases after test initiation.

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

6.7.1. If conductivity is <2,000 µmhos/cm (corrected to 25°C) or the salinity is <1 ppth, freshwater organisms should probably be used rather than Menidia beryllina. Check with FDEP District personnel and check the Facility's NPDES permit for bioassay requirements.

6.7.2. Salinity will be too low in freshwater samples to measure on the meter, use the following web site to convert conductivity to salinity: https://www.hamzasreef.com/Contents/Calculators/SalinityConversion.php

6.7.3. If the salinity is >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 ppth and an ion imbalance is suspected, salinity adjustment is required to 7 ppth. If the permit requires a specific salinity, or the district personnel request a

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specific salinity, the saltwater tests can be performed at that salinity. Salinity adjustments are made per SOP TA-07.13.

6.7.4. If the salinity is between 6 and 20 ppth, then add well water to the Carolina dish containing the fish to begin their acclimation to a lower salinity. If the salinity is above 20 ppth, add a higher salinity of ASW to the Carolina dish containing the fish to begin their acclimation to a higher salinity. Fish are ordered at the predicted test salinity to prevent a need for large changes in salinity.

6.7.5. 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.

6.7.6. The EPA document recommends that organisms should not be subjected to a change of more than 3 ppth salinity in 3 hours. If the salinity is not known until the time of sample receipt, then acclimate organisms as closely as possible to the EPA rate. Organisms 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.

6.8. 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 field data sheet supplied by sampler for field TRC and atypical field measurements.

6.8.1. If TRC is ≥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. 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.

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

6.10. Using a graduated cylinder, pour a minimum of 500 mL of ASW into each control replicate chambers.

6.11. Pour a minimum of 500 mL of 100% sample into each of the 100% sample concentration chambers.

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6.12. Measure the desired volumes of ASW 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 500 mL from the 2000 mL cylinder to a 500 mL cylinder. Empty the 500 mL cylinder into test chamber A; repeat for chambers B, C and D for each concentration. Rinse cylinders with deionized water between concentrations. Repeat for each test concentration.

6.12.1. For each sample tested, use four 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.

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

6.12.3. Example of sample volumes (0.5 dilution) for four 500 mL replicates of each concentration of a chronic toxicity test:

Concentration	**ASW**	**Sample**	**Total Volume**
Control	2000 mL	0 mL	2000 mL
6.25%	1875 mL	125 mL of 100%	2000 mL
12.5%	1750 mL	250 mL of 100%	2000 mL
25%	1500 mL	500 mL of 100%	2000 mL
50%	1000 mL	1000 mL of 100%	2000 mL
100%	0 mL	2000 mL of 100%	2000 mL

6.13. Perform QA standard reference toxicant (SRT) tests concurrently with facility tests, using potassium chloride (other toxicants can be used such as copper chloride). If additional Menidia chronic tests are run during the month, only one passing SRT must be run. If the test fails, it must be repeated until it passes and corrective actions must be taken.

6.13.1. Example of KCl concentrations (toxicant) and ASW volumes for chronic SRT test. SRTs are performed at 20 ppth salinity: Toxicant and concentrations can change as needed by the lab.

Concentration	ASW	**Toxicant**	**Total Volume**
Control	2000 mL	0 mL	2000 mL
0.0625 g/L	1875 mL	125 mL of 1 g/L	2000 mL
0.125 g/L	1750 mL	250 mL of 1 g/L	2000 mL
0.25 g/L	1500 mL	500 mL of 1 g/L	2000 mL
0.5 g/L	1000 mL	1000 mL of 1 g/L	2000 mL
1 g/L	0 mL	2000 mL of 1 g/L KCl	2000 mL

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6.14. Pour aliquots from all A replicates into the appropriate 30 mL 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 the *Chronic Bioassay Bench Sheet*. Record any comments pertaining to the condition or appearance of the sample. During the following seven days, rotate through all the replicates and take parameters before and after renewing the sample.

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

6.16. 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.

6.17. Test initiation (0-hour and 0-Day):

6.17.1. Using a wide bore pipette, load two (20% of test organisms per replicate) fish into each test chamber. Repeat the loading process until ten fish have been loaded into each chamber. Verify count over a light table. Record counts in appropriate columns on the *Chronic Bioassay Bench Sheet*.

6.17.2. (10 fish per replicate) x (4 replicates per test concentration) = 40 fish per concentration tested.

6.17.3. A person other than the loader should verify the number of fish in each replicate.

6.18. Prepare *Artemia* nauplii and feed at a rate per step 6.15.1 (6.15.2- 6.15.3 only for alternative method). Feed after initial loading and after daily renewal. Do not feed on test day 7. Use the labeled calibrated repeater pipet to feed fish 2 mL *Artemia* per replicate. Without contacting test liquid or chamber, gently dispense the food along the inner wall of the chamber to minimize disturbance to the fish and prevent loss or cross contamination.

6.18.1. See Step 6.15.2 for feeding instructions. Adjust amount fed as necessary to compensate for mortality and/or dissolved oxygen issues. Typically, the FDEP feeds 2 mL of concentrated artemia per replicate. If there are issues related to feeding, the step below can be followed.

6.18.2. For days 0-2, concentrate < 24-hour old Artemia (SOP TA-01.03) and place 4 mL of concentrate in a 100 mL beaker containing 20-30 mL of ASW. Bring the volume to 80 mL. Stir to mix, then pipet 2 mL to each replicate; or feed ~0.1 mL of concentrated Artemia per replicate. Adjust as needed for mortality or DO problems.

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6.18.3. For days 3-6, concentrate < 24-hour old Artemia (SOP TA-01.03) and place 6 mL of concentrate in a 100 mL beaker containing 20-30 mL of ASW. Bring the volume to 80 mL. Stir to mix, then pipet 2 mL to each replicate, or feed ~0.15 mL of concentrated *Artemia* per replicate. Adjust as needed for mortality or DO problems.

6.19. Use the test randomizer located on the shelf above the stand-up parameter station to randomly place the test chambers in the proper incubator.

6.19.1. Use covers to prevent accidental contamination and minimize evaporation.

6.20. Measure alkalinity and hardness if appropriate (SOP TA-06.08), total residual chlorine (SOP TA-06.22) and total ammonia (SOP TA-06.35) of sample, salinity adjusted sample (as needed) and control water. Record each in the corresponding logbook along with control water parameters in LIMS.

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

7. INTERIM READINGS AND RENEWALS

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

7.1.1. 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).

7.1.1.1. 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. Fish are recorded as live if they move when lightly prodded or if any external or internal organs of the organism are still active.

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

7.2. Pour aliquots from the appropriate replicate(s) after renewal into the appropriate 30 mL cup. Measure pH, temperature, DO, conductivity, and salinity (appropriate replicate for each concentration) and record readings in the appropriate columns in *Chronic Bioassay Bench Sheet*.

7.3. Feed fish with *Artemia* nauplii concentrate daily after the renewal. Prepare *Artemia* and adjust volume per step 6.15. Return chambers to incubator and cover to reduce evaporation.

8. TEST TERMINATION

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

8.2. On day 7 (t = 168 hr ± 6 hr), count and record number of surviving and dead fish. Remove and discard dead fish. 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 Sheets*. Record any comments pertaining to the appearance or condition of the fish.

8.2.1. Counting is done over a light table. Phys/chem readings are taken from the aliquot to minimize disturbance to the fish.

8.3. Pour the contents of Control replicate A onto a fine mesh net held over the waste bucket. mmediately euthanize the fish by immersing them in ice water in the Carolina dish until there is no more movement and they are fully euthanized.

8.4. When the fish are dead, gently rinse them with DI water. Use forceps and place each fish into the respective weigh boat. Return weigh boat to the Carolina dish.

8.5. Repeat steps 8.3 and 8.4 for each replicate.

8.6. Dry the weight boats containing the fish for 6 hours (minimum) at 105°C or 24 hours at 60°C in the sediment drying oven the day of test termination. Store the Carolina dish in the desiccator before and after they are dried.

8.7. Refer to SOP TA-06.21.

8.8. Dry for at least 24 hours at 60°C.

8.9. Fish can also be dried for 8 hours (minimum of 6 hours) at 105°C.

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

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9. STATISTICAL ANALYSIS

9.1. Complete the weight page in the *Chronic Bioassay Bench Sheets Pimephales promelas and Menidia beryllina* logbook.

9.2. Enter test data into statistical program to obtain LC50, NOEC, LOEC, and IC25 as per SOP TA-07.17.

9.2.1. Refer to data analysis in EPA-821-R-02-014. TSK, Dunnetts, and ICP programs can be used to analyze data. TOXCALC®, CETIS® or 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. Log sample receipt, temperature and condition into *Chronic 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, test chamber size, test solution volume, food type and food batch numbers. Access scanned Field Sheets via the Receiving folder, \\floridadep\data\DEAR\Labs\Receiving to obtain sampling information.

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

10.3. *Organism Log-In Bench Sheets* logbook. Record per SOP TA-04.20.

10.4. FDEP Laboratory Information Management System (LIMS). Record per SOP TA-06.43.

10.5. Record information in appropriate logbooks (*Chronic Bioassay Bench Sheets* and LIMS calibration logbooks).

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

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

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

10.7.2. 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.

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10.7.3. 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.).

10.8. Enter the NOEC, LOEC, 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.

11. QUALITY CONTROL

11.1. Refer to FDEP Laboratory Quality Assurance Manual for corrective action and handling out-of-control data. Refer to EPA-821-R-02-014 Test Method 1006.0 for test conditions and acceptability criteria.

11.2. Sample should be held at 0-6°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.

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

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 temperatures from method specifications may be contributing to toxicity.”

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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 will 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. 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 decision for additional testing), 3) Section administrator is notified, and 4) a nonconformance report (NCR) is submitted describing the problem and the corrective action.

11.8.1. To create NCR, open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to https://floridadep.gov/dear/quality-assurance/content/dep-sops for sample collection and preservation, and the Laboratory Quality Assurance Manual for corrective action and handling out-of-control data.

11.9. Perform QA standard reference toxicant (SRT) tests monthly or concurrently as needed (see step 6.12). If an SRT fails the test acceptability criteria or control chart

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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.

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 FDEP Laboratory Quality Assurance Manual for sample disposal

12.3. Refer to the FDEP Health and Safety Manual.

12.4. Read MSDS information for reagent handling and disposal.

13. REFERENCES

13.1. FDEP Laboratory Quality Assurance Manual

13.2. FDEP Health and Safety Manual

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 Edition, Test Method 1006.0. October 2002*

13.4. DEP SOP FS 1000, *General Sampling*

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

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

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

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

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

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

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

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 Test Kit*

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13.15. DEP SOP TA-06.35, *Measuring Total Ammonia using HACH Ammonia Nitrogen Test Kits for Freshwater and Saltwater Samples*

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

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

13.18. DEP SOP TA-06.44, *YSI MultiLab 4010-1 Meter Calibration and YSI IDS ProOBOD DO Probe Maintenance*

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

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

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

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

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

APPENDIX OF SIGNIFICANT CHANGES

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04/17/2026 Section 11: Quality Control, was expanded to further address test/culture temperature requirements (section 11.4) and control chart failures (section 11.9) to address the 2026 audit deficiencies.

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