Ceriodaphnia dubia Acute Toxicity Testing Method

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Ceriodaphnia dubia Acute Toxicity Testing Method

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 821-R-02-012 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition, Test Method 2002.0. It is used to estimate the acute toxicity of freshwater effluents and receiving waters on *Ceriodaphnia dubia* (water fleas), using < 24-hour old neonates in either a 48-hour static exposure or a 96-hour static renewal exposure test. Tests conducted by this procedure are BSY-AS-FCD and BSY-AD-FCD.

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 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 waters are placed into test chambers. Test organisms are collected, fed and held for observation prior to loading into test vessels. 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 survival of the organisms. Statistical endpoints are obtained from the EPA WET Statistics Excel spreadsheet per SOP TA-07.17. Results are verified and entered in the laboratory information management system (LIMS).

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

2.3. Interferences:

2.3.1. 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; pH <6.0 SU or >9.0 SU; salinity > 1 part per thousand; ammonia, chlorine, debris, indigenous organisms that may attack or be confused with the test organisms.

3. EQUIPMENT AND SUPPLIES

3.1. *C. dubia* neonates (< 24 hr. old)

3.1.1. Obtain neonates per SOP TA-05.02 and feed YCT and *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) (1:1) at least 2 hours prior to test initiation per SOP TA-02.00.

3.2. 30 mL plastic test cups,

3.2.1. Five cups per concentration plus five cups for controls are needed for each sample tested. Cups are rinsed with DI water prior to use. Four cups are used for both effluent and receiving water samples to contain the test

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organisms for each test concentration; the fifth cup contains the surrogate for parameters for each concentration.

3.2.2. Minimum cup size is 30 mL. Minimum sample volume is 15 mL. The culture cups are filled to 20 mL.

3.3. Plexiglass tray to hold cups.

3.4. Moderately Hard Water (MHWCD)

3.4.1. Prepare in advance per SOP TA-03.15.

3.5. 100- and 250-mL Glass cylinders, to pour up test concentrations.

3.6. Sample to be tested

3.6.1. For a valid test: a) 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 collection period for a composite sample) to test initiation. See method manual for exceptions to the above requirement.

3.7. 500 mL Flask, for mixing test concentrations.

3.8. Plastic transfer pipet, large bore

3.8.1. Used to transfer animals from neonate cups to test cups.

3.9. Meters to measure physical-chemical parameters:

3.9.1. pH (Calibrate per SOP TA-06.43)

3.9.2. Dissolved Oxygen (DO) (Calibrate per SOP TA-06.39)

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

3.9.4. Conductivity (Calibrate per SOP TA-06.36)

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

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

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

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

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

3.11. Thermo-Scientific incubator (#1, #4, or #5) with temperature and lighting control.

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

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3.12. Computer with access to LIMS and the EPA WET Excel spreadsheet for statistical analysis

4. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

4.2. 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.

4.2.1. The remainder of the sample is stored in the Biology Walk-in cooler for solution renewal.

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

5. TEST INITIATION

5.1. The day before the test, place new 30-mL test cups in Plexiglas tray. Label each column of trays to identify the test concentration and facility sampled. Fill each cup with deionized (DI) water.

5.1.1. Place tape across front of tray and use waterproof marker to designate columns. Five cups for each set of control replicates are needed for each sample tested, plus five cups per concentration for each sample tested. DI water is usually placed in cups to detect leaks and to rinse the cups. Cups can be rinsed three times with DI water just prior to use rather than soaking in DI water overnight.

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

5.2.1. Measure TRC per SOP TA-06.22. If TRC ≥0.1 mg/L, and non-chlorine toxicity is to be measured, dechlorinate a subsample (volume needed to initiate the test) of the sample using sodium thiosulfate per SOP TA.9.18. Setup 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.

5.3. Measure the alkalinity, hardness, and total ammonia of the sample.

5.3.1. Measurements are made per SOP TA-06.08 and TA-06.33, respectively.

5.4. Calibrate meters per respective SOP. Record calibration readings in the *LIMS logbook.* Check meters and record values at the end of day.

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

5.5.1. If pH is outside the acceptable range of 6.0-9.0 SU, then an additional test with a pH-adjusted subsample 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.

5.6. In the same sample cup, take an initial D.O. measurement per the SOP. Record value in Bioassay Sample Log-in logbook and LIMS TOX_DO logbook.

5.6.1. If the D.O. is ≤ 4.0 mg/L or ≥ 9.0 mg/L, aerate the 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 the DO begins to fall, set up gentle aeration (<100 bubbles per minute) 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.

5.7. In the same sample cup with the sample, take an initial conductivity measurement. Record value in the Bioassay Sample Log-in Logbook and LIMS TOX_COND_SAL. Measurements can be rounded to nearest 5 µmhos or 0.1 mmhos.

5.7.1. If the conductivity is above 2,000 µmhos/cm, then discern whether the reading is a result of salinity or high mineral content (i.e., what is the source of the sample?). If the reading results from salinity, then *C. dubia* is not the appropriate test organism for this effluent sample.

5.7.2. This rationale does **NOT** apply to the SRT test.

5.8. ***For Screening Tests:*** Empty DI water from the pre-rinsed cups prior to filling with MHWCD and 100% sample. Measure 20 mL of MHWCD into each of the control replicate cups. Measure 20 mL aliquots of the 100% sample into each of the five 100% sample concentration cups.

5.8.1. Include five cups for each set of control and test replicates are needed for each sample tested. The following table shows suggested sample volumes for each cup of the acute screening toxicity test. Diluent is synonymous with the control water.

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

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5.9. ***For Definitive tests (0.5 dilution factor):*** Empty DI water from the soaking cups prior to filling with MHWCD and sample test concentrations. Measure 20 mL of MHWCD into each of the five control replicate cups. Measure 20 mL aliquots of the 100% sample into each of the five 100% sample concentration cups.

5.9.1. Include five cups per concentration in a dilution series for each sample tested. We generally use a 0.5 dilution for definitive tests. The following table shows sample volumes used when mixing a solution for an acute definitive toxicity test.

Concentration	Diluent	Sample	Total Volume
Control	100 mL	0 mL	100 mL
6.25 %	100 mL	100 mL of 12.5% sample	200 mL
12.5%	100 mL	100 mL of 25% sample	200 mL
25 %	100 mL	100 mL of 50% sample	200 mL
50%	100 mL	100 mL of 100%sample	200 mL
l00%	0 mL	100 mL	100 mL

5.10. ***For the 50% concentration*****:** Measure and pour 100 mL of diluent (MHWCD) and 100 mL of 100% sample in the 500-mL flask and swirl to mix. Then dispense 20 mL of the solution into each of the five cups labeled for the 50% concentration.

5.11. ***For the 25% concentration*****:** Add 100 mL of the diluent to the 50% concentration remaining in the flask (should be 100 mL) mix and dispense 20 mL to each of the five 25% concentration cups.

5.12. ***For the 12.5% concentration*****:** Add 100 mL of the diluent to the 25% concentration remaining in the flask (should be 100 mL) mix and dispense 20 mL to each of the five 12.5% concentration cups.

5.13. ***For the 6.25% concentration*****:** Add 100 mL of the diluent to the 12.5% concentration remaining in the flask (should be 100 mL) mix and dispense 20 mL to each of the five 6.25% concentration cups.

5.14. Perform QA standard reference toxicant (SRT) tests monthly (or concurrent with bioassay if bioassays performed less frequently; a concurrent SRT with one bioassay can be used as the SRT for a series of tests as long as the time interval does not exceed 30 days prior to the start of a bioassay test). Standard reference toxicant (SRT) tests will be performed monthly since the *C. dubia* are cultured in-house. Potassium chloride is used for the toxicant (prepared per SOP TA-09.01). Toxicant can be changed as needed.

5.15. Measure and mix the listed volumes for each concentration. Dispense 20 mL of each concentration to each of the appropriate cups (total of 5 cups per conc.). Discard the excess. Test concentrations can be checked by measuring the conductivity of the prepared concentrations. Total volumes are greater than the actual amount needed for each concentration in case of spillage.

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Example of concentrations for KCl SRT.

Concentration	Diluent	SRT solution 1 g/L KCl	Total Volume
Control	100 mL	0 mL	100 mL
0.3 g/L	70 mL	30 mL of 1 g/L sample	100 mL
0.4 g/L	60 mL	40 mL of 1 g/L sample	100 mL
0.5 g/L	50 mL	50 mL of 1 g/L sample	100 mL
0.6 g/L	40 mL	60 mL of 1 g/L sample	100 mL
0.7 g/L	30 mL	70 mL of 1 g/L sample	100 mL

5.16. Place one cup from each set of the 5 replicate cups (containing test solutions) onto a second tray from which physical parameters will be measured.

5.16.1. A separate tray of cups prevents buffers and standards from accidentally being splashed into the test cups.

6. TEST LOADING, COUNTING, AND RECORDING

6.1. Feed test organisms at least 2 hours prior to loading.

6.2. After test concentrations are poured, measure the pH, temperature, DO, and conductivity of the solutions in the "parameter" cups on the second tray. Record values in the appropriate bench sheet. Record any comments pertaining to the condition or appearance of the sample. Empty the parameter cups into the sink and flush the drain with water. Fill with water and store in the incubator for interim readings.

6.3. With a Pasteur pipet (or clean plastic transfer-pipet), load one *C. dubia* neonate into each of the four test cups of the controls and each test concentration (load at a rate of 20%. 1 neonate per round). Repeat loading the organisms one per cup until a total of five organisms are loaded into each cup.

6.3.1. (5 *C. dubia* per replicate) x (4 replicates per test concentration) = 20 *C. dubia* per concentration tested.

6.4. An analyst other than the analyst who originally loaded the test organisms should verify that five organisms are in each cup. The verification analyst should add or remove organisms as necessary to meet the five organisms per cup requirement. Place the tray of cups into the incubator and cover the tray with clear Plexiglas. Record counts. Record date and time of test initiation.

6.4.1. Loading and verification can be done at the dissecting scope if desired; otherwise it can be done at a light table.

6.4.2. Record counts in appropriate columns on *Acute Bioassay Bench Sheet.*

6.4.3. The time and date of test initiation (organisms loaded) is recorded on the test bench sheet.

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6.5. Cover with plexiglass and store tray in appropriate incubator.

7. INTERIM READINGS

7.1. On test day 1 [t = 24 hr. and any other interim readings (~24-hour intervals) if test duration is >48 hr.], count number of surviving organisms. . Record counts in appropriate logbook.

7.1.1. Record counts in appropriate columns on acute bioassay bench sheets in laboratory notebook.

7.2. Measure the pH, temperature, DO, and conductivity of the solutions in the "parameter" cups on second tray. Record values in the appropriate bench sheet. DO NOT TAKE ANY METER READINGS IN CUPS CONTAINING ORGANISMS ON DAY 1 (t = 24 hr., or any other interim time) unless mortality in a cup = 100%. Replicate A is used for parameters on 48-hour before renewal and 96-hour. The meter cup (labeled M) is used for parameters at all other times.

7.3. **FOR CUPS WITH 100% MORTALITY ONLY,** measure the physical-chemical parameters and record in the appropriate spaces on the *Acute Bioassay Bench Sheet*.

7.4. Replace cover and place tray in incubator

8. TESTS WITH DURATION >48 HOURS

8.1. On test day 2 (t = 48 hr.), feed C. dubia 2 hours prior to renewal. Count and record number of surviving animals.

8.1.1. Record counts in appropriate columns on acute bioassay bench sheets in laboratory notebook. **DO NOT TAKE ANY METER READINGS IN THESE CUPS** prior to organism transfer.

8.2. Prepare test cups and "new" test solutions following steps found under "Test Initiation." Measure and record DO, pH, temperature, and conductivity in the "parameter" cups containing "new" test solutions.

8.2.1. 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 phys-chem 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 as well as alkalinity and hardness in the sample used for test renewal.

8.2.2. 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.

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8.3. A minimum of 2 hours after feeding, use a transfer pipet to carefully transfer surviving test organisms (**adults only**-in the rare event that neonates produced by the original organisms are present at 48 hours) from cups containing "old" test solution to corresponding cups containing "new" test solution.

8.3.1. Record any additional mortality in the appropriate spaces on the bioassay bench sheets. Keep the transfer of "old" test solution to a minimum. Release test organisms under the surface of the test solution.

8.4. Measure and record DO, pH, temperature, and conductivity in cup (A) containing "old" solution (after organisms have been removed).

8.5. Perform steps in section 7, “Interim Readings” at ~24 hr. intervals until test termination.

9. TEST TERMINATION

9.1. On termination day, (t = 48 hr. (±2 hr.) or t = 96 hr. (±4 hr.)) count number of surviving organisms (**adults only** as it is not uncommon for the original test organisms to produce neonates by 72-96 hours). Record counts. Record any comments pertaining to the condition of the test organisms. **After counting is completed**, measure pH, temperature, DO, and conductivity of the solutions in the cups. Record test termination date and time.

9.1.1. Measurements for pH, temperature, DO, and conductivity are to be made (cup A) at test termination. Organisms can be present in the cups when final parameters are measured.

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

9.2. Pour contents of all test cups into sink and flush drain with water. Dispose of cups in trashcan and store empty tray.

9.2.1. Refer to the FDEP Laboratory Quality Manual for waste disposal procedures.

9.3. Enter test results into statistical program to obtain LC_{50}, per SOP TA-07. 17 for definitive tests.

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

9.4. Enter test results into LIMS.

9.4.1. Results are entered into LIMS by one person and verified by a second person per SOP BG-01.

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10. DATA ARCHIVAL

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

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

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

10.2.2. If the correction requires an explanation, write a comment and reference the error with a letter. Initial and date the explanation. Do not attempt to put all information (correction and comments) into a small space.

10.2.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

10.3. Record information in appropriate logbooks (*Acute Bioassay Bench Sheet* and LIMS calibration logbooks).

10.4. FDEP Laboratory Information Management System (LIMS)

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

10.5.1. 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.

10.6. Enter the LC50, NOEC, and LOEC 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 the FDEP Laboratory Quality Manual for corrective action and handling out-of-control data. Refer to EPA 821-R-02-013 Test Method 1002.0 for test conditions and acceptability criteria.

11.2. Perform QA Standard Reference Toxicant (SRT) tests monthly or concurrently if bioassays are performed less frequently. The SRT must be done 30 days prior to the start of a facility bioassay. FDEP Laboratory uses Potassium chloride as the standard toxicant. Other toxicants can be used. Concentrations and toxicants can be modified as needed. Diluent is synonymous with control water.

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

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

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

11.4.2. Incubator light intensity maintained at ~50-100 ft-candles for a photoperiod of 16-hour light:8-hour dark.

11.5. 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. 4) A non-conformance report (NCR) is submitted describing the problem and the corrective action.

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

12. SAFETY/HAZARDOUS WASTE MANAGEMENT

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

12.2. Refer to the FDEP Laboratory Health and Safety Plan.

12.3. Refer to the FDEP Laboratory Quality Manual for sample disposal.

12.4. Read MSDS information for reagent handling and disposal.

13. REFERENCES

13.1. DEP Laboratory Quality Manual

13.2. DEP Laboratory Health and 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 2002.0.

13.4. DEP SOP FS 1000, *General Sampling*

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

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

13.7. DEP SOP TA-03.15, *Preparation of Synthetic Moderately Hard Freshwater*

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13.8. DEP SOP TA-05.02, *Production of Ceriodaphnia dubia Young for Chronic and Acute Toxicity Testing*

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

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

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

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

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

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-06.36, *Calibration and Use of the Fisherbrand Traceable Bench Conductivity Meter for Conductivity and Salinity*

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

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

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

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

APPENDIX OF CHANGES

07/25/2008 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 # 9 comment.

07/10/2009 Deleted (Based on *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edition, EPA-821-R-21-012 Test Method 2002.0) from title. Updated Quality manual reference.

05/17/2010 Added 'CAUTION Individuals allergic to latex should not use latex gloves. A suitable substitute should be found and used. Deleted 'Vinyl gloves can be substituted for individuals allergic to latex in Safety Section comments. Updated the reference to the Biology Quality Manual. Revised "example of SRT concentrations" table in comment for Methods Step 14. Deleted '3ppt' from Step 14.

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

01/25/2014 Reformatted SOP.

02/11/2015 Formatted for 508 compliance.

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01/06/2016 Updated links.

03/09/2016 Updated SOPs and references.

08/16/2016 Corrected SRT volumes for 1.25 ppth.

08/16/2017 Changed SRT from NaCl and KCL. Added SOP for HACH kit. Changed vial(s) to cup(s).

07/18/2018 Updated links. Changed incubator numbers. Added information for secondary verification when loading organisms.

07/12/2019 Updated SOP's and references.

08/19/2020 Updated various sections according to new universal language. Additions made to Scope and Application, Summary of Method, Sample Collection, Preservation and Handling, Data Archival, and Quality Control sections. Updated language in Test Initiation section.

07/27/2021 Updated for universal language and grammar clarity. Added a table of contents due to length of SOP.

08/01/2022 Updated links, updated reference SOPs, and checked grammar.

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