Menidia beryllina Acute Toxicity Testing Methods

DEPARTMENT OF ENVIRONMENTAL PROTECTION
LABORATORY
BIOLOGY PROGRAM
TALLAHASSEE, FLORIDA

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1. SCOPE AND APPLICATION

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 2006.0. It is used to estimate the acute toxicity of saltwater whole effluent and receiving waters on *Menidia beryllina* (inland silverside), using 9- to 14-day old organisms in a 48-hour static exposure or in a 96-hour static renewal exposure. Tests conducted using this procedure are BSY-AS-MFS and BSY-AD-MFS.

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 100 µm screen to remove debris and/or indigenous organisms. Sample dilutions and control waters 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. Physical-chemical parameters are measured and recorded daily. Survival/mortality is 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). 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. Acute bioassay test duration generally does not exceed 96 hours.

2.2 For this test, it is presumed that the salinity requirement of 5-32 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, on hands, glassware, sample hardware, and testing equipment; dissolved oxygen <4.0 mg/L; pH <6.0 SU 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.

2.4 Test acceptability criteria: 90% or greater survival in the controls. Method modification allows reduction of organisms per chamber from 10 to 5 and increase in replicates/concentration from 2 to 4.

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3 EQUIPMENT AND SUPPLIES

3.1 *M. beryllina* test organisms (9–14 days old, less than or equal to 24-hr range in age) acclimated to test salinity prior to test initiation.

3.7.1 Test organisms are purchased and maintained per SOP TA-04.20. *M. beryllina* test organisms are fed live *Artemia* nauplii at least 2 hours prior to test initiation.

3.2 1 L Test chambers (glass jars), autoclaved. Test chambers can be dried in a drying oven for eight hours at 105 °C rather than being autoclaved.

3.3 Bioassay laboratory grade Forty Fathoms ® Artificial Sea salts or Crystal Sea ® Marine Artificial Sea salts.

3.4 10 mL Volumetric pipets, with Pipettor/bulb

3.7.1 One pipet per sample, plus one for the control.

3.5 250 mL, 500 mL, and 1 L Graduated cylinders (as needed)

3.7.1 One per sample is needed for initially measuring test concentrations, and later for measuring aliquots of each test concentration into test chambers.

3.6 1 L, 2 L, and 4 L Erlenmeyer flasks (as needed)

3.7.1 One per sample is needed for uniform mixing of test concentrations.

3.7 8" Carolina dish

3.7.1 Transfer fish from shipping container to Carolina dish for easier loading of bioassay chambers.

3.8 Glass pipet fitted with rubber bulb

3.7.1 Used to transfer fish from Carolina dish to test chambers.

3.9 Meters to measure physical/chemical parameters:

3.7.1 pH (Calibrate per SOP TA-06.43.)

3.7.2 Dissolved oxygen (DO) (Calibrate per SOP TA-06.39.)

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

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

3.10 Hach® kits to measure physical/chemical parameters:

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

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

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

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

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3.11 Thermo-Scientific Incubator (Precision Model 818 (# 1))

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

3.7.2 Other incubators or the water bath can be used to regulate test temperature.

3.12 30mL, disposable plastic cups (comet cups) w/ parameter tray

3.7.1 Aliquots from the chambers are poured into corresponding comet cup and parameters are measured in the aliquots. This is done to reduce disturbance of the test organisms, and to minimize risk of possible contamination.

3.13 *Artemia* nauplii (<24 hr old)

3.7.1 Prepare in advanced per SOP TA-01.03.

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

3.14 Computer with access to the Laboratory Information Management System (LIMS) per SOP TA-06.42

3.15 *Bioassay Sample Log-In* logbook

3.16 *Organism Log-In Benchsheets Acute* logbook

3.17 *Acute Bioassay Bench Sheets* logbook

4 REAGENTS AND STANDARDS

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

4.7.1 NSW or ASW (refer to SOP TA-03.02) diluted to the salinity of the sample can be used as the control. In a test where salts are added to the sample to accommodate the salinity requirements of the test organisms, NSW or ASW is diluted to the original sample salinity and then using salts is adjusted to the test salinity. This is used as a 'salt' control'. It is in addition to the 'normal' test control. Salt adjustments are made per SOP TA-07.13.

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 (time of last sample collect for grab samples or the end of the 24-hour collection period for composite samples) to test initiation. The remainder of each sample is stored in the Biology Walk-in cooler for solution renewal the following day or days.

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4.2 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, B, C, etc.), test concentration, and facility. Place tape across chamber beneath the chamber ID. Use a waterproof marker to write on the tape.

6.7.1 Minimum of two chambers for 20 organisms per concentration including controls for effluent samples and a minimum of four chambers for 40 organisms per concentration including controls for receiving water samples.

6.7.2 Minimum test chamber size is 250 mL and minimum test volume is 200 mL. The FDEP Laboratory generally uses 1 L chambers and 500 mL of sample. The FDEP Laboratory uses four replicates per concentration for screening tests and two replicates per concentration for definitive tests. Reduce test volume (not < 200 mL) and chamber size when there is inadequate sample for "regular" testing.

6.2 Calibrate meters per respective SOP and record calibration readings in LIMS.

6.3 Fill a disposable plastic cup with sample and take initial pH measurement per SOP TA-06.37. Record value the *Bioassay Sample log-in* logbook and LIMS TOX_pH.

6.7.1 If the pH is outside the acceptable range of 6.0–9.0 SU, adjust pH of sample per SOP TA-08.06. Record pH adjustment and/or measurement in the appropriate bench sheet. Continue with the test as described in this SOP. A screen test with a 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.

6.4 In the same plastic cup containing the sample, take an initial DO measurement per SOP TA-06.39. Record value in *Bioassay Sample Log-in* logbook and LIMS TOX_DO.

6.7.1 If the DO is ≤ 4.0 mg/L or $\underline{\geq}$ 9.0 mg/L, aerate the entire sample to as near saturation as practical 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/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.

6.7.2 Check the pH again after sample aeration. Aeration can cause pH to change i.e. equilibrate). Based on this second pH reading, refer to 6.3.1 to decide if the sample needs to be pH adjusted, and a screen test performed.

6.5 In the same plastic cup containing the sample, measure the initial conductivity, temperature, and salinity of the sample. Record value in the *Bioassay Sample Log-*

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in logbook and LIMS TOX_COND_SAL. Conductivity measurements can be rounded to nearest 5 µmhos or 0.1 mmhos.

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

6.5.1.1 If district personnel request a saltwater test for a freshwater effluent, then the saltwater tests can be performed in addition to the freshwater tests.

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: Aquarium Calculators (hamzasreef.com) (in the "Unit Conversion" tab).

6.6 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 necessary. If the salinity is between 1 and 6 ppth and an ion imbalance ***is*** suspected, salinity adjustment is required to 7 ppth. Salinity adjustments are made per SOP TA-07.13.

6.7.1 If the permit requires a specific salinity, or the district personnel request a specific salinity, the saltwater tests can be performed at that salinity.

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

6.7.1 The EPA document recommends that *Menidia* should not be subjected to a change of more than 3 ppth salinity in 3 hours. The *Menidia* are cultured by the vendor and held in 20 ppth. The salinity should be changed by no more than 3 ppth per hour. If time is limited, the first adjustment can be 5 ppth if the salinity of the *Menidia* water is at least 20 ppth. The greater salinity adjustments should be made in the first few hours, and the salinity adjustments should lessen in the hours closest to test initiation. The *Menidia* should have one hour after the last salt adjustment for final acclimation before being loaded into the test. If possible, adjustment of test organism salinity should begin several days prior to test initiation. If organisms are supplied from a vendor, request that the vendor begin salinity adjustments prior to shipping.

6.8 Measure total residual chlorine (TRC) in the sample per SOP TA-06.22. Record the result in the LIMS TOX_CHLORINE and on the bioassay bench sheets. Check the 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 by the sodium thiosulfate method per SOP TA-09.18. Setup a concurrent screening test using the 100% sample that has not been dechlorinated and a control that contains the the same amount of sodium thiosulfate added to the sample. 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 ***Screening Test:*** Measure 500 mL of control water into each of the control replicate chambers. Measure 500 mL aliquots of the 100% sample into the 100% test concentration chambers.

6.7.1 Suggested sample volumes (per 1 L test chamber and 500 mL sample volume/chamber) for an *acute screening* toxicity test with 4 replicates per concentration:

Diluent is synonymous with control water.

Concentration	Diluent	Sample	Total Volume
Control	500 mL/chamber	0 mL	2000 mL
100%	0 mL	500 mL/chamber	2000 mL

6.10 ***Definitive Test (0.5 dilution):*** Measure and pour 500 mL of control water into each of the control replicate chambers. Measure and pour 500 mL aliquots of the 100% sample into each of the 100% sample concentration chambers.

6.7.1 The sample volumes below are an example for four 500 mL replicates of each concentration for an *acute definitive* toxicity test:

Concentration	Diluent	Sample	Total Volume
Control	2000 mL	0 mL	2000 mL
6.25%	2000 mL	2000 mL of 12.5% sample	4000mL
12.50%	2000 mL	2000 mL of 25% sample	4000 mL
25%	2000 mL	2000 mL of 50% sample	4000 mL
50%	2000 mL	2000 mL of 100% sample	4000 mL
l00%	0 mL	2000 mL	2000 mL

6.7.2 Different volumes and number of replicates can be used as long as the minimum requirements are met.

6.11 ***For the 50% Concentration:*** Measure and pour 2000 mL of diluent and 2000 mL of 100% sample in the 4000 mL flask. Swirl to mix. Then, using the 500 mL graduated cylinder measure and dispense 500 mL of the solution into each of the replicate chambers for the 50% concentration.

6.12 ***For the 25% Concentration:*** Add 2000 mL of the diluent to the 2000 mL of 50% concentration remaining in the flask, mix and dispense 500 mL to each of the 25% concentration chambers.

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6.13 ***For the 12.5% Concentration:*** Add 2000 mL of the diluent to the 2000 mL of 25% concentration remaining in the flask, mix and dispense 500 mL to each of the 12.5% concentration chambers.

6.14 ***For the 6.25% Concentration:*** Add 2000 mL of the diluent to the 2000 mL of 12.5% concentration remaining in the flask, mix and dispense 500 mL to each of the 6.25% concentration chambers.

6.15 Perform QA standard reference toxicant (SRT) tests monthly, using potassium chloride (toxicant can be changed as needed) for the SRT.

6.7.1 An example of sample volumes for an *acute definitive* potassium chloride SRT test, using the toxicant made per SOP TA-09.01: Concentrations/volumes can be modified as needed. Diluent is synonymous with control water. "SRT" refers to 2 g/L KCl needed to achieve desired concentration. Total volume refers to volume used in test.

Concentration	Diluent	SRT	Total Volume
Control	2000 mL	0 mL	2000 mL
0.25 g/L	1500 mL	500 mL of 2 g/L KCl	2000 mL
0.5 g/L	1250 mL	750 mL of 2 g/L KCl	2000 mL
1 g/L	1000 mL	1000 mL of 2 g/L KCl	2000 mL
1.5 g/L	500 mL	1500 mL of 2 g/L KCl	2000 mL
2 g/L	0 mL	2000 mL of 2 g/L KCl	2000 mL

6.16 Pour aliquots from replicate A of each concentration into 30 mL vials held within a parameter tray. Measure pH, temperature, DO, and conductivity/salinity from the aliquots. Record all readings. Record any comments pertaining to the condition or appearance of the sample.

6.7.1 Conductivity readings can be rounded to the nearest 5 µmhos or 0.1 mmhos. Salinity will be too low in freshwater samples to measure on the meter, use the following web site to convert conductivity to salinity: Aquarium Calculators (hamzasreef.com) (in the "Unit Conversion" tab) or read the salinity directly from the Fisherbrand Traceable Bench Conductivity meter. Readings for pH, DO, and temperature shall be read to one decimal place. Take measurements from lowest to highest toxicant concentration.

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

6.17 Using a glass pipet fitted with a rubber bulb, load 20% of test organisms per replicate into each test chamber. Repeat the loading until a minimum of **five** fish (four chambers concentration) or **ten** fish (two chambers per concentration) have been loaded into each chamber. Load 2 organisms at a time for 10 organisms/replicate and 1 organism at a time for 5 organisms/replicate

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6.18 Verify the count over a light table. A person other than the loader should verify the number of test organisms in each replicate. Record the counts in the appropriate columns on the *Acute Bioassay Bench Sheet.* Feed organisms while holding at least 2 hours prior to the test by adding 0.2 mL *Artemia* nauplii. Fish in test chambers are fed again after loading and taking dilution water parameters.

6.19 Place chambers together (random order determined by rolling dice which corresponds to randomizer sheets) in incubator and cover with clear Plexiglas. Covers prevent accidental contamination and minimize evaporation.

6.20 Measure alkalinity and total ammonia of sample (per SOP TA-06.08 and TA-06.35 or TA-06.33, respectively).

7 INTERIM READINGS

7.1 On day 1 (t = 24 hr and any other interim readings if test duration is > 48 hr), count and record the number of surviving fish. Remove dead fish. Pour aliquots into the vials within the parameter tray, and measure pH, temperature, DO, conductivity and salinity, daily. Record readings.

7.7.1 All counting is done over a light table. Fish are recorded as live if they move when lightly prodded. Record counts and physical/chemical readings in appropriate columns in *Acute Bioassay Bench Sheet.*

7.7.2 Although not a requirement, the FDEP Laboratory generally takes aliquots from the replicates at the times specified below:

Number of replicate concentrations	Time	Replicate
4	0 hours	A
	24 hours	B
	48 hours	C
	72 hours	D
	96 hours	A
2	0 hours	A
	24 hours	B
	48 hours	A
	72 hours	B
	96 hours	A

If a replicate is not available (all dead in the replicate the previous day), the next sequential replicate is used.

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7.2 Return to incubator when done and cover chambers.

7.3 If test duration is > 48 hr repeat steps 7.1-7.2 section at 72 hr.

8 TESTS WITH DURATION > 48 HOURS

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

8.2 Use a large bore plastic pipette to remove any accumulated dead *Artemia* or other waste. Collect aliquots of "old" solution for parameters; then, use a screened siphon to carefully remove all but ~ 250 mL of "old" test solution. Remove as much debris as possible during this process. Be careful there aren't any test organisms stuck to the bottom of the siphon before moving to the next replicate. Count and record the number of surviving and dead organisms; remove dead organisms.

8.7.1 Counting is done over a light table. Physical/chemical readings (temperature, pH, DO, and conductivity) are taken in the appropriate replicate chamber for each concentration per comment for *Interim Readings* step 7.1.

8.7.2 Record counts in appropriate columns on *Acute Bioassay Bench Sheets*.

8.3 Use the 500 mL cylinder to carefully pour approximately 250mL of "new" test solutions into the appropriate test chambers.

8.7.1 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. Measure the same physical/chemical parameters on the "new" test solution as was measured on the original sample on Day 0. If chlorine or ammonia were not detected in the original sample, it is generally not necessary to check for these components in the sample used for test renewal.

8.7.2 The table below shows the breakdown of waters needed for renewals.

Concentration	Diluent	Sample	Total Volume
Control	1000 mL	0 mL	1000 mL
6.25%	937.5 mL	62.5 mL	1000 mL
12.5%	875 mL	125 mL	1000 mL
25%	750 mL	250 mL	1000 mL
50%	500 mL	500 mL	1000 mL
100%	0 mL	1000 mL	1000 mL

8.4 Collect aliquots of "new" solution (from test chambers) into disposable plastic cups. Measure and record DO, pH, temperature, and conductivity in each of the plastic cups 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.

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8.6 Perform Step 7.1 at 24-hr intervals until test termination.

8.7.1 Feed only 0.2 mL concentrated *Artemia* nauplii immediately after renewal.

9 TEST TERMINATION

9.1 On termination day (t = 48 hr ± 2 hr; t = 96 hr ± 4 hr; or, whatever the test duration is), count the number of surviving fish. Measure parameters of the solutions in the chambers or from aliquots poured from chambers into disposable plastic cups. Record all counts and readings. Record any comments pertaining to the appearance or condition of the fish. Record test termination date and time.

9.7.1 Test may be terminated prior to the intended duration if 100% mortality has occurred in the test concentrations AND control mortality is ≤ 10%.

9.7.2 Counting is done over a light table. Physical/chemical readings (temperature, pH, DO, and conductivity) are taken in the appropriate replicate chamber for each concentration per comment for *Interim Readings* step 7.1.

9.7.3 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 Enter test results into statistical program to obtain LC_{50}.

9.7.1 Per SOP TA-07.17 for definitive tests. 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. For example, some ocean outfalls are permitted to be tested at 30% effluent, which would be the "100%" test concentration.

9.4 Enter test results into LIMS.

9.7.1 Data is verified by a second person prior to uploading to LIMS per SOP BG-01.

9.7.2 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 Record information in appropriate logbooks (*Acute Bioassay Bench Sheets*).

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10.3 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.

10.7.1 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

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.).

10.4 DEP Laboratory Information Management System (LIMS)

10.5 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.

10.6 *Bioassay Sample Log-In* log book. Record per SOP TA-06.14.

10.7 *Organism Log-In Benchsheets* log book. Record per SOP TA-04.20.

10.8 Log sample receipt, temperature, and condition into *Acute Bioassay Bench Sheets* log book. 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, and food type, and food batch numbers. Access scanned Field Sheets via \\fldep1\dear\Labs\BizhubScans\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 2006.0 for test conditions and acceptability criteria.

11.2 Perform QA standard reference toxicant (SRT) tests monthly or concurrently.

11.3 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.

11.7.1 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

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Laboratory Quality Manual for corrective action and handling out-of-control data.

11.4 Samples should 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.

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

11.6 Incubator is maintained at the selected test temperature of 25 ± 1°C.

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

11.7.1 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.

11.7.2 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.

11.7.3 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.

11.7.4 If results do indicate toxicity in a sample, the acceptability of the test will depend on the experience and professional judgement of the laboratory analysts and the reviewing staff of the regulatory authority. If the temperature deviation from test specifications is suspected of contributing to the toxicity of a test, report as “No Result”. If after review, the temperature deviation from test specifications is not suspected of contributing to toxicity, J-qualify the result and add the comment, “Estimated value. There is added uncertainty due to deviation of test solution temperatures from method specifications.”

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 Manual for sample disposal.

12.4 Read MSDS information for reagent handling and disposal.

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13 REFERENCES

13.1 DEP Laboratory Quality Manual, DEP Laboratory Quality Assurance Manual and SOPs | Florida Department of Environmental Protection

13.2 DEP Laboratory Health and Safety Plan, https://www.floridadep.net/resources/health-safety-manual

13.3 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 2006.0.

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-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-06.08, Measuring Total Alkalinity and Hardness

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

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

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

13.13 DEP SOP TA-06.33, Measuring Total Ammonia using HACH Ammonia Nitrogen Test Kits for Freshwater and Saltwater Samples

13.14 DEP SOP TA-06.35, Measuring Total Ammonia using the HACH Ammonia Nitrogen Pocket Colorimeter II Test Kit

13.15 DEP SOP TA-06-43, Calibration and Use of Fisherbrand Accumet AB315 pH/mV Meter

13.16 DEP SOP TA-06.39, Thermo Scientific Orion Stat A213 Benchtop RDO/DO Meter Calibration and DO Measurement

13.17 DEP SOP TA-07.13, Salinity Adjustment of Samples: Toxicity Bioassay

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

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

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APPENDIX OF CHANGES

07/28/2008 Moved *Scope and Application*, *Summary of the Method* and *Interferences* from comments to body of SOP. Added *References* and *Appendix of Changes*. Removed SOP TA-06.06 from Materials # 11 comment. Inserted (refer to SOP TA-03.02) in Materials # 3 comment.

07/10/2009 Replaced “freshwater” with “marine” in *Scope and Application*. Added “if possible” to *Test Initiation* Step #8 comment. Added “temperature” and replaced “all chambers” with “in the appropriate replicate chamber for each concentration” per comment for *Interim Readings* Step 1. Updated Quality manual reference.

05/18/2010 Added ‘CAUTION Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used’ and deleted ‘Vinyl gloves can be substituted for individuals allergic to latex’ in Safety Section comments. Updated the reference to the Biology Quality Manual.

04/26/2011 Changed Biology Quality Manual reference to Bureau Quality Manual.

12/05/2013 Reformatted SOP.

02/11/2015 Formatted for 508 compliance.

01/06/2016 Updated links.

03/09/2016 Updated links and References.

03/10/2017 Updated ammonia SOP information in section 3 and the reference section

02/23/2018 Updated links.

03/05/2018 Updated SRT toxicant information

02/21/2019 Updated SOPs and references. Corrected other minor errors for grammar, punctuation, and clarity.

01/06/2020 Updated SOPs and links.

02/12/2021 Updated to reflect universal language and corrected for grammar and punctuation.

01/31/2022 Updated to reflect universal language, fixed minor grammatical errors, updated a link to function properly, added a table of contents page.

02/09/2023 Updated Links, adjusted bullets to line up properly, checked for grammar.

02/05/2024 Checked for grammar. Added how to properly euthanize fish. Updated links. Added LIMS SOP.

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

04/19/2024 Added section 9.7.1 and added the same information to 2.1.

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