Pimephales promelas and *Cyprinella leedsi* Chronic Toxicity Testing Methods

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Pimephales promelas and *Cyprinella leedsi* Chronic Toxicity Testing Methods

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 1000.0. It is used to estimate the chronic toxicity of freshwater whole effluent and receiving water on *Pimephales promelas* (fathead minnow) and *Cyprinella leedsi* (bannerfin shiner) using < 24-hour old (or < 48 hours if shipped) organisms in a 7-day static-renewal exposure. Tests conducted by this procedure are BSY-CS-FFS and BSY-CD-FFS.

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 screen to remove debris and/or indigenous organisms. Sample dilutions and control waters are placed into test chambers. Test organisms are collected, fed and held for observation prior to loading into test chambers. Tests are incubated in laboratory incubators during the duration of the test. Physl-chem parameters are measured and recorded daily. Survival/mortality is recorded daily. The test endpoints are survival and growth (measured as dry weight) (IC25) of the organisms. Statistical endpoints are obtained from the EPA WET Statistics Excel spreadsheet per SOP TA-07.17. Results are entered in the Laboratory Information Management System (LIMS). If the reported result is less than the top concentration tested (i.e. <100%), add “Upper and lower confidence intervals are, ##.#% and ##.#%, respectively.” to the comment field when entering into LIMS.

2.2. Test acceptability criteria: 80% or greater survival in the controls and average dry weight per surviving control organism equal to or greater than 0.25 mg.

2.3. Currently, *C. leedsi* has not been approved by EPA for NPDES compliance testing. The certification request (submitted ~1997) was inactivated in 2011 at the request of the Biology Program.

2.4. Interferences: Refer to EPA-821-R-02-013 Section 11. 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; and indigenous organisms that may attack or be confused with the test organisms.

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3. EQUIPMENT AND SUPPLIES

3.1. *P. promelas* or *C. leedsi* test organisms (< 48 hours old).

3.1.1. Test organisms are fed live *Artemia* nauplii at least 2 hours prior to test initiation. The FDEP Laboratory currently purchases test organisms. Refer to SOP TA-04.20.

3.2. 1 L Test chambers, autoclaved, 4 per test concentration.

3.2.1. Test chambers can be air dried or dried in a drying oven for eight hours at 90 °C rather than being autoclaved.

3.2.2. A test with 5 test concentrations and a control, using 4 replicates per treatment would require 24 chambers.

3.2.3. A minimum of four replicates per concentration is required.

3.2.4. The minimum test chamber size is 500 mL (recommended minimum) and the minimum volume per replicate is 250 mL (recommended minimum). A loading of 10 organisms per replicate is recommended and a minimum of 40 organisms per concentration is required.

3.2.5. We generally use 1L test chambers and 500 mL of sample. Adjust chamber size and replicate volume as necessary to accommodate available sample.

3.3. 1000 mL Volumetric flasks (as needed)

3.3.1. One set for toxicant/sample and one for diluent

3.4. 100 mL, 500 mL, 1000 mL and 2000 mL, Graduated cylinders (as needed).

3.4.1. For measuring amount of toxicant/sample or diluent needed for each concentration. One set each for toxicant, sample, and diluent.

3.5. 1 L, 2 L, 4 L and 6 L Erlenmeyer flasks (as needed).

3.5.1. For mixing test dilutions and holding toxicant, sample, and diluent.

3.6. 1mL and 10 mL Serological pipettes, graduated.

3.6.1. Pipettor for volumetric pipettes.

3.7. Magnetic stir bars and stirrer.

3.7.1. For mixing toxicant.

3.8. Carolina dish, 8" diameter.

3.8.1. Transfer fish from holding container to Carolina dish for easier loading of bioassay chambers.

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3.9. Droppers and glass tubing with fire polished edges, 4 mm ID, for transferring organisms.

3.9.1. Nets or Nytex® screen cannot be used to transfer organisms as the screen can injure them.

3.10. Meters to measure phys-chem parameters:

3.10.1. pH (Calibrate per SOP TA-06.43).

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

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

3.10.4. Conductivity/salinity (Calibrate per SOP TA-06.36).

3.11. Hach® kits to measure:

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

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

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

3.11.4. Ammonia (Measure per SOP TA-06.35).

3.12. Incubator should maintain selected test temperature (25 ± 1°C recommended) and a photoperiod of 16-hours light to 8-hours dark. Light intensity should be 50-100 ft candles. Test temperature must not deviate (maximum minus minimum) more than 3°C in the test chambers for the duration of the test.

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

3.13. 30 mL disposable plastic cups, with parameter tray.

3.13.1. For aliquots of concentrations for parameters.

3.14. Fine mesh fish net.

3.14.1. To aid in euthanizing fish at test termination.

3.15. Ice bath.

3.15.1. Use 8’’ Carolina dish with ice and cold water. For euthanizing fish during test termination.

3.16. Waste bucket.

3.17. Live *Artemia* nauplii (prepare per SOP TA-01.03).

3.18. Plastic transfer pipette.

3.18.1. Used for feeding.

3.19. Drying oven.

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3.19.1. Capable of sustaining 100□ C for 8 hours or 60□ C for 24 hours. Refer to SOP TA-06.21.

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

3.21. *Chronic Bioassay Bench Sheet* logbook.

4. REAGENTS AND STANDARDS

4.1. Dilution water - MHW (prepared per SOP TA-03.15).

4.2. Sample to be tested.

5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

5.1.1. For a valid test: a) the sample should be held at 0-6°C prior to test initiation, and b) sample holding time should not exceed 36 hours from time of collection (time of last sample collect for grab samples or the end of the 24-hour period for composite samples) 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. These samples typically arrive on Tuesday, Thursday and Saturday in one gallon opaque plastic jugs, or other Receiving approved containers.

5.1.3. The remainder of each sample is stored in the Biology Walk-in cooler for solution renewal the following day(s).

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

6. TEST INITIATION

6.1. Place clean test chambers onto laboratory cart. Label each chamber to identify the replicate (A–D), test concentration, and facility sampled.

6.1.1. Place tape across front of chamber, beneath jar ID, and use waterproof marker to identify. Four chambers for each set of control replicates needed for each sample tested, plus four chambers per concentration for each sample tested.

6.2. Calibrate meters and record calibration readings.

6.2.1. Calibrate meters per respective SOPs. Record calibration readings in LIMS (report results in accordance with SOP TA-06.42).

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6.3. Fill a 30 mL disposable plastic cup with sample and take initial pH measurement per SOP TA-06.43. Record value in the *Bioassay Sample log-in* logbook and LIMS TOX_pH logbook.

6.3.1. 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. 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.

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

6.4.1. If DO is < 4.0 mg/L or > 9.0 mg/L, aerate the entire sample to near saturation prior to test initiation. Record aeration (initial and final DO as well as 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/min) to ALL test replicates for duration of test using micropipettes and the aeration manifolds. No action is required if DO increases after test initiation.

6.5. In the same plastic cup containing the sample, measure the conductivity and temperature of the sample.

6.5.1. If conductivity is above 2,000 µmhos/cm or the salinity is more than 1 ppt, then freshwater organisms are probably not the appropriate test organisms for this effluent sample. Check with FDEP District personnel and also check the Facility's NPDES permit for bioassay requirements.

6.6. Measure total residual chlorine (TRC) in the sample per SOP TA-06.22. Record the result in the LIMS TOX_CHLORINE logbook and on the *Chronic Bioassay Bench Sheet*. Check the 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-09.18. 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. Pour up the test by measuring the desired volume of sample/toxicant and diluent with a graduated cylinder (add the final amount of each solution with a 10 mL

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pipette when nearing the desired volume, to prevent over pouring) and mixing them in the dedicated mixing flask. Return mixed dilution to the graduated cylinder (we generally use the plastic 2000 mL cylinder with the handle for easy pouring) and pour into test chambers A through D for the matching concentration. Repeat for each concentration.

6.7.1. For each sample tested, use four chambers per concentration in a dilution series chosen based on previous knowledge of toxicity level and permit requirements. There are typically 5 concentrations assigned by the permit and a control water.

6.7.2. Example of sample volumes for four 500 mL replicates of each concentration (0.5 dilution) of a chronic toxicity test (most common permit concentration requirements):

6.7.2.1. There are two methods for pouring up facility samples.

6.7.2.2. The first method listed below only works for 0.5 dilutions. You begin with the top concentration and dilute by half for each successive dilution (see table for specifics).

6.7.2.3. The second method listed below works with any concentration and begins with the lowest dilution and goes up in concentration. These dilutions are calculated as follows:

6.7.2.3.1. Total Vol. X Concentration decimal = Sample Vol.

6.7.2.3.2. Ex. 2000 mL X 0.0625 = 125 mL sample needed for 6.25%

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Concentration	Diluent	Sample	Total Volume
Control	2000 mL	0 mL	2000 mL
l00%	0 mL	2000 mL of 100%	2000 mL
50%	2000 mL	2000 mL of 100%	4000 mL
25%	2000 mL	2000 mL of 50%	4000 mL
12.50%	2000 mL	2000 mL of 25%	4000 mL
6.25%	2000 mL	2000 mL of 12.5%	4000 mL
Alternate pour up method			
Concentration	**Diluent**	**Sample**	**Total Volume**
Control	2000 mL	0 mL	2000 mL
6.25%	mL	125 mL	2000 mL
12.50%	mL	250 mL	2000 mL
25%	mL	500 mL	2000 mL
50%	mL	1000 mL	2000 mL
l00%	0 mL	2000 mL	2000 mL

6.8. Perform QA standard reference toxicant (SRT) tests concurrently with bioassays (or monthly if performed 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), using 2 g/L potassium chloride toxicant (prepared per SOP TA-09.01) (other toxicants can be used).

6.8.1. Example of sample volumes for a chronic SRT test with potassium chloride as the toxicant (note the different dilution concentrations used here vs the facility test):

Concentration	Diluent	Toxicant (2 g/L KCl in MHW)	Total Volume
Control	2000 mL	0 mL	2000 mL
0.125 g/L	1875 mL	125 mL	2000 mL
0.25 g/L	1750 mL	250 mL	2000 mL
0.5 g/L	1500 mL	500 mL	2000 mL
0.75 g/L	1250 mL	750 mL	2000 mL
1 g/L	1000 mL	1000 mL	2000 mL

6.9. Pour a subsample from all A replicates into the disposable plastic cups in the parameter tray. Measure pH, DO, temperature (can be measured from test chamber), and conductivity of the solutions from the subsamples. Record all readings. Record any comments pertaining to the condition or appearance of the sample. On the following seven days, rotate through each of the replicates in order and take parameters before and after renewing the sample.

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6.9.1. Conductivity readings can be rounded to the nearest 5 µmhos or 0.1 mmhos. Readings for pH, DO, and temperature are read to one decimal place. Take measurements from lowest to highest toxicant concentration.

6.9.2. Record all readings in the appropriate columns on the *Chronic Bioassay Bench Sheet*.

6.10. Using a wide bore pipette, load two (20% of test organisms per replicate) fish into each test chamber. Repeat the loading process until ten fish have been loaded into each replicate. Verify count over a light table. Record counts in appropriate columns on the *Chronic Bioassay Bench Sheet*.

6.10.1. (10 fish per replicate) x (4 replicates per test concentration) = 40 fish per concentration tested

6.10.2. A person other than the loader should verify the number of fish in each replicate.

6.10.3. Record counts and phys-chem readings in appropriate columns in the *Chronic Bioassay Bench Sheet*.

6.11. Use a plastic transfer pipette to feed fish 0.2 mL *Artemia* nauplii concentrate (800-1100 *Artemia*) once in the morning 2 hours prior to renewals, and again after renewals (feed per SOP TA-2.00).

6.11.1. Feed once in test chambers on Day 0 (initiation day, all fish are fed 2 hours prior to loading in test chambers) and then twice on succeeding days. *Artemia* nauplii concentrate is prepared based on SOP TA-01.03.

6.12. Place chambers (use randomization template determined by a dice roll and maintain initial pattern for test duration) in incubator and cover with clear Plexiglass, place sealed pipettes between test chambers and Plexiglass to allow for air movement. Covers prevent accidental contamination and minimize evaporation.

6.13. Measure alkalinity, hardness, and total ammonia of sample, control water (control water measurements are done when the water is made and not usually during the test), and fish water.

6.13.1. Measure per SOP TA-06.08 and TA-06.35, respectively.

7. INTERIM READINGS

7.1. Every 24 hours, pour subsamples of the appropriate replicates into disposable plastic cups. Measure pH, temperature (can be taken from test chamber), DO, and conductivity in the subsamples before renewing the test solutions and record readings. Count and record the number of fish in each replicate. Starting with the controls and then going in order of increasing toxicant concentration, clean out dead *Artemia* nauplii at the bottom of each jar. Carefully siphon at least half of the solution in each chamber through a fine mesh net attached to a plastic hose into the

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waste bucket. Try to keep the fish within the chamber. Replace the contents of each chamber with respective fresh concentrations of the sample.

7.1.1. All counting is done over a light table. Fish are recorded as live if they move when lightly prodded. Record counts and phys-chem readings in appropriate columns in the Chronic Bioassay Bench Sheet.

7.1.2. Check the excess mesh net below the end of the hose for trapped fish before siphoning the next jar. If a fish is stuck, gently move the mesh around the water to release the fish.

7.2. Pour subsamples from the appropriate replicate after renewal. Measure pH, temperature (can be measure in the test chamber), DO, and conductivity after renewing the test solutions and record readings.

7.2.1. Subsamples are poured to minimize disturbance to the fish.

7.3. Feed fish 0.2 mL *Artemia* nauplii concentrate once in the morning two hours prior to renewals, and again after the renewals. Cover chambers when done and return to the incubator.

8. TEST TERMINATION

8.1. On day 7 (t = 168 hours ± 6 hours), count the number of surviving fish. Remove and discard any dead fish. Pour subsamples and measure parameters of the solutions. 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. Counting is done over a light table. Phys-chem readings are taken from the subsample to minimize disturbance to the fish.

8.1.2. Record counts, readings, date, and time in appropriate columns in *Chronic Bioassay Bench Sheet*.

8.2. Make weigh boats two days before test termination per SOP TA-06.21.

8.3. Pour the contents of Control replicate A onto a fine mesh net held over the waste bucket. Immediately euthanize the fish by immersing the fish in ice water in the Carolina dish. Keep the fish immersed for two minutes after the last movement is noted in the fish.

8.4. When the fish are euthanized, take a pair of forceps and place each fish into the respective weigh boat. One weigh boat per test chamber is used. If all fish from one test chamber died and were removed during the test (prior to euthanasia), leave that weigh boat empty.

8.5. Repeat steps 7.3-7.4 for all test concentrations and replicates.

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8.6. The day after test termination, dry the weigh boats containing the fish in the sediment drying oven (oven #1). Store the Carolina dish in the desiccator before and after they are dried. Refer to SOP TA-06.21 for weighing chronic tests.

8.6.1. Dry for at least 24 hours at 60°C.

8.6.1.1. Fish can also be dried for 8 hours (minimum 6 hours) at 100°C.

8.7. Let the dish cool in the desiccator and then place it next to the 5-place balance to acclimate to the room conditions for at least 30 minutes. Record the weights in the *Weights for Chronic Tests* logbook.

9. STATISTICAL ANALYSIS

9.1. Complete the weight page in the *Chronic Bioassay Bench Sheet* logbook.

9.2. Enter test results into statistical program to obtain NOEC, LOEC and IC25 per SOP TA-07.17.

9.3. Enter test results into LIMS.

9.3.1. Data is verified by a second person prior to being uploaded into LIMS.

9.3.2. If the reported result is less than the top concentration tested (i.e. <100%), add “Upper and lower confidence intervals are, ##.#% and ##.#%, respectively.” to the comment field when entering into LIMS.

10. DATA ARCHIVAL

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

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

10.3. Record information in appropriate logbooks (*Chronic Bioassay Bench Sheet*).

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

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

10.4.2. 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.

10.4.3. 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.).

10.5. DEP Laboratory Information Management System (LIMS)

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10.6. Log sample receipt, temperature, and condition into Chronic Bioassay Bench Sheet 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.

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

11. QUALITY CONTROL

11.1. 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.

11.2. Perform QA standard reference toxicant (SRT) tests monthly or concurrently. 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 be documented, the outlier should be removed from the future calculations of the control chart.

11.3. If a problem occurs during sample processing or testing, the following steps are performed: 1) lab supervisor is notified, 2) client is notified (client makes decision for additional testing), 3) section administrator is notified, and 4) a nonconformance report (NCR) is submitted describing the problem and the corrective action.

11.4. To create NCR: open LIMS and go to Laboratory/Administrative/Non-Conformance Report. Refer to FS 1000 for sample collection and preservation, and the Laboratory Quality Assurance Manual for corrective action and handling out-of-control data.

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

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

11.7. 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

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minimum) during the test period. Verify continuous temperature of equipment using a NIST- traceable thermometer. Apply correction factors as needed for accuracy.

11.7.1. Temperature Excursion Response:

11.7.1.1. 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.”

11.7.1.2. 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.”

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

11.7.1.4. 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.

11.8. If controls fail to meet minimum acceptability criteria, the test will be invalidated, and the analyses rerun as soon as possible/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. Note that based on the nature of the statistics, it can be expected for one in 20 points to 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.

11.9. The subsection 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.

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11.10. Analysis of samples which do not meet preservation requirements (temperature ≤ 6 ºC for shipped samples (overnight) and temperature decreasing (from field measurement) 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.

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 DEP Laboratory Quality Assurance Manual for sample disposal.

12.3. Refer to the DEP Laboratory Health and Safety Manual.

12.4. Read MSDS information for reagent handling and disposal.

13. REFERENCES

13.1. DEP Laboratory Quality Assurance Manual

13.2. DEP Health and Safety Manual

13.3. 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 1000.0*

13.4. MSDS Information Sheets

13.5. DEP SOP FS 1000, *General Sampling*

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

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

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

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

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

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

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

13.13. DEP SOP TA-06.21, *Weighing Chronic Tests*

13.14. DEP SOP TA-06.22, *Measuring Total Residual Chlorine Using the Hach® Pocket Colorimeter™ II Test Kit*

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13.15. DEP SOP TA-06.35, *Measuring Total Ammonia Using the Hach® Ammonia Nitrogen Pocket Colorimeter™ II Test Kit*

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

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-06.42, *LIMS Entry for Laboratory Parameters*

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

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

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

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

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

APPENDIX OF SIGNIFICANT CHANGES

02/12/2015 Formatted for 508 compliances.

02/01/2021 Updated SOP for universal language and grammar accuracy.

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

04/17/2026 Section 11: Quality Control, was expanded to further address test/culture temperature requirements (section 11.7) and control chart failures (section 1) to address the 2026 audit deficiencies.

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