Measurement Quality Objectives for Chronic Freshwater Toxicity Test Methods

The following Measurement Quality Objectives establish recommendations and requirements for chronic freshwater toxicity testing conducted for Surface Water Ambient Monitoring Program (SWAMP) projects. Non-SWAMP projects should meet the minimum requirements established in the fourth edition of the U.S. EPA guidance document *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (821/R-02/013).

Table 1. Laboratory Quality Control for Chronic Freshwater Toxicity Test Methods

Negative Control	Frequency of Analysis	Measurement Quality Objective	Data Quality Indicator or Reasoning
Laboratory Control Water	Laboratory control water, consistent with the appropriate U.S. EPA test method, must be used with each analytical batch.	Laboratory control water must meet all test acceptability criteria for the species of interest.	Evaluates the health and sensitivity of the test organisms.
Additional Control Water for Manipulated Samples	Additional controls are required whenever manipulations are performed on one or more of the ambient samples within each analytical batch.	Both controls must meet test acceptability criteria, but if the secondary control is significantly different from the primary control, then the secondary control should be used for further statistical analysis in the determination of sample toxicity.	Evaluates the effects of manipulations upon the test organisms.
Additional Control Water for Unmanipulated Samples	Additional controls can be used for samples that have parameters near the tolerance threshold of the organism.	Must meet test acceptability criteria to be used for statistical comparisons. Does not have to be significantly different from the primary control for statistical comparisons.	Evaluates the effects of parameters near the tolerance threshold of the organism.
Positive Control	**Frequency of Analysis**	**Measurement Quality Objective**	**Data Quality Indicator or Reasoning**
Reference Toxicant Tests	One reference toxicant test per analytical batch is required when using organisms that are either commercially-supplied or wild-caught. Monthly reference toxicant testing is required for laboratories utilizing in-house cultures.	The last plotted data point (LC50 or EC50) should be within 2 standard deviations of the cumulative mean (n=20). Reference toxicant tests that fall outside of recommended control chart limits are evaluated to determine the validity of associated tests. A reference toxicant test outside of the 2 standard deviations does not invalidate the associated test results.	Used to assess intra-laboratory precision.

Table 2. Laboratory Quality Control Corrective Actions for Chronic Freshwater Toxicity Test Methods

Negative Control	Recommended Corrective Action
Laboratory Control Water	Laboratories must begin retesting affected samples and the associated control within 7 days of test failure or after resampling. The laboratory should try to determine the source of the control failure, document the investigation, and record the steps taken to prevent a recurrence.
Additional Control Water	Additional controls for manipulated samples must meet test acceptability criteria for the test to be valid.
Positive Control	**Recommended Corrective Action**
Reference Toxicant Tests	If the LC50 exceeds ± 2 standard deviations of the running mean of the last 20 reference toxicant tests, the laboratory should investigate sources of variability, take actions to reduce identified sources of variability, and may perform an additional reference toxicant test during the same month.

Table 3. Field Quality Control for Chronic Freshwater Toxicity Test Methods

Quality Control	Frequency of Analysis	Measurement Quality Objective	Data Quality Indicator or Reasoning
Field Blanks	Based on project requirements.	No statistical difference between the laboratory control and the field blank within an analytical batch.	Used to measure bias introduced during sample collection and handling.
Bottle Blanks	Based on project requirements.	No statistical difference between the laboratory control and the bottle blank within an analytical batch.	Used to measure bias introduced during washing procedures prior to collection.

Table 4. Field Quality Control Corrective Actions for Chronic Freshwater Toxicity Test Methods

Quality Control	Recommended Corrective Action
Field Blanks	If contamination of the field blanks and associated samples is known or suspected, the laboratory should flag the affected data. The project coordinator should be notified so that the sampling team can identify the contamination source(s) and perform corrective actions prior to the next sampling event.
Bottle Blanks	If contamination of the bottle blanks and associated samples is known or suspected, the laboratory should flag the affected data. The project coordinator should be notified so that the laboratory or vendor can identify the contamination source(s) and perform corrective actions prior to the next sampling event.

Table 5. Sample Handling for Chronic Freshwater Toxicity Test Methods

Container	Sample Receipt Temperature	Sample Preservation	Holding Time
Amber glass (recommended)	0 – 6 °C (required)	Wet or blue ice in field; 0 – 6 °C refrigeration in laboratory (do not freeze); dark at all times (required)	<48 hours (required)

Table 6. 6-8-Day Chronic Freshwater *Ceriodaphnia dubia* Survival and Reproduction Toxicity Test

Test Acceptability Criteria	≥80% mean survival in controls; 60% of the surviving control females must produce 3 broods with an average of 15 or more young per female (required)
Test Type	Static renewal (required)
Age at Test Initiation	<24 hours old and all released within an 8-hour period (required)
Replication at Test Initiation	10 (required minimum)
Organisms per Replicate	1 (assigned using blocking by known parentage; required)
Food Source	YCT and *S. capricornutum* (or comparable food; required)
Temperature Range	25 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required)
Renewal Frequency	100% daily renewal (required)
Test Duration	Until 60% or more of surviving control females have 3 broods (maximum test duration is 8 days; required)
Endpoints	Survival and reproduction (required)
Conductivity	100 – 1,900 µS/cm; substitute with *H. azteca* if conductivity is >2,500 µS/cm (recommended)
Light Intensity	10 – 20 µE/m^2/s or 50 – 100 ft-c (recommended)
Photoperiod	16 hours of ambient laboratory light, 8 hours dark (recommended)
Test Chamber Size	20 – 40 mL (recommended)
Replicate Volume	15 mL (recommended)
Feeding Regime	0.1 mL of YCT and 0.1 mL of *S. capricornutum* per test chamber daily (recommended)
Minimum Sample Volume	2.5 L for one-time grab sample (recommended)
Laboratory Control Water	Moderately hard water prepared in accordance with U.S. EPA protocols (recommended)
Initial Water Chemistry	1 DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement (required)
Renewal Water Chemistry	2 DO measurements (1 in old solution and 1 in new solution); 1 pH and temperature measurement (required)
Final Water Chemistry	1 DO, pH, conductivity, ammonia, and temperature measurement (required)
Initial DO Range	4.0 mg/L – 100% saturation (recommended)

Table 7. 10-Day Chronic Freshwater *Chironomus dilutus* Survival and Growth Toxicity Test

Test Acceptability Criteria	≥80% mean survival in the controls, and an average of ≥0.60 mg ash-free dry weight for surviving individuals (required)
Test Type	Static renewal (required)
Age at Test Initiation	7 – 10 days old, post hatch, and ≤0.12 mg/individual (ash-free dry weight; required)
Replication at Test Initiation	4 (required minimum)
Organisms per Replicate	10 (required minimum)
Food Source	Flake fish food (required)
Temperature Range	23 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required)
Renewal Frequency	80% renewal on days 2, 4, 6, and 8 (required)
Test Duration	10 days (required)
Endpoints	Survival and growth (required)
Conductivity	<12‰ salinity (recommended)
Light Intensity	100 – 1,000 lux (recommended)
Photoperiod	16 hours of ambient laboratory light, 8 hours dark (recommended)
Test Chamber Size	300 mL (recommended)
Test Chamber Substrate	5 mL of clean sand (recommended)
Replicate Volume	200 mL (recommended)
Feeding Regime	2 mg for days 1 – 3; 4 mg for days 4 – 6; and 6 mg for days 7 – 9 (recommended)
Minimum Sample Volume	2.5 L for one-time grab sample (recommended)
Laboratory Control Water	Culture water, well water, surface water, site water, or reconstituted water (recommended)
Initial Water Chemistry	1 DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement (required)
Renewal Water Chemistry	2 DO measurements (1 in old solution and 1 in new solution); 1 pH, conductivity, and temperature measurement (required)
Final Water Chemistry	1 DO, pH, conductivity, ammonia, and temperature measurement (required)
Initial DO Range	2.5 mg/L – 100% saturation (recommended)

Table 8. 10-Day Chronic Freshwater *Hyalella azteca* Survival and Growth Toxicity Test

Test Acceptability Criteria	≥80% mean survival in the controls, and measurable growth (required)
Test Type	Static renewal (required)
Age at Test Initiation	7 – 14 days old (required)
Replication at Test Initiation	4 (required minimum)
Organisms per Replicate	10 (required minimum)
Food Source	YCT (required)
Temperature Range	23 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required)
Renewal Frequency	80% renewal on day 2, 4, and 6 (required)
Test Duration	10 days (required)
Endpoints	Survival and growth (required)
Conductivity	<15‰ salinity (recommended)
Light Intensity	10 – 20 µE/m^2/s or 50 – 100 ft-c (recommended)
Photoperiod	16 hours of ambient laboratory light, 8 hours dark (recommended)
Test Chamber Size	300 mL (recommended)
Replicate Volume	100 mL (recommended)
Feeding Regime	1.5 mL every other day after water renewals (recommended)
Minimum Sample Volume	2.5 L for one-time grab sample (recommended)
Laboratory Control Water	Moderately hard water prepared in accordance with U.S. EPA protocols (recommended)
Initial Water Chemistry	1 DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement (required)
Renewal Water Chemistry	2 DO measurements (1 in old solution and 1 in new solution); 1 pH and temperature measurement (required)
Final Water Chemistry	1 DO, pH, conductivity, ammonia, and temperature measurement (required)
Initial DO Range	2.5 mg/L – 100% saturation (recommended)

Table 9. 7-Day Chronic Freshwater *Pimephales promelas* Survival and Growth Toxicity Test

Test Acceptability Criteria	≥80% mean survival in the controls, and an average of ≥0.25 mg dry weight for surviving individuals (required)
Test Type	Static renewal (required)
Age at Test Initiation	Newly-hatched larvae <24 hours old; if shipped, <48 hours old with a 24-hour age range (required)
Replication at Test Initiation	4 (required minimum)
Organisms per Replicate	10 (required minimum)
Food Source	Newly-hatched *Artemia* nauplii (<24 hours old; required)
Temperature Range	25 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required)
Renewal Frequency	80% daily renewal (required)
Test Duration	7 days (required)
Endpoints	Survival and growth (required)
Conductivity	100 – 1,900 µS/cm; substitute with alternate species if conductivity is >6,000 µS/cm (e.g. *A. affinis*; recommended)
Light Intensity	10 – 20 µE/m^2/s or 50 – 100 ft-c (recommended)
Photoperiod	16 hours of ambient laboratory light, 8 hours dark (recommended)
Test Chamber Size	500 mL (recommended)
Replicate Volume	250 mL (recommended)
Feeding Regime	On days 0 – 6, feed 0.1 g of newly hatched *Artemia* nauplii 3 times daily at 4-hour intervals or, as a minimum, 0.15 g twice daily at 6-hour intervals (at the beginning of the work day prior to renewal, and at the end of the work day following renewal); sufficient nauplii are added to provide an excess (recommended)
Minimum Sample Volume	7 L for one-time grab sample (recommended)
Laboratory Control Water	Moderately hard water prepared in accordance with U.S. EPA protocols (recommended)
Initial Water Chemistry	1 DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement (required)
Renewal Water Chemistry	2 DO measurements (1 in old solution and 1 in new solution); 1 pH and temperature measurement (required)
Final Water Chemistry	1 DO, pH, conductivity, ammonia, and temperature measurement (required)
Initial DO Range	4.0 mg/L – 100% saturation (recommended)

Table 10. 96-Hour Chronic Freshwater *Selenastrum capricornutum* Growth Toxicity Test

Test Acceptability Criteria	Mean cell density of at least 1 X 10^6 cells/mL in the controls, and variability (CV%) among control replicates less than or equal to 20% with EDTA addition; mean cell density of at least 2 X 10^5 cells/mL in the controls, and variability among control replicates less than or equal to 20% without EDTA addition (required)
Test Type	Static non-renewal (required)
Age at Test Initiation	4 – 7 days old (required)
Replication at Test Initiation	4 (required minimum)
Organisms per Replicate	10,000 cells per mL (recommended minimum)
Food Source	Not applicable
Temperature Range	25 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required)
Test Duration	96 hours (required)
Endpoint	Growth (required)
Conductivity	<1,500 µS /cm; substitute with alternate species if conductivity is >3,000 µS /cm (recommended)
Light Intensity	86 ± 8.6 µE/m2/s or 400 ± 40 ft-c (recommended)
Photoperiod	Continuous Illumination (“cool white” fluorescent lighting; recommended)
Test Chamber Size	125 mL – 250 mL (recommended)
Replicate Volume	50 mL – 100 mL (recommended)
Nutrient Media	Media prepared in accordance with U.S. EPA protocols (recommended)
Shaking Rate	100 cpm continuous, or twice daily by hand (recommended)
Minimum Sample Volume	1 L for one-time grab sample (recommended)
Laboratory Control Water	Moderately hard water prepared in accordance with U.S. EPA protocols (recommended)
Initial Water Chemistry	1 DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement (required)
Daily Water Chemistry	1 pH and temperature measurement (required)
Final Water Chemistry	1 DO, pH, conductivity, ammonia, and temperature measurement (required)
Initial DO Range	4.0 mg/L – 100% saturation (recommended)