

Chronic Freshwater Toxicity Testing

Terms appearing in the tables are defined in the [Surface Water Ambient Monitoring Program Quality Assurance Program Plan](#), which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control¹: Chronic Freshwater Toxicity Testing

Negative Controls	Frequency of Analysis	Control Limits
Laboratory Control Water	Laboratory control water consistent with Section 7 of the appropriate EPA method/manual must be tested with each analytical batch.	Laboratory control water must meet all test acceptability criteria (please refer to Section 7 of the appropriate EPA method/manual) for the species of interest.
Conductivity/Salinity Control Water	A conductivity or salinity control must be tested when these parameters are above or below the species tolerance.	Follow EPA guidance on interpreting data and refer to tables below for tolerance ranges.
Additional Control Water	Additional method blanks are required whenever manipulations are performed on one or more of the ambient samples within each analytical batch (e.g., pH adjustments, continuous aeration).	There must be no statistical difference between the laboratory control water and each additional control water within an analytical batch.
Sediment Control	Sediment control consistent with Section 7 of the appropriate EPA method/manual must be tested with each analytical batch of sediment toxicity tests.	Sediment control must meet all data acceptability criteria (please refer to Section 7 of the appropriate EPA method/manual) for the species of interest.
Positive Controls	Frequency of Analysis	Control Limits
Reference Toxicant Tests	Reference toxicant tests must be conducted monthly for species that are raised within a laboratory, or per analytical batch for commercially-supplied or field-collected species.	Last plotted data point (LC50 or EC50) must be within 2 SD 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. An out of control reference toxicant test result does not necessarily invalidate associated test results. More frequent and/or concurrent reference toxicant testing may be advantageous if recent problems have been identified in testing.

¹Unless method specifies more stringent requirements.

In special cases where the criteria listed in the above tables cannot be met, EPA minimum criteria may be followed. The affected data should be flagged accordingly.

Test data are reviewed to verify that the test acceptability criteria for a valid test have been met. Any test not meeting the minimum test acceptability criteria is considered invalid. All invalid tests should be repeated with the newly collected sample. If this is not possible, the test should be repeated with an archived sample and all tests must be properly flagged.

Deviations from the summary of recommended test conditions must be evaluated on a project-specific basis to determine the validity of test results. Depending on the degree of the departure and the objective of the test, deviations from recommended conditions may or may not invalidate a test result. Before rejecting or accepting a test result as valid, the reviewer should consider the degree of the deviation and the potential or observed impact of the deviation on the test result. For example, if dissolved oxygen is measured below 4.0 mg/L in one test chamber, the reviewer should consider whether any observed mortality in that test chamber corresponded with the drop in dissolved oxygen.

Table 1: Quality Control¹: Chronic Freshwater Toxicity Testing (continued)

Field Quality Control	Frequency of Analysis	Control Limits
Sample Duplicate	5% of total project sample count	Recommended acceptable RPD<20%
Field Blanks	Based on project requirements	No statistical difference between the laboratory control water (or sediment control) and the field blank within an analytical batch
Bottle Blanks	Based on project requirements	No statistical difference between the laboratory control water and the equipment blank within an analytical batch

¹Unless method specifies more stringent requirements.

In special cases where the criteria listed in the above tables cannot be met, EPA minimum criteria may be followed. The affected data should be flagged accordingly.

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Table 2: Corrective Action: Chronic Freshwater Toxicity Testing

Negative Controls	Corrective Action
Laboratory Control Water	If tested with in-house cultures, affected samples and associated quality control must be retested within 24 hours of test failure. If commercial cultures are used, they must be ordered within 16 hours of test failure for the earliest possible receipt. Retests must be initiated within 30 hours of receipt, depending on the need for organism acclimation. The laboratory should try to determine the source of the control failure, document the investigation, and document the steps taken to prevent a recurrence.
Conductivity/Salinity Control Water	Affected samples and associated quality control must be flagged.
Additional Control Water	Based on the objectives of the study, a water sample that has similar qualities to the test sample may be used as an additional control. Results that show statistical differences from the laboratory control should be flagged. The laboratory should try to determine the source of variation, document the investigation, and document the steps taken to prevent a recurrence. This is not applicable for TIE method blanks.
Sediment Control	Based on the objectives of the study, a sediment sample that has similar qualities to the test sample may be used as an additional control. Results that show statistical differences from the laboratory control should be flagged. The laboratory should try to determine the source of variation, document the investigation, and document the steps taken to prevent a recurrence.
Positive Controls	Corrective Action
Reference Toxicant Tests	If the LC50 exceeds +/- two standard deviations of the running mean of the last 20 reference toxicant tests, the test should be flagged.
Field Quality Control	Corrective Action
Field Duplicate	For duplicates with a heterogeneous matrix, results that do not meet SWAMP criteria should be flagged. The project coordinator should be notified so that the sampling team can identify the source of variation and perform corrective action prior to the next sampling event.
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 action prior to the next sampling event.
Equipment 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 action prior to the next sampling event.

Table 3: Chronic Freshwater Testing: 7-Day Survival and Growth *Pimephales promelas* Toxicity Test

Method Recommendation	
EPA/821/R-02/013 (Test Method 1000.0) or validated and SWAMP-approved alternative method	
Data Acceptability Requirements	
<i>Parameter</i>	<i>Criteria</i>
Test Acceptability Criteria ¹	80% or greater survival in controls and an average dry weight per original organism in control chambers equals or exceeds 0.25 mg
Data Qualification	
<i>Test Conditions</i>	<i>Required</i>
Test Type	Static renewal
Age at Test Initiation	Newly-hatched larvae <24 hours old. If shipped, <48 hours old with a 24-hour age range
Replication at Test Initiation	4 (minimum)
Organisms/Replicate	10 (minimum)
Food Source	Newly-hatched <i>Artemia</i> nauplii (<24 hours old)
Renewal Frequency	Daily
Test Duration	7 days
Endpoints	Survival and growth (biomass)
<i>Test Conditions</i>	<i>Recommended²</i>
Temperature Range	25 ± 1.0 °C (±3 °C required)
Light Intensity	10 – 20 µE/m ² /s or 50 – 100 ft-c
Photoperiod	16 hours of ambient laboratory light, 8 hours dark
Test Chamber Size	>500 mL or per method specific requirements
Replicate Volume	>250 mL or per method specific requirements
Feeding Regime	2 or 3 times per day
Laboratory Control Water	Moderately hard water prepared in accordance with EPA protocols
Minimum Sample Volume	7 L for one-time grab sample
<i>Sensitivity</i>	<i>Performance Criteria</i>
Reference Toxicant Testing	See Table 2
Water Chemistry	
<i>Test Parameter</i>	<i>Required Frequency</i>
Initial Water Chemistry	One DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement per sample and per dilution
Daily Water Chemistry	One initial DO, one final DO, and one final pH measurement per sample
Final Water Chemistry	One DO, pH, and temperature measurement per sample and per dilution
<i>Test Parameter</i>	<i>Recommended Criteria</i>
Initial DO Range	4.0 mg/L - 100% saturation
Initial pH Range	6.0 - 9.0
Conductivity Controls	Include appropriate controls when sample conductivities are 0 – 100, or above 1900 µS/cm
Sample Handling/Collection	
<i>Test Parameter</i>	<i>Recommended Conditions</i>
Relevant Media	Water column
Sample Container Type	Amber glass
Sample Preservation	Wet or blue ice in field, 0 - 6 °C refrigeration in laboratory, dark at all times
Sample Receipt Temperature	0 - 6 °C
Holding Time	<48 hours @ 0 - 6 °C; dark

¹Test data are reviewed to verify that test acceptability criteria (TAC) requirements for a valid test have been met. Any test not meeting these criteria is considered invalid. All invalid tests must be repeated with a newly collected sample.

²Deviations from the summary of recommended test conditions must be evaluated on a project-specific basis to determine the validity of test results. Depending on the degree of the departure and the objective of the test, deviations from recommended conditions may or may not invalidate a test result.

Table 4: Chronic Freshwater Testing: 6-8 Day Survival and Reproduction *Ceriodaphnia dubia* Toxicity Test

Method Recommendation	
EPA/821/R-02/013 (Test Method 1002.0) or validated and SWAMP-approved alternative method	
Data Acceptability Requirements	
<i>Parameter</i>	<i>Criteria</i>
Test Acceptability Criteria ¹	≥80% survival in controls and an average of 15 or more young per surviving female. 60% of the surviving control females must produce three broods.
Data Qualification	
<i>Test Conditions</i>	<i>Required</i>
Test Type	Static renewal
Age at Test Initiation	<24 hours old and all released within an 8-h period
Replication at Test Initiation	10 (minimum)
Organisms/Replicate	One (assigned using blocking by known parentage)
Food Source	YCT and <i>Selenastrum</i> or comparable food
Renewal Frequency	Daily
Test Duration	6-8 days (when 60% surviving females produces 3 rd brood)
Endpoints	Survival and reproduction
<i>Test Conditions</i>	<i>Recommended²</i>
Temperature Range	25 ± 1 °C (±3 °C required)
Light Intensity	10 – 20 µE/m ² /s or 50 – 100 ft-c
Photoperiod	16 hours of ambient laboratory light, 8 hours dark
Test Chamber Size	20 - 40 mL
Replicate Volume	>15 mL
Feeding Regime	Daily
Laboratory Control Water	Moderately hard water prepared in accordance with EPA protocols
Minimum Sample Volume	2 L for one-time grab sample
<i>Sensitivity</i>	<i>Performance Criteria</i>
Reference Toxicant Testing	See Table 2
Water Chemistry	
<i>Test Parameter</i>	<i>Required Frequency</i>
Initial Water Chemistry	One DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement per sample and per dilution
Daily Water Chemistry	One initial DO, one final DO, and one final pH measurement per sample
Final Water Chemistry	One DO, pH, and temperature measurement per sample and per dilution
<i>Test Parameter</i>	<i>Recommended Criteria</i>
Initial DO Range	4.0 mg/L - 100% saturation
Initial pH Range	6.0 - 9.0
Conductivity Controls	Include appropriate controls when sample conductivities are 0 – 100, or >1900 µS/cm. Substitute with <i>Hyalella azteca</i> if conductivity is >2500.
Sample Handling/Collection	
<i>Test Parameter</i>	<i>Recommended Conditions</i>
Relevant Media	Water column
Sample Container Type	Amber glass
Sample Preservation	Wet or blue ice in field, 0 - 6 °C refrigeration in laboratory, dark at all times
Sample Receipt Temperature	0 - 6 °C
Holding Time	<48 hours@ 0 - 6 °C; dark

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Table 5: Chronic Freshwater Testing: 96-Hour Growth *Selenastrum capricornutum* Toxicity Test

Method Recommendation	
EPA/821/R-02/013 (Test Method 1003.0) or validated and SWAMP-approved alternative method	
Data Acceptability Requirements	
<i>Parameter</i>	<i>Criteria</i>
Test Acceptability Criteria ¹	Mean cell density of at least 1×10^6 cells/mL in the controls and variability (CV%) among control replicates less than or equal to 20% (non-EDTA: Mean cell density of at least 2×10^5 cells/mL in the controls; and variability (CV%) among control replicates less than or equal to 20% (required))
Data Qualification	
<i>Test Conditions</i>	<i>Required</i>
Test Type	Static non-renewal
Age at Test Initiation	4 - 7 days
Replication at Test Initiation	4 (minimum)10,000 cells/mL (recommended)
Organisms/Replicate	10,000 cells/mL (recommended)
Food Source	n/a
Renewal Frequency	None
Test Duration	96 h
Endpoints	Growth
<i>Test Conditions</i>	<i>Recommended²</i>
Temperature Range	25 ± 1 °C (+/- 3 °C required)
Light Intensity	86 ± 8.6 $\mu\text{E}/\text{m}^2/\text{s}$ OR 400 ± 40 ft-c
Photoperiod	Continuous Illumination ("cool white" fluorescent lighting)
Test Chamber Size	125 mL or 250 mL
Replicate Volume	50 mL or 100 mL
Feeding Regime	None
Nutrient Media	Media prepared in accordance with EPA protocols
EDTA Addition	EDTA required per method
Laboratory Control Water	Moderately hard water or stock culture medium prepared in accordance with EPA protocols
Minimum Sample Volume	1 L for one-time grab sample
<i>Sensitivity</i>	<i>Performance Criteria</i>
Reference Toxicant Testing	See Table 2
Water Chemistry	
<i>Test Parameter</i>	<i>Required Frequency</i>
Initial Water Chemistry	One DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature measurement per sample and per dilution
Daily Water Chemistry	One pH measurement per sample
Final Water Chemistry	One DO, pH, and temperature measurement per sample and per dilution
<i>Test Parameter</i>	<i>Recommended Criteria</i>
Initial DO Range	4.0 mg/L - 100% saturation
Initial pH Range	6.0 - 9.0
Conductivity Controls	Include appropriate controls when sample conductivities exceed 1500 $\mu\text{S}/\text{cm}$
Sample Handling/Collection	
<i>Test Parameter</i>	<i>Recommended Conditions</i>
Relevant Media	Water column
Sample Container Type	Amber glass
Sample Preservation	Wet or blue ice in field, 0 - 6 °C refrigeration in laboratory, dark at all times
Sample Receipt Temperature	0 - 6 °C
Holding Time	<48 hours @ 0 - 6 °C; dark

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²Deviations from the summary of recommended test conditions must be evaluated on a project-specific basis to determine the validity of test results. Depending on the degree of the departure and the objective of the test, deviations from recommended conditions may or may not invalidate a test result.