Cyprinella leedsi and *Pimephales promelas* Acute Toxicity Testing

DEPARTMENT OF ENVIRONMENTAL PROTECTION
LABORATORY
BIOLOGY PROGRAM
TALLAHASSEE, FLORIDA

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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 2000.0. It is used to estimate the acute toxicity of whole effluents and receiving waters (freshwater aqueous samples) with the Bannerfin Shiner, *Cyprinella leedsi,* and the Fathead Minnow, *Pimephales promelas*, using 1- to 14-day old organisms in a 48-hour static exposure or in a 96-hour static renewal exposure. Tests conducted by this procedure are BSY-AS-FFS and BSY-AD-FFS.

2. SUMMARY OF METHOD

2.1 Samples are received and prepared in the laboratory. The physical-chemical (phys-chem) parameters of the sample are measured. Samples are adjusted, as needed, to meet permit or method requirements. Sample is filtered through a 100 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. Physical-chemical parameters are measured and recorded daily. Survival/mortality are recorded daily. The test endpoint is mortality of the organisms. Statistical endpoints are obtained from the EPA WET Statistics Excel spreadsheet per SOP TA-07.17. 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 “Upper and lower confidence intervals are, ##.#% and ##.#%, respectively.” to the comment field when entering into LIMS.

2.2 Interferences: Refer to EPA-821-R-02-012, Section 9. Includes: toxic substances introduced by contaminants in dilution water or on hands, glassware, sample hardware, and testing equipment; dissolved oxygen <4.0 mg/L; 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.

2.3 Test acceptability criteria: 90% or greater survival in the controls. Method modification allows reduction of organisms per chamber from 10 to 5 and increase in chambers/concentration from 2 to 4.

3 EQUIPMENT/SUPPLIES AND REAGENTS/STANDARDS

3.1 *C. leedsi* or *P. promelas* (1–14 days old, less than or equal to 24-hr range in age).

3.1.1 Test organisms are obtained from vendors. Refer to SOP TA-04.20.

3.1.2 Fish are fed live *Artemia* nauplii at least 2 hours prior to test initiation. 10 fish are used per concentration replicate, unless the test method is

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modified to double the minimum number of replicates to reduce the number of organisms per replicate.

3.2 1 L Test chambers (glass jars)

3.3 Synthetic Moderately Hard Water (MHW)

3.3.1 Prepare in advance per SOP TA-03.15.

3.4 30 mL disposable plastic cups with parameter tray

3.4.1 Used to hold aliquots collected from test solutions for measuring physical/chemical parameters. A parameter tray is prepared by placing clean cups in a pre-cut plexiglass tray. Label tape is placed in front of the cups. The tape is labeled with the appropriate concentration per vial.

3.5 Sample to be tested

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

3.6 500 mL, 1000 mL and /or 2000 mL Graduated cylinders

3.6.1 Used for mixing test concentrations.

3.7 1000 mL, 2000 mL and 4000 mL Erlenmeyer flasks

3.7.1 Used for mixing test concentrations.

3.8 8" Plastic Carolina dish

3.8.1 Transfer fish from the bag they were shipped into Carolina dish for easier loading of bioassay chambers. Used to hold purchased organisms prior to test initiation.

3.9 Glass pipet with rubber bulb

3.9.1 For transferring fish from Carolina dish to test chambers.

3.10 Large bore plastic transfer pipette

3.11 Meters to measure physical/chemical parameters:

3.11.1 pH (Calibrate per SOP TA-06.43)

3.11.2 Dissolved Oxygen (DO) (Calibrate per SOP TA-06.39)

3.11.3 Conductivity (Calibrate per SOP TA-06.36)

3.11.4 Digital thermometer (Calibration check per SOP TA-06.13)

3.12 Hach® kits to measure physical/chemical parameters:

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

3.12.2 Alkalinity (Measure per SOP TA-06.08)

3.12.3 Hardness (Measure per SOP TA-06.08)

3.12.4 Ammonia (Measure per SOP TA-06.33 or SOP TA-06.35)

3.13 Thermo Scientific Incubator (Incubator #1, #5 or #7)

3.13.1 Incubator should maintain selected test temperature (20 ± 1°C or 25 ± 1 °C recommended) and a photoperiod of 16 h light: 8 h 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.

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

4 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

4.1 See SOP FS 1000 for sample collection and preservation.

4.1.1 For a valid test: a) the sample should be held at 0-6°C prior to test initiation, and b) sample holding time should not exceed 36 hours from time of collection (time of last sample collection for grab samples, or the end of the 24-hour collection period for composite samples) to test initiation. Sample is collected and used upon receipt. Sample typically arrives on Tuesday in 4L opaque high density polyethylene plastic sampling containers.

4.2 See SOP TA-06.14 for Bioassay Sample Log-in procedure.

5 TEST INITIATION

5.1 Place appropriate number of clean test chambers onto laboratory cart. Label each chamber to identify the replicate (A, B, C, D, etc.), test concentration, and facility. Include the test species on the label when testing multiple species/samples. Place tape on the front of chamber (beneath chamber ID). Use waterproof marker to identify.

5.1.1 The method specifies a minimum of two chambers and 20 organisms per concentration including controls for effluent samples and a minimum of four chambers and 40 organisms per concentration including controls for receiving water samples. Test chambers can be dried in a drying oven for eight hours at 100 ± 5°C rather than being autoclaved.

5.1.2 Minimum test chamber size is 250 mL and minimum test volume is 200 mL. The FDEP Laboratory generally uses 1 L chambers and 500 mL of sample per chamber. The FDEP Laboratory generally uses four chambers

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per concentration for screening tests, and *C. leedsi* facility and SRT tests; and two chambers per concentration for *P. promelas* facility and SRT tests (4 chambers can be used here, too). Test volumes may be reduced as needed in accordance with the method to accommodate lower than excepted sample volumes.

5.1.3 When using this method to run facility samples, test concentrations differ between tests using *Cyprinella Leedsi* and those that use *Pimephales Promelas*, the corresponding concentrations are:

Cyprinella Leedsi	CTRL	12.5%	25%	50%	75%	100%
Pimephales Promelas	CTRL	6.25%	12.5%	25%	50%	100%

5.1.4 When conducting SRT tests, test concentrations differ between tests using *Cyprinella Leedsi* and those that use *Pimephales promelas*, the corresponding concentrations are:

Cyprinella Leedsi	CTRL	0.125	0.25	0.50	0.75	1.0
Pimephales Promelas	CTRL	0.5	0.75	1.0	1.5	2.0

5.1.4.1 The toxicant used in both SRT tests is 2 g/L KCl in MHW, see SOP TA-09.01.

5.2 Verify the calibration of meters in accordance with their respective SOP and manufacturer manual. Record calibration readings in LIMS.

5.3 Measure the total residual chlorine (TRC) in 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.

5.3.1 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. The control used in the screen should have the same amount of sodium thiosulfate (as used to dechlorinate the sample) added. Record chlorine result, amount of sodium thiosulfate used, and final chlorine result (after dechlorination) in the logbook and in the designated section in LIMS (in accordance with SOP TA-06.42).

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

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5.4.1 If the pH is outside the acceptable range of 6.0–9.0 SU, an additional test with a pH-adjusted subsample will be run. Adjust pH of sample per SOP TA-08.06. The test with the non-adjusted sample is then analyzed as a screen test, regardless of whether the pH-adjusted aliquot is used for a screen or definitive test.

5.5 In the 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.

5.5.1 If the DO 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 initial and final DO, aeration rate, and aeration 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 solutions for the duration of test using micropipettes and the aeration manifold. No action is required if DO increases after test initiation.

5.5.2 Measure pH again after aeration. Aeration can cause chemical changes to the sample which alter the pH. If pH has changed after aeration, refer to 5.4.1.

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

5.6.1 If the conductivity is above 2,000 µmhos/cm, try to 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. leedsi* or *P. promelas* are likely not the appropriate test species for this effluent sample. This rationale does **NOT** apply to SRT tests.

5.7 ***Screening Test:*** Measure and pour 500 mL of MHW water into each of the control replicate test chambers. Measure and pour 500 mL aliquots of the 100% sample into the 100% test concentration test chambers.

5.7.1 Minimum of two chambers for each set of control and test replicates needed for each sample tested. Test method can be modified to double the test chambers and decrease the number of organisms per replicate. The following table shows suggested sample volumes (per replicate in a 1L test chamber) for *acute screen* toxicity test. Diluent is synonymous with control water.

Concentration	Diluent Volume	Sample	Total
Control	500 mL	0 mL	500 mL
100%	0 mL	500 mL	500 mL

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5.8 ***Definitive Test:*** For this test type you can make the concentrations using either the "Individual Concentration" or the "Half-dilution" method. Initiation day uses 2 L of each concentration to fill all replicates.

5.8.1 For the individual concentration method, the analyst pouring up the waters will use the following formula to find the amount of sample needed for each concentration:

5.8.1.1 $$\frac{\%\ concentration}{100\%} \times volume\ needed$$

5.8.1.2 The breakdown for "Individual Concentration" pour-up method is shown in the table below:

Note: The numbers given in this Section of the SOP are for 4 test chambers. If running a test using 2 test chambers per dilution, the volumes must be halved.

Concentration	Diluent	Sample	Total Volume
Control	2000 mL	0 mL	2000 mL
6.25%	1875 mL	125 mL	2000 mL
12.5%	1750 mL	250 mL	2000 mL
25%	1500 mL	500 mL	2000 mL
50%	1000 mL	1000 mL	2000 mL
75%	500 mL	1500 mL	2000 mL
100%	0 mL	2000 mL	2000 mL

5.8.2 For "Half-dilution" pour-up method concentrations, the analyst pouring up the waters will follow these steps to obtain each concentration:

Note: The numbers given in this Section of the SOP are for 4 test chambers. If running a test using 2 test chambers per dilution, the volumes must be halved.

Note: The 100% and 75% (if using) dilutions will need to be poured up separately from the ones using the "half-dilution" method.

5.8.2.1 ***For the 50% Concentration****:* Measure and pour 2000 mL of diluent (MHW) and 2000 mL of 100% sample in a 4000 mL flask. Swirl to mix. Then, using the 500 mL graduated cylinder measure and dispense 500 mL of the solution into each of the four replicate chambers for the 50% concentration.

5.8.2.2 ***For the 25% Concentration***: Add 2000 mL of the diluent to the 2000 mL of 50% concentration remaining in the flask, mix, and dispense 500 mL to each of the four 25% concentration chambers.

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5.8.2.3 ***For the 12.5% Concentration***: Add 2000 mL of the diluent to the 2000 mL of 25% concentration remaining in the flask, mix, and dispense 500 mL to each of the four 12.5% concentration chambers.

5.8.2.4 ***For the 6.25% Concentration***: Add 2000 mL of the diluent to the 2000 mL of 12.5% concentration remaining in the flask, mix, and dispense 500 mL to each of the four 6.25% concentration chambers.

5.9 Pour an aliquot from replicate A of each test concentration into the appropriately labeled parameter tray cup. Measure pH, DO, temperature, and conductivity of the solution in each cup. Record readings. Record any comments pertaining to the condition or appearance of the sample.

5.9.1 Conductivity readings can be rounded to the nearest 5 µmhos or nearest 0.1 mmhos (depending on significant figures). Readings for pH, DO, and temperature shall be read to one decimal place. Due to the possibility of rapid cooling of solutions in the cups from the temperature in Room B246, test temperatures can be measured directly from the test replicate chambers. The electronic thermometer is best for this measurement. It should be rinsed with DI water between each concentration.

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

5.10 Using a glass pipet with rubber bulb, load fish into the jars. If using 4 jars, load 1 fish at a time until a total of 5 fish per jar have been loaded. If using 2 jars, load 2 fish per jar until a total of 10 fish per jar have been loaded. There should be 20 fish per dilution. When loading, 20% of total organisms can be added at one time in each test chamber. Adjust loading as necessary (add or remove test organisms). Record counts in appropriate columns on the *Acute Bioassay Bench Sheet*.

5.11 Verify that the correct number of fish has been loaded within each replicate. A person other than the loader should verify the organisms per replicate. The verification person should remove malformed/injured/dead organisms and replace as needed with healthy organisms.

5.12 Place chambers together in the incubator and cover with clear Plexiglass. Chamber order is determined by rolling the dice located in the laboratory drawer and selecting the corresponding randomizer template (numbers 1-6). This randomizer template will be used throughout the test. Plexiglass covers prevent accidental contamination and minimize evaporation.

5.13 Measure alkalinity, hardness, and total ammonia of sample. Measurements made per SOPs TA-06.08, and TA-06.33/TA-06.35, respectively. Record measurements on the *Acute Bioassay Bench Sheet*.

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6 TESTS WITH DURATION >48 HOURS

6.1 On test day 2 (t = 48 hour), add 200 µL of concentrated *Artemia* nauplii (per TA-01.03) at least 2 hours prior to test renewal.

6.2 Using a plastic transfer pipette, clean any accumulations of waste (i.e. dead *Artermia* from the bottom of the test jar. Collect about 20 mL of pre-renewal solution for parameters from replicate C (if replicate C is unavailable, select the next available replicate; then, use a screened siphon to carefully remove ~250 mL of pre-renewal water from the test chamber. The siphon can be moved around the jar to remove as much debris as possible during this process. When siphoning, before moving on to the next jar, ensure that there are not any test organisms stuck to the bottom of the siphon. Count and record number of surviving organisms; remove dead organisms. Record counts in appropriate columns on the *Acute Bioassay Bench Sheet*.

6.2.1 All counting is done over a light table. Fish are recorded as live if they move when lightly prodded or gill movement is seen. Record all counts and readings in appropriate columns on the *Acute Bioassay Bench Sheet*.

6.3 Use the designated mixing cylinders to carefully pour fresh test solutions into the appropriate test chambers.

6.3.1 Fresh test solutions are prepared from sample bottles received on test-day 0 that have been stored at 0-6°C.

6.3.2 As noted in the initiation section, the person pouring up concentrations can use either the individual or half-dilution method.

6.3.2.1 The table below shows the breakdown of waters needed for the "Individual Concentration" renewal pour-up method for 4 jars (total volumes may differ if there are fewer test chambers in use):

Concentration	Diluent	Sample	Total Volume
Control	1000 mL	0 mL	1000 mL
6.25%	937.5 mL	62.5 mL	1000 mL
12.5%	875 mL	125 mL	1000 mL
25%	750 mL	250 mL	1000 mL
50%	500 mL	500 mL	1000 mL
75%	250 mL	750 mL	1000 mL
100%	0 mL	1000 mL	1000 mL

6.4 Collect about 20 mL of after-renewal solution (from test chamber C, if test chamber C is not available move to next available replicate) into clean parameter cup. Measure and record DO, pH, temperature, and conductivity in each of the cups containing the after-renewal solution.

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6.5 Verify that the number of surviving organisms per replicate is the same as the survival counts prior to renewal. Record the after-renewal counts on the bench sheet.

7 INTERIM READINGS

7.1 On day 1 (t = 24 hr) and any other interim readings if test duration is >48 hr (i.e. day 3 (t=72 hours)), count number of surviving fish, remove any dead fish, and record counts. Collect about 20 mL of each test concentration (from test chambers) into clean parameter cups. The FDEP laboratory typically collects parameter water from replicate B on day 1 and replicate C on day 2 (or A if 2 test chambers) and so on. Measure and record DO, pH, temperature (can be taken directly in the test chamber), and conductivity in each of the cups.

7.1.1 All counting is done over a light table. Fish are recorded as live if they move when lightly prodded or gill movement is seen. Record all counts and readings in appropriate columns on the *Acute Bioassay Bench Sheet*.

7.1.2 The phys-chem readings are aliquoted from the test chambers in order to minimize disturbance to the fish and reduce the possibility of contaminating the test solutions.

8 TEST TERMINATION

8.1 On termination day (typically t = 48 hr ± ~2 hr or t = 96 hr ± ~4 hr), count and record the number of surviving fish. Collect about 20 mL of each test concentration (from test chambers) into clean parameter cups. Measure and record DO, pH, temperature (can be taken directly in the test chamber), and conductivity in each of the cups. Record any comments pertaining to the appearance or condition of the fish. Record test termination date and time.

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

8.1.2 Counting is done over a light table. Phys-chem readings (pH, DO, and conductivity) are taken from a parameter vial. Temperature can be taken directly from the test chambers. At 96-hours, collect aliquots from the next replicate (usually A).

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

8.2 Pour contents of all chambers into a bucket with ice or fill the sink with ice. Triple rinse and then fill the jars with DI water and place them on the dirty glassware cart. Refer to Laboratory Quality Manual for waste disposal procedures.

8.3 Enter test results into the statistical program to obtain LC50 per SOP TA-7.17 for definitive tests.

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8.3.1 LC50s for screening tests are reported in LIMS as "100 L" if mortality is < 50% in the 100% sample test solution (written in the logbook as >100%); "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 highest test concentration does not have to be 100% sample but does have to comply with the percent effluent designated in the facility's NPDES permit.

8.4 Enter test results into LIMS.

8.4.1 The result is verified by a second person prior to being uploaded into LIMS per SOP BG-01.

8.4.2 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.

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.

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

9.3 FDEP Laboratory Information Management System (LIMS).

9.4 Record information in appropriate logbooks (*Acute Bioassay Bench Sheet*).

9.5 Record information in the *Bioassay Sample Log-In* logbook, per SOP TA-06.14.

9.6 Log sample receipt, temperature, and condition into *Acute Bioassay Bench Sheet* logbook. Fill in information requested at top of bench sheet 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.

9.7 Enter the LC50, EC50, NOEC, LOEC, and/or IC20 and IC25 results from the EPA WET Excel spreadsheet into the appropriate logbook, appropriate computer file,

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and LIMS results editor. A second person must verify data prior to uploading into LIMS.

9.8 Endpoint results for SRT tests are entered into laboratory control charts, located at \\floridadep\data\DEAR\Labs\Lab5\Biology\BenchCommon\ToxCommon\Bioassay Charts and Tables

10 QUALITY CONTROL

10.1 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.

10.2 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.

10.3 Samples should be held at 0-6 degrees C prior to test initiation and between renewals (exception for 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.

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

10.5 The method recommends tests and test cultures be maintained at 25±1°C (A. bahia tests are recommended to be maintained at 26±1°C). The method requires test temperatures not deviate more than 3° during the test period (max-min).

10.5.1 Because of the imprecise nature and thermal cycling of laboratory incubators, the Tox lab strives to maintain tests and cultures at 25±1°C but follow the 3° requirement as a test acceptability criterion. The incubators are set to alert if the temperature exceeds the range by a degree on either side for an hour. Temperature fluctuations below this threshold are minor enough to not impact the test/culture water temperatures significantly.

10.5.2 To ensure accuracy of test and culture temperatures within the incubators, the Tox lab has set correction factors to the continuous monitoring temperature probes within the incubators to ensure the temperature they are recording is as close to the

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actual test/culture water temperature as possible. The correction factors were established by taking an average of the test/culture temperatures on the shelf the monitor is on with an NIST-certified thermometer and correcting against the temperature the continuous monitoring probe was recording.

10.5.3 In the case that a test exceeds the three-degree test acceptability criterion, a comment must be added to the data as follows:

10.5.3.1 Test temperature >3°, no toxicity seen: "Temperature of test solutions deviated from method specifications."

10.5.3.2 Incubator temperature >3°, no toxicity seen: "Continuous temperature monitoring in surrogate test vessels deviated from method specifications."

10.5.3.3 Test temperature >3°, with toxicity seen: ["J" qualify] "Estimated value. Deviation of test solution temperatures from method specifications may be contributing to toxicity."

10.6 If controls fail to meet minimum acceptability criteria, the test will be invalidated, and the analyses re-run 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 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.

For SRT results, if all test acceptability criteria are met, but the results fall outside of the acceptable range of the control charts (2 standard deviations from the average endpoint over the last 20 tests), the lab supervisor should be notified immediately. Minor or rare deviations outside of the acceptable range for an SRT do not automatically mean it has failed as some deviation in live organisms is expected.

10.7 The 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

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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.

10.8 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.

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

10.10 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.

11 SAFETY/HAZARDOUS WASTE MANAGEMENT

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

11.2 Refer to the DEP Laboratory Health and Safety Plan.

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

11.4 Read MSDS information for reagent handling and disposal.

12 REFERENCES:

12.1 DEP Laboratory Quality Assurance Manual

12.2 DEP Health and Safety Manual

12.3 EPA 821-R-02-012 *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th Edition, Test Method 2000.0*

12.4 MSDS Information Sheets

12.5 DEP SOP FS 1000, General Sampling

12.6 DEP SOP BG-01, Data-Entry Verification

12.7 DEP SOP TA-01.03, Artemia nauplii Culture

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12.8 DEP SOP TA-03.15, Preparation of Synthetic Moderately Hard Freshwater

12.9 DEP SOP TA-04.20, Receiving and Maintaining Purchased Test Organisms

12.10 DEP SOP TA-06.08, Measuring Total Alkalinity and Hardness

12.11 DEP SOP TA-06.13, Thermometer Accuracy Check and Use

12.12 DEP SOP TA-06.14, Bioassay Sample Log-In Procedure

12.13 DEP SOP TA-06.22, Measuring Total Residual Chlorine *U*sing the Hach® Pocket Colorimeter™ II Test Kit

12.14 DEP SOP TA-06.33, Measuring Ammonia *U*sing HACH® Ammonia Nitrogen Test Kits for Freshwater and Saltwater Samples

12.15 DEP SOP TA-06.35, Measuring Total Ammonia *U*sing the HACH® Ammonia Nitrogen Pocket Colorimeter™ II Test Kit

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

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

12.18 DEP SOP TA-06.42, LIMS Entry for Laboratory Parameters

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

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

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

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

12.23 DEP SOP TA-09.18, Dechlorination Procedure using Sodium Thiosulfate

APPENDIX OF CHANGES

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