Ceriodaphnia dubia Chronic Toxicity Testing Method

DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF LABORATORIES BIOLOGY SECTION
TALLAHASSEE FLORIDA

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Ceriodaphnia dubia Chronic Toxicity Testing Method

1. SCOPE AND APPLICATION

1.1. This method is based on EPA 821-R-02-013 *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, 4th Edition, Test Method 1002.0. It describes the procedure used to estimate the chronic toxicity of whole effluent and receiving waters (freshwater aqueous samples) on <24-hour old (and ≤ 8-hour range in age) *Ceriodaphnia dubia* (water flea) neonates during a 7-day static renewal exposure. Tests covered by this procedure are BSY-CS-FCD and BSY-CD-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 60 µm filter to remove debris and/or indigenous organisms. Sample dilutions and control water are placed into test chambers. Test organisms are collected, fed, and held for observation prior to loading into test chambers. Organisms are then loaded into test chambers. Tests are incubated in laboratory incubators. While tests are in progress, survival/mortality, reproduction, and phys-chem parameters are measured and recorded daily before and after renewal. Tests are checked at ~24-hour intervals. The test endpoints are survival and reproduction. Results are entered into the Laboratory Information Management System (LIMS). If the reported result is less than the top concentration tested (i.e. <100%), add the comment, “Upper and lower confidence intervals are, ##.#% and ##.#%, respectively.” when entering into LIMS.

2.2. Test acceptability criteria: 80% or greater survival in the controls, 60% or more of surviving control females must have produced three broods, and the average number of young per surviving female must be 15 or greater. Test duration is until a minimum of 60% of surviving females have three broods. The maximum test duration is 8 days for these criteria to be met. This presumes all criteria for a valid test are met.

2.3. Adjust sample as needed to meet permit requirements. Adjust pH (TA-08.06) and dissolved oxygen concentration; dechlorinate (TA-09.18), etc.

2.4. Interferences: Refer to EPA 821-R-02-013 Section 12.3. Includes toxic substances introduced by contaminants in dilution water or on hands, glassware, sample hardware, and testing equipment. Dissolved oxygen <4.0 mg/L or >9.0 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.

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2.5. Definitions:

2.5.1. Neonates: *C. dubia* less than 24-hours old

2.5.2. Diluent: Control water

2.5.3. Sample 1: Original (1st) sample taken from the facility; used for initial test set-up (0-hr and 24-hr)

2.5.4. Sample 2: Sample collected 48 hours after the original sample; used for renewals (48-hr and 72-hr after test initiation)

2.5.5. Sample 3: Sample collected 96 hours after the original sample; used for renewals (96-hr, 120-hr, 144-hr and 168-hr (if needed) after test initiation)

3. EQUIPMENT AND SUPPLIES

3.1. 30 mL disposable plastic cups filled with deionized (DI) water and soaked for a minimum of 12 hours

3.1.1. (Ten replicate cups) + (one parameter cup) =11 cups per test concentration.

3.2. 60-place Plexiglass tray to hold test cups and one smaller tray to hold parameter cups. One tray per sample/facility tested.

3.3. 250 mL Autoclaved, graduated cylinder for making test dilutions

3.4. 1000 mL Autoclaved, Erlenmeyer flask for mixing test dilutions

3.5. 6000 mL Autoclaved, Erlenmeyer flask to hold effluent sample

3.6. 60 µm mesh to filter sample

3.7. 1 mL and 10 mL Serological pipettes, graduated

3.8. Pipette pump for serological pipettes

3.9. Plastic transfer pipette

3.10. Magnetic stir bars and stirrer

3.11. 0.2 mL Calibrated pipettor

3.12. Pipette tips

3.13. YCT and algal suspension combined 1:1 for daily feeding (YCT prepared per SOP TA-01.01; algal suspension of R. subcapitata prepared per SOP TA-01.04.)

3.14. Magnifying glass

3.15. Random loading sheet and a die

3.16. Effluent samples to be tested (recommended to be warmed to 25 ± 1°C)

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3.17. Meters to measure:

3.17.1. pH calibrated per SOP TA 06.43

3.17.2. Dissolved oxygen (DO) calibrated per SOP TA-06.44

3.17.3. Temperature using digital thermometer calibrated per SOP TA-06.13

3.17.4. Conductivity/salinity calibrated per SOP TA-06.36

3.18. Hach® kits to measure:

3.18.1. Total residual chlorine (TRC) per SOP TA-06.22

3.18.2. Alkalinity per SOP TA-06.08

3.18.3. Hardness per SOP TA-06.08

3.18.4. Ammonia per SOP TA-06.35

3.19. *C. dubia* neonates meeting age requirements, obtained per SOP TA-05.02

3.20. Thermo Scientific Incubator (Incubator #1 or #5)

3.20.1. Incubator should maintain test temperature at 25 ± 1°C and a photoperiod of 16 hours light and 8 hours dark. Light intensity should be ~50-100 ft. candles. Sample temperatures in cups must not deviate more than 3°C for the duration of the test.

3.21. Computer with access to LIMS

3.22. *Bioassay Sample Log-in* logbook

3.23. *Chronic Bioassay Bench Sheets for Ceriodaphnia dubia* logbook

4. REAGENTS AND STANDARDS

4.1. Moderately Hard Freshwater (MHWCD), prepared per SOP TA-03.15.

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

5.1.1. Sample criteria for use in a test: a) The sample should be held at 0-6°C prior to test initiation or, if hand delivered the day of collection, be cooling; b) Sample holding time should not exceed 36-hr 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.

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

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

5.1.2.2. Any variation in sample collection (from above) is not necessarily cause for test invalidation, but the DEP Toxicity Coordinator should always be notified/consulted for approval/acceptability.

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

6. TEST INITIATION

6.1. Using tape and a waterproof marker, label the Plexiglass tray containing soaked 30mL test cups with test concentration and facility samples.

6.2. Calibrate meters and record readings in appropriate LIMS logbook daily during test. Check meters at end of day and record values.

6.3. Filter sample through 60 µm mesh into 6000 mL Erlenmeyer flask.

6.4. Fill a 30 mL disposable plastic cup with 20 mL effluent sample and take an initial pH measurement per SOP TA-06.43. Record value in *Bioassay Sample Log-in* logbook and LIMS TOX_PH logbook.

6.4.1. If pH is outside acceptable range of 6.0–9.0, then an additional test with a pH-adjusted aliquot will be run. Adjust pH of sample per SOP TA-08.06. Record pH adjustment and/or measurement in the appropriate bench sheet. The test with the non-adjusted sample is then analyzed as a screening test, regardless of whether the pH adjusted aliquot is used for a screening or definitive test.

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

6.5.1. 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 the test using micropipettes and the aeration manifold. No action is required if DO increases after test initiation. In same sample cup, measure the conductivity and temperature of the sample.

6.6. Measure total residual chlorine (TRC) in sample per SOP TA-06.22. Record value in the LIMS TOX_CHLORINE logbook and on the appropriate bioassay bench sheets.

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

6.6.1. 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 using sodium thiosulfate per SOP TA.9.18. Set up a concurrent screening test using the 100% sample that has not been dechlorinated and a control. Record chlorine result, amount of sodium thiosulfate used, and final chlorine result after dechlorination in the logbook and in the designated section in LIMS.

6.7. Measure alkalinity, hardness, and total ammonia of sample per SOP TA-06.08 and TA-06.35. Record in the appropriate LIMS logbook.

6.8. Preferred method for Definitive tests:

6.8.1. Warm sample to 25 ± 1ºC.

6.8.2. Empty DI water from soaking cups prior to filling with diluent (MHWCD) and sample test concentrations.

6.8.3. Using a graduated cylinder, pour 20 ± 1 mL of diluent into each of the eleven control replicate cups, including the parameter cup.

6.8.4. Pour 20 ± 1 mL aliquots of the 100% sample into each of the eleven 100% sample concentration cups, including the parameter cup.

6.8.5. Measure the desired volumes of diluent and sample into a 1000 mL Erlenmeyer flask using two graduated cylinders. Swirl flask by hand to mix until homogenized. Measure 250 mL of the mixture back into a 250 mL cylinder. Transfer 20 mL from the 250 mL cylinder into test replicate cup A; repeat for the remaining replicates for that concentration, including the parameter cup. Rinse cylinders with deionized water between concentrations. Repeat for each test concentration.

6.8.5.1. For each sample tested, use ten replicates per concentration (plus a parameter cup) in a dilution series based on the Facility's NPDES permit, instruction from the appropriate DEP ROC or, if applicable, previous knowledge of toxicity level. Usually, a minimum of 5 test concentrations plus the control are used.

6.8.6. Place the eleventh cup of each concentration in a separate, small tray to measure physical parameters. A second tray is used to prevent possible contamination of the test cups.

6.8.7. Example of sample volume (0.5 dilution) below for ten 20mL replicates and one parameter cup per replicate of each concentration in the *C. dubia* chronic toxicity test:

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Concentration	Diluent	Sample	Total Volume
Control	250 mL	0 mL	250 mL
6.25%	250 mL	250 mL of 12.5%	500 mL
12.5%	250 mL	250 mL of 25%	500 mL
25%	250 mL	250 mL of 50%	500 mL
50%	250 mL	250 mL of 100%	500 mL
100%	0 mL	250 mL	250 mL

6.9. Perform QA Standard Reference Toxicant (SRT) tests monthly or concurrently if bioassays are performed less frequently. The SRT must be done within the same month or 30-day period as the facility bioassay.

6.9.1. DEP Laboratory uses potassium chloride (KCl) as the standard toxicant. Other toxicants can be used. An example of sample volumes for acute definitive KCl SRT test, using the toxicant made per SOP TA-09.01, is below. Concentrations and toxicants can be modified as needed. Diluent is synonymous with control water.

6.9.2. Example of KCl (1 g/L toxicant in MHWCD, see SOP TA-09.01) concentrations and diluent volumes for chronic SRT test:

Concentration	Diluent	Toxicant	Total Volume
Control	250 mL	0 mL	250 mL
0.3 g/L	210 mL	90 mL of 1 g/L KCl	300 mL
0.4 g/L	180 mL	120 mL of 1 g/L KCl	300 mL
0. 5 g/L	150 mL	150 mL of 1 g/L KCl	300 mL
0.6 g/L	120 mL	180 mL of 1 g/L KCl	300 mL
0.7 g/L	90 mL	210 mL of 1 g/L KCl	300 mL

6.10. Using calibrated 0.2 mL pipettor, or calibrated dropper, feed 0.2 mL of combined 1:1 YCT and algal suspension per test cup (recommended).

6.10.1. Feeding is done every day during transfer of organisms. Record feeding time in bench sheets comment section or in other designated space.

6.11. Measure pH, temperature, DO, and conductivity of the solutions in the parameter cups (eleventh cup) in the separate tray.

6.11.1. Phys-chem parameters on “new sample” should be taken before transfer of organisms to ensure that concentrations were poured correctly. This allows the chance to interpret and correct mistakes without causing harm to the organisms.

6.11.2. Record all readings, and any comments pertaining to the condition or appearance of the sample, in their appropriate columns on the

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Parameter page of the *Chronic Bioassay Bench Sheets for Ceriodaphnia dubia Definitive Tests* logbook.

6.11.2.1. Conductivity measurements are reported to 3 significant figures. pH, DO, and temperature measurements should be read to one decimal place. Take measurements from lowest to highest toxicant/sample concentration.

6.11.3. Empty the phys-chem cups into the sink, rinse cups, and flush the drain with water. Fill cups with DI water to soak overnight for use the next day.

6.12. Test initiation (0-hour and Day-0)

6.12.1. At a light table with a plastic pipette, randomly load one *C. dubia* neonate from one chronic culture cup (collected specifically for chronic test from adults for neonates) into replicate A of each test concentration (1 control cup plus 5 dilutions for a total of 6 cups). Repeat for the remaining 9 sets (B-J) of replicate cups using a different cup of neonates per set of replicates (generally 10 neonate cups are used). If necessary, the same cup of neonates can be used to load more than 1 set of replicates but the cup needs to have a minimum of 12 neonates.

6.12.2. Use the random loading pattern table found in the lab to load the neonates across the concentrations. It is based on the one found in the EPA chronic manual. This randomizes what order the neonates get loaded into the replicates.

6.12.3. In the logbook, record which cup was used to load each row.

6.13. A person other than the loader should verify that there is one neonate in each replicate for test initiation.

6.14. Place the tray of cups in the incubator and cover the tray with clear Plexiglass.

6.14.1. Covers prevent accidental contamination and minimize evaporation.

6.15. Record counts and the date and time of test initiation on the appropriate pages of the *Chronic Bioassay Bench Sheets for Ceriodaphnia dubia Screening Tests* logbook.

7. INTERIM READINGS AND RENEWALS

7.1. Every day at 24-hour (± 3 hours) intervals, perform applicable Steps 6.2-6.11.3 to prepare new test cups/concentrations, transfer *C. dubia*, take parameters, feed, etc.

7.1.1. On days when a new set of effluent sample water arrives, follow sections 6.2 through 6.8 for phys/chem readings. When two or more jugs of sample water were collected on the same day, only temperatures must be recorded from all jugs in *Bioassay Sample Log-in* logbook. The remaining phys-chem parameters (pH, DO, and conductivity) should be

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performed and recorded from only one jug in *Bioassay Sample Log-in* logbook.

7.1.2. Parameters of the "before renewal" and "after renewal" solutions are taken daily. All before-renewal parameters should be taken using "A" replicates. If the "A" replicate has already been used or removed due to mortality, measure the next available, alphabetically sequential aliquot of the same concentration.

7.2. On days 1 until test termination (see 2.2), retrieve the previous day's cups from the incubator, count and record the number of surviving *C. dubia* and record the number of neonates on the Reproduction pages of the logbook.

7.2.1. Use a magnifying glass over a light table to see the small neonates.

7.2.1.1. Neonates will typically be present on days 3-8.

7.3. With a plastic transfer pipette, transfer the original *C. dubia* adult from previous tray into the corresponding test cup on the new tray.

7.3.1. Transfers MUST be performed prior to measuring physical parameters in cups from the previous day.

7.3.2. Verify count using the magnifying glass and light table.

7.4. Place new tray of cups into incubator and cover tray with clear Plexiglass.

7.5. Repeat applicable steps 6.2 -7.3.2 daily until test termination criteria (60% of surviving control females have 3rd brood) is met, then proceed to Step 8. DO NOT CONTINUE TEST BEYOND 8 DAYS.

8. TEST TERMINATION

8.1. Count number of neonates produced and record in appropriate columns on bench sheet.

8.1.1. Adults and neonates MUST be counted prior to measuring physical parameters in cups from the previous day, but they don't need to be removed to take the final measurements.

8.1.2. At test termination, all observations on numbers of offspring should be completed within 2 hours. The rapid rate of development of *C. dubia* means that an extension of more than a few hours in the test period could be a significant part of the brood production cycle of the animals and allow production of additional broods.

8.1.2.1. Adults not producing neonates by end of the test are placed on a slide and examined to determine if they are male or female usually by a member of the invertebrate taxonomy group. Refer to EPA-821-R-02-013 Section 13.10.9-13.13.2.2.

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8.2. Measure pH, DO, and conductivity of the solutions and record in appropriate columns on bench sheet.

8.3. Record any comments pertaining to the condition of the test animals.

8.4. Record test termination date and time.

8.5. Pour contents of all test cups into sink and flush drain with water.

8.6. Dispose of cups into a trashcan and put tray in storage area.

8.7. Enter test results into the EPA WET Excel spreadsheet to obtain the NOEC, LOEC, or IC25. Refer to SOP TA-07.17. Data transcription (paper to electronic) should be verified by another analyst (Windows text to speech function can be used).

8.8. 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.

9. DATA ARCHIVAL

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

9.2. 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.

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

9.2.2. 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.

9.2.2.1. 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.).

9.3. Upon sample receipt, log sample receipt, temperature, and condition into *Chronic Bioassay Bench Sheets* logbook. Fill in information requested at top of bench sheets as completely as possible prior to test initiation. Include full name of facility, address, contact person, date and time collected, field TRC, LIMS sample and job numbers, test chamber ID, test chamber size, test solution volume, food type, and food batch numbers. Access scanned Field Sheets via \\floridadep\data\DEAR\Labs\Receiving to obtain sampling information. Initial TRC, alkalinity, and hardness measurements should be recorded in their respective LIMS logbooks.

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9.4. Record meter calibration readings in the LIMS logbooks and appropriate bench sheets.

9.5. During sample prep and analysis, parameters and organism data should be entered in the *Chronic Bioassay Bench Sheets for Ceriodaphnia dubia Screening Tests* logbook.

9.6. Enter the NOEC, LOEC, and IC25 results from the EPA WET Excel spreadsheet (Refer to Step 8.7.) into the appropriate logbook, computer file, and LIMS results editor. 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. A second person must verify data prior to uploading into LIMS.

10. QUALITY CONTROL

10.1. Refer to DEP Laboratory Quality Assurance 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. Sample should be held at 0-6°C prior to test initiation and between renewals. Exception for hand delivered sample on day of collection—sample should be cooling relative to collection temperature.

10.2. Sample holding time should not exceed 36 hours from time of collection until initial use of sample.

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

10.4. Tests and cultures shall be maintained at 25 ± 1°C (26 ± 1°C for *A. bahia*, where applicable). Test temperature shall not deviate by more than 3°C (maximum minus minimum) during the test period. Verify continuous temperature of equipment using a NIST- traceable thermometer. Apply correction factors as needed for accuracy.

10.4.1. Temperature Excursion Response:

10.4.1.1. If the test solution temperature deviation (maximum minus minimum) exceeds 3°C and no toxicity is observed, document the deviation and add the comment, "Temperature of test solutions deviated from method specifications."

10.4.1.2. If the incubator monitoring temperature deviation (maximum minus minimum) exceeds 3°C and no toxicity is observed, document the deviation and add the comment, "Continuous temperature monitoring in surrogate test vessels deviated from method specifications."

10.4.1.3. If the test solution temperature deviation (maximum minus minimum) exceeds 3°C and toxicity is observed, document the

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deviation, qualify results as estimated (“J”), and add the comment, “Estimated value. Deviation of test solution temperatures from method specifications may be contributing to toxicity.”

10.4.1.4. If the temperature deviation (maximum minus minimum) exceeds 3°C and the test does not meet method acceptability criteria, report results as “No Result” and notify the client that the test should be repeated.

10.5. If controls fail to meet minimum acceptability criteria, the test will be invalidated, and the analyses rerun as soon as possible or the schedule allows for SRT tests. Facility tests are rerun at the discretion of the district toxicity coordinator. If the test organism sensitivity falls outside the acceptable range (±2 standard deviations of the mean) during the standard reference toxicant (SRT) tests, the SRT test will be repeated until an acceptable test result is achieved and the cause of the aberration is determined. Test results will be reported as usual, not invalidated, if there is a successful (within the acceptable range) SRT for that test organism within 30 days. Based on the nature of the statistics, it can be expected for 1 in 20 points will fall outside the acceptable range (±2 standard deviations of the mean). If the SRT shows that the test species are overly sensitive, but the facility test results in a >100% effluent value, the result should still be reported with no qualifier.

10.6. The Tox Environmental Manager is responsible for assessing and approving corrective actions. Samples which exceed holding time criteria (36 hours) by no more than eight (8) hours will be analyzed, the results will be qualified, the client will be notified, and a non-conformance report will be prepared. Samples which exceed holding time by more than eight (8) hours shall not be analyzed. The client will be notified and given the option of cancelling the job or rescheduling.

10.7. Analysis of samples which do not meet preservation requirements (temperature ≤ 6°C for shipped samples (overnight) and temperature decreasing (from field measurement and in hand delivered samples) is determined on a case-by-case basis. Generally, samples that are to be used for compliance purposes will not be analyzed; samples that are for information only will be processed. All data from samples not meeting temperature requirements will be qualified, non-conformance reports generated, and the client notified.

10.8. Perform QA Standard Reference Toxicant (SRT) tests monthly for in-house cultured *C. dubia* . If an SRT fails the test acceptability criteria or control chart limits, it must be repeated as soon as possible to get a passing test within the month timeframe. If two or more consecutive tests do not fall within control chart limits, the SRT must be repeated immediately and corrective action must be taken. If two or more tests fall outside of the control chart limits in 20 samples, the SRT must be repeated immediately and corrective action must be taken. When the cause of the outlier can

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be documented, the outlier should be removed from the future calculations of the control chart.

10.9. 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) Program administrator is notified. 4) A non-conformance report (NCR) is submitted describing the problem and the corrective action.

10.9.1. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report.

10.10. 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.

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 DEP Laboratory Quality Assurance Manual for sample disposal.

11.3. Refer to the Laboratory Health and Safety Manual.

11.4. Read MSDS information for reagent handling and disposal.

12. REFERENCES

12.1. EPA 821-R-02-013, *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th Edition, Test Method 1002.0*

12.2. DEP SOP TA-01.01, *YCT (Yeast, Cereal Leaves, Flake-Fish Food, or Trout Chow) Preparation*

12.3. DEP SOP TA-01.04, *Raphidocelis subcapitata Culture for Ceriodaphnia dubia Food*

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

12.5. DEP SOP TA-05.02, *Production of Ceriodaphnia dubia for Chronic Toxicity Testing*

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

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

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

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

12.10. DEP SOP TA-06.35, *Measuring Total Ammonia using the Hach Ammonia Nitrogen Pocket Colorimeter II Test Kit*

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

12.12. DEP SOP TA-06.44, *Standard Operating Procedure for: YSI MultiLab 4010-1 Meter Calibration and YSI IDS ProOBOD DO Probe Maintenance*

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

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

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

12.16. DEP SOP TA-09.01, *Potassium Chloride Reference Toxicant Stock Solutions*

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

12.18. FS 1000, *General Sampling*

12.19. DEP Laboratory Quality Assurance Manual

12.20. DEP Laboratory Health and Safety Manual

12.21. MSDS Information Sheets

APPENDIX OF SIGNIFICANT CHANGES

02/11/2015 Formatted for 508 compliance.

04/17/2026 Section 10: Quality Control, was expanded to further address test/culture temperature requirements (section 10.4) and control chart failures (section 10.8) to address the 2026 audit deficiencies.

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