# *Menidia beryllina* Acute Toxicity Testing Methods

## 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 effluents and receiving waters (marine aqueous samples) to the inland silverside, *Menidia beryllina*, using 9 (nine) to 14 (fourteen) 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 and samples are manipulated as needed. Samples and controls are placed into test vessels. Test organisms are fed and held for observation prior to loading into test vessels. Organisms are loaded into test vessels. Tests are incubated in laboratory incubators or water bath. Tests are checked at ~24-hour intervals for mortality. Mortalities are recorded daily. Phys/chem parameters are measured daily. The test endpoint is mortality of the organisms. Results are entered into the laboratory information management system (LIMS).

2.2. Acute bioassay test duration generally does not exceed 96 hours.

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. Method modification allows reduction of organisms per chamber from 10 to 5 and increase in replicates/concentration from 2 to 4.

## 3. EQUIPMENT AND SUPPLIES

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

3.1.1. *M. beryllina* test organisms are fed live *Artemia* nauplii at least 2 hours prior to test initiation. Test organisms are currently purchased. Refer to SOP TA-04.20.

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



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3.3. Natural Seawater (NSW) or Artificial Seawater (ASW)

3.3.1. 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 diluted to the original sample salinity and then adjusted with salts to the test salinity is used as a 'salt' control (in addition to the control). Salt adjustments made per SOP TA-07.13.

3.4. Bioassay laboratory grade Forty Fathoms ® Artificial Sea salts, Crystal Sea ® Marine Artificial Sea salts or hypersaline brine (HSB).

3.4.1. Used to adjust NSW or ASW to match higher sample salinities without adding salt crystals. Salinity adjustments are made using SOP TA-07.13. HSB can be made per EPA-821-R-02-012 Section 7.3.7.

3.5. Volumetric pipets, 10 mL, with Pipettor/bulb

3.5.1. One pipet per sample tested, plus one for the control.

3.6. Sample warmed to 20–25 °C

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

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

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

3.8.1. One per sample needed for uniform mixing of test concentrations.

3.9. 8" Carolina dish

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

3.10. Glass pipet fitted with rubber bulb

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

3.11. Meters to measure physical/chemical parameters:

3.11.1. pH (Calibrate per SOP TA-06.37)

3.11.2. Dissolved oxygen (DO) (Calibrate per SOP TA-06.04.)

3.11.3. Digital thermometer (Temperature) (Calibrate per SOP TA-06.13.)

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

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

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

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

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



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3.13. 3.12 Ammonia (Measure per SOP TA-06.35 or SOP TA-06.33)

3.14. Percival Scientific Incubator (Incubator # 1 or # 7)

3.14.1. Incubator should maintain selected test temperature (20 ± 1°C or 25 ± 1 °C (recommended)) and a photoperiod of 16h light: 8h dark. Light intensity should be 50-100 ft candles or ambient laboratory intensity. Test temperature must not deviate (maximum minus minimum) more than 3 °C in the test vessels for the duration of the test.

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

3.15. Cups, 30 mL plastic w/ parameter tray

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

3.16. Live *Artemia* nauplii (Prepare per SOP TA-01.03.)

## 4. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

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

4.2 See SOP TA-06.14 *Bioassay Sample Log-in Procedure* for bioassay sample log-in.

## 5. TEST INITIATION

5.1. 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.

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

5.1.2. 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.



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5.2. Calibrate meters per respective SOP and record calibration readings in the *Daily Physical Chemistry Calibration Log Book*.

5.3. Fill a 30 mL plastic cup with sample and take initial pH measurement.

5.3.1. If the pH is outside the acceptable range of 6.0–9.0 SU, then an additional test with a pH-adjusted aliquot will be run. The test with the original sample is then analyzed as a screening test, regardless of whether the pH-adjusted aliquot is used for a screening or definitive test. Refer to SOP TA-08.06 for pH adjustment.

5.4. In the same plastic cup containing the sample, take an initial DO measurement.

5.4.1. If the DO is ≤ 4.0 mg/L or ≥ 9.0 mg/L, aerate the entire sample to as near saturation as practical prior to test initiation. Samples with a potential DO problem generally show a downward trend in DO during the first few hours of the test. Monitor the DO closely. If the DO begins to fall, set up gentle aeration (< 100 bubbles/min) to ALL test solutions for the duration of test using micropipettes and the aeration manifold.

5.5. In the same plastic cup containing the sample, measure the conductivity and temperature of the sample. Check the conversion table to determine salinity.

5.5.1. If the conductivity is below 2,000 µmhos/cm or 1 ppth, then *Menidia* is likely not the appropriate test organism for this effluent sample. Refer to the facility's NPDES Permit for test requirements.

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

5.5.2. Salinity (based on conductivity and temperature) can be determined from online sources.

5.6. If the salinity is between 1 and 6 ppth and ***no*** ion imbalance is suspected or over 6 ppth, no salinity adjustment is necessary. If the salinity is between 1 and 6 ppth and an ion imbalance ***is*** suspected, then artificial sea salt should be added to increase the apparent salinity by 1 ppth.

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

5.7. If the salinity is between 6 and 20 ppth, then add well water to the Carolina dish holding the fish to begin their acclimation. Fish are tested at the original salinity of sample.

5.7.1. The *Menidia* are cultured and held in 20 ppth. Decrease the salinity in the holding dish toward the sample salinity by adding well water. Increase the salinity by adding hypersaline brine or a higher salinity saltwater. 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



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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.

5.8. If the salinity is above 20 ppth, then add hypersaline brine to the Carolina dish holding the fish to begin their acclimation.

5.8.1. Increase the salinity in holding dish by adding hypersaline brine to acclimate. ASW or NSW having greater salinity than the holding dish can also be used to increase salinity of water in the holding dish.

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

5.9.1. Measure TRC per SOP TA-06.22. If TRC > 0.1 mg/L and non-chlorine toxicity is to be measured, dechlorinate a portion of the sample by the sodium thiosulfate method per SOP TA-09.18 and test dechlorinated replicates of all chosen test concentrations concurrently with additional chlorinated (100% sample ONLY) replicates.

5.10. ***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.

5.10.1. 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 |

5.11. ***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.

5.11.1. 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 |



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| Concentration | Diluent | Sample | Total Volume |
|---|---|---|---|
| 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 |

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

5.12. ***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.

5.13. ***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.

5.14. ***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.

5.15. ***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.

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

5.16.1. 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 500 µg/L copper solution for the < 500 µg/L concentrations. 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 |

5.17. 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.



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5.17.1. Conductivity readings can be rounded to the nearest 5 µmhos or 0.1 mmhos. Check the conversion table to determine salinity 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 concentration.

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

5.18. 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

5.19. 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 jars are fed again after loading and taking dilution water parameters.

5.20. Place chambers together (random order) in incubator and cover with clear Plexiglas. Covers prevent accidental contamination and minimize evaporation

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

## 6. INTERIM READINGS

6.1. 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.

6.1.1. 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.*

6.1.2. 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 |



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| | | |
|---|---|---|
| | 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.

6.2. Return to incubator when done and cover chambers.

6.3. If test duration is > 48 hr, repeat steps6.1-6.2 section at 72 hr.

## 7. TESTS WITH DURATION > 48 HOURS

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

7.2. 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 the number of surviving and dead organisms; remove dead organisms.

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

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

7.3.1. 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. 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.

7.3.2. 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.

7.4. 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.



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7.5. 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.

7.6. Perform Step 6.1 at 24 hr intervals until test termination.

7.6.1. Feed only 0.1 mL concentrated *Artemia nauplii* immediately after renewal. Resume regular feeding at 72 hours.

## 8. TEST TERMINATION

8.1. On termination day (t = 48 hr ±~ 2 hr or 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 parameter vials. Record all counts and readings. Record any comments pertaining to the appearance or condition of the fish. Record test termination date and time.

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

8.1.2. 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 5.1.

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

8.2. Pour contents of all chambers into the sink and flush drain with water. Place glassware to be washed on appropriate cart.

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

8.3.1. 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.

8.4. Enter test results into LIMS.

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

## 9. DATA ARCHIVAL

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



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9.2. Record information in appropriate logbooks (*Acute Bioassay Bench Sheet* and *Daily Physical Chemistry Calibration Log Book*).

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

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

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

9.3.3. 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.).

9.4. DEP Laboratory Information Management System (LIMS)

9.5. 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.

## 10. QUALITY CONTROL

10.1. Refer to DEP Laboratory Quality Manual for corrective action and handling out-of-control data.

10.2. 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. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

11.2. Refer to the DEP Laboratory Health and Safety Plan.

11.3. Refer to the DEP Laboratory Quality Manual for sample disposal.

11.4. Read MSDS information for reagent handling and disposal.



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## 12. REFERENCES

12.1. DEP Laboratory Quality Manual, http://publicfiles.dep.state.fl.us/dear/labs/lab_qualitymanual_18.pdf

12.2. DEP Laboratory Health and Safety Plan, http://publicfiles.dep.state.fl.us/DEAR/DEAR%20Safety/FDEP%20Health%20%20Safety%20Plan%20B%20-%20June%202016.pdf

12.3. 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.

12.4. DEP SOP FS 1000, *General Sampling*

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

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

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

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

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

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

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

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

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

12.14. DEP SOP TA-06.35, *Measuring Total Ammonia using the HACH Ammonia Nitrogen Pocket Colorimeter II Test Kit*DEP SOP TA-06.37, *Calibration and Use of Fisherbrand FE150 pH Benchtop Meter*

12.15. DEP SOP TA-06.39, *Thermo Scientific Orion Stat A213 Benchtop RDO/DO Meter Calibration and DO Measurment*

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

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

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

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

12.20. 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.



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