Salinity Adjustment of Samples: Toxicity Bioassay

1. SCOPE AND APPLICATION

1.1. This method is based on EPA-821-R-02-012 *Method for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. It is used to adjust the salinity of bioassay diluents and test solutions as needed to meet salinity requirements of test methods and NPDES permits. Tests conducted by this procedure are BSY-AS-MMY, BSY-AD-MMY, BSY-CS-MMY, BSY-CD-MMY, BSY-AS-MFS, BSY-AD-MFS, BSY-CS-MFS, and BSY-CD-MFS.

2. SUMMARY OF THE METHOD

2.1. Sample salinity is measured. Required salinity is found in the permit summary. Artificial sea salts are added to the test solution(s) to bring the sample to the required test salinity.

2.2. When a salinity adjustment is necessary for a test solution, a salinity-adjusted control must be tested along with the salinity-adjusted test solution and the regular control, to check for any toxicity due to the addition of artificial salts. The salinity-adjusted control is adjusted using the same steps as for the bioassay test solution salinity. The salinity-adjusted control can be artificial seawater (ASW) diluted to the salinity of the original test solution then salinity adjusted in the same manner as the sample. If the salinity of a freshwater whole effluent is being adjusted, well water can be used to prepare the salinity-adjusted control. ASW can be used for the salinity adjustment of test solutions that are tested at less than 100% concentration.

3. EQUIPMENT AND SUPPLIES

3.1. Magnetic stirrer

3.2. Magnetic stir bar, 50mm, 1 per bioassay sample

3.3. Magnetic stir bar retriever

3.4. 4 L Erlenmeyer flask, 1 per screening bioassay sample

3.5. 6 L Erlenmeyer flask, 1 per definitive bioassay sample

3.6. Squeeze bottle with DI water (used to rinse probes)

3.7. 200 mL rinse water cup

3.8. Waste container

3.9. Thermo Scientific Orion Star A213 Benchtop RDO/DO Meter – Calibrate and use per SOP TA-06.39.

3.10. Conductivity to salinity conversion tables

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3.11. Fisherbrand Traceable Bench Conductivity Meter – Calibrate and use per SOP TA-06.36

3.12. Latex or non-latex gloves

3.13. Computer with access to LIMS

3.14. *Bioassay Sample Log-in* book

4. REAGENTS AND STANDARDS

4.1. Bioassay laboratory grade Forty Fathoms ® Artificial Sea salts, Crystal Sea ® Marine Artificial Sea salts or similar brand

4.2. Deionized water

4.3. Bioassay sample(s) (also called 'test solution')

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

6. METHODS

6.1. Refer to the *Bioassay Sample Log-in* book to determine which test solutions may need to have their salinity adjusted. Refer to NPDES permits and/or consult with district personnel for required test salinity.

- If the test solution salinity is less than 1 ppth, freshwater test species should be used unless NPDES permit requirements or requested tests dictate otherwise.
- If the test solution salinity is between 1 and 6 ppth and there are no permit specifications, use artificial sea salts to increase the salinity to 6 ppth for the mysid test. No adjustment is necessary for the *Menidia* test.
- If the test solution salinity is greater than 6 ppth, no salinity adjustments are necessary unless specified in the facility's NPDES permit, the EPA test method, or requested by District personnel.

6.2. Determine test solution salinity: **A**) measure the salinity directly with the Fisherbrand Traceable Bench Conductivity Meter; or **B**) measure the test solution temperature and conductivity and calculate the conductivity needed to obtain the desired salinity. Use the conversion tables to calculate the salinity.

Note: This site: https://www.hamzasreef.com/Contents/Calculators/SalinityConversion.php can be used for salinity conversions from conductivity and temperature measurements.

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6.3. Pour 2200 mL of sample into the 4 L Erlenmeyer flask for a screening bioassay. Pour 4000 mL of sample into the 6 L Erlenmeyer flask for a definitive (0.5 dilution) bioassay.

6.3.1. Place the 50 mm stir bar in the flask and gently stir the test solution.

6.3.2. Suspend the conductivity meter probe (pre-rinsed with DI water) in the flask ensuring that the tip of the probe is in the test solution.

6.3.3. Place a small amount of Crystal Sea Marine Mix ® bioassay grade artificial sea salts into the flask. Allow the salt to mix/dissolve in the test solution.

6.3.3.1. The amount of salt that needs to be added can be calculated by subtracting the calculated salinity from the desired salinity and multiplying the difference by the test solution volume in liters. Weigh this amount of sea salt (in grams) and add the salt to the appropriate volume of the test solution. Allow the salt to dissolve. Make minor adjustmentsin accordance with steps 6.4.3, 6.5, and 6.6.

6.3.3.2. Conversion note: 1 g of artificial sea salt ≈ 1 ppth/L.

6.3.3.2.1. **Example**: Most permits require mysid tests be run at 20 ppth and *Menidia* tests run at 5 ppth if the sample is <5 ppth. If a sample is 3 ppth, two different sample mixtures are made.

1) Mysid: (20 ppth – 3 ppth)(4 L)= 68 g of sea salt required.

2) *Menidia:* (5 ppth – 3 ppth)(4 L)= 8 g of sea salt required.

6.4. Measure salinity per Step 6.2 (A). If the adjusted salinity is more than 0.5 ppth greater than the desired salinity, add unsalted sample to decrease the salinity to the desired range (± 0.5 ppth of desired salinity). To increase salinity, repeat step 6.4.3 for sea salts or step 6.3 for HSB until the salinity of the test solution is in the appropriate range.

6.5. Continue the test setup per the appropriate SOP.

7. DATA ARCHIVAL

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

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

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

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

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Do not attempt to put all information (correction and comments) into a small space.

7.2.1.3. 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.).

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

7.3.1. Log sample receipt, temperature, and condition into Acute Bioassay Bench Sheets logbook. Fill in information requested at top of bench sheets as completely as possible prior to test initiation. Include full name of facility, address, contact person, date and time collected, field TRC, LIMS sample and job numbers, test chamber ID, test chamber size, test solution volume, food type, and food batch numbers. Access scanned Field Sheets via \\Labs\Receiving to obtain sampling information.

8. QUALITY CONTROL

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

8.2. For NPDES compliance testing, do not use hypersaline brine (HSB) to adjust the salinity of test solutions or diluents because using HSB precludes the testing of 100% test solution. Use artificial sea salts unless otherwise stipulated (in NPDES permit).

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

8.4. To create an NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to DEP SOPs | Florida Department of Environmental Protection for sample collection and preservation, and the Laboratory Quality Manual for corrective action and handling out-of-control data.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

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

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

9.4. Read MSDS information for reagent handling and disposal.

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10. REFERENCES

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

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

10.3. 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 2002.0.*

10.4. DEP SOP FS 1000, *General Sampling*

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

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

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

Appendix of Significant Changes:

05/14/2008	Added Scope and Application, Summary of the Method and Appendix of Changes.
03/24/2009	Added References section.
02/13/2015	Formatted for 508 compliance.

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