

# Acute 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 Control<sup>1</sup>: Acute Freshwater Toxicity Testing**

Negative Controls	Frequency of Analysis	Control Limits
<b>Laboratory Control Water</b>	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.
<b>Conductivity/Salinity Control Water</b>	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.
<b>Additional Control Water</b>	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.
<b>Sediment Control</b>	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
<b>Reference Toxicant Tests</b>	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.

<sup>1</sup>Unless 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 Control<sup>1</sup>: Acute Freshwater Toxicity Testing (continued)**

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

<sup>1</sup>Unless 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.

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**Table 2: Corrective Action: Acute Freshwater Toxicity Testing**

<b>Negative Controls</b>	<b>Corrective Action</b>
<b>Laboratory Control Water</b>	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.
<b>Conductivity/Salinity Control Water</b>	Affected samples and associated quality control must be flagged.
<b>Additional Control Water</b>	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.
<b>Sediment Control</b>	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.
<b>Positive Controls</b>	<b>Corrective Action</b>
<b>Reference Toxicant Tests</b>	If the LC50 exceeds +/- two standard deviations of the running mean of the last 20 reference toxicant tests, the test should be flagged.
<b>Field Quality Control</b>	<b>Corrective Action</b>
<b>Field Duplicate</b>	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.
<b>Field Blanks</b>	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.
<b>Equipment Blanks</b>	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: Acute Freshwater Testing: 96-Hour Survival *Ceriodaphnia dubia* Toxicity Test**

<b>Method Recommendation</b>	
EPA/821/R-02/012 (Test Method 2002.0) or validated and SWAMP-approved alternative method	
<b>Data Acceptability Requirements</b>	
<i>Parameter</i>	<i>Criteria</i>
Test Acceptability Criteria <sup>1</sup>	≥90% survival in the controls
<b>Data Qualification</b>	
<i>Test Conditions</i>	<i>Required</i>
Test Type	Static renewal
Age at Test Initiation	<24hours
Replication at Test Initiation	4 (minimum)
Organisms/Replicate	5 (minimum)
Food Source	YCT and <i>Selenastrum</i> or comparable food
Test Duration	96 hours
Renewal Frequency	100% Daily Renewal
Feeding Regime	Feed while holding prior to test and 2 hours prior to test solution renewal
Endpoints	Survival
<i>Test Conditions</i>	<i>Recommended<sup>2</sup></i>
Temperature Range	25 ± 1 °C (±3 °C required)
Light Intensity	10 – 20 µE/m <sup>2</sup> /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
Laboratory Control Water	Moderately hard water 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
<b>Water Chemistry</b>	
<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.
<b>Sample Handling/Collection</b>	
<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

<sup>1</sup>Test 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.

<sup>2</sup>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.

**Table 4: Acute Freshwater Testing: 96-Hour Survival *Hyalella azteca* Toxicity Test**

<b>Method Recommendation</b>	
EPA/821/R-02/012 or validated and SWAMP-approved alternative method	
<b>Data Acceptability Requirements</b>	
<i>Parameter</i>	<i>Criteria</i>
Test Acceptability Criteria <sup>1</sup>	≥90% survival in controls
<b>Data Qualification</b>	
<i>Test Conditions</i>	<i>Required</i>
Test Type	Static renewal
Age at Test Initiation	7 – 14 days old
Replication at Test Initiation	4 (minimum)
Organisms/Replicate	10 (minimum)
Food Source	YCT
Renewal Frequency	80% renewal on Day 2
Test Duration	96 hours
Endpoints	Survival
<i>Test Conditions</i>	<i>Recommended<sup>2</sup></i>
Temperature Range	23 ± 1.0 °C (±3 °C required)
Light Intensity	10 – 20 μE/m <sup>2</sup> /s or 50 – 100 ft-c
Photoperiod	16 hours of ambient laboratory light, 8 hours dark
Test Chamber Size	300 mL
Replicate Volume	100 mL water
Feeding Regime	1.5 mL YCT every other day
Laboratory Control Water	Moderately hard water prepared in accordance with EPA protocols
Minimum Sample Volume	1L for one time grab sample
<i>Sensitivity</i>	<i>Performance Criteria</i>
Reference Toxicant Testing	See Table 2
<b>Water Chemistry</b>	
<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
Renewal 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	2.5 mg/L - 100% saturation
Initial pH Range	6.0 - 9.0
Conductivity Controls	Include appropriate controls when sample conductivities are 0 – 100, or >10,000 μS/cm
<b>Sample Handling/Collection</b>	
<i>Test Parameter</i>	<i>Recommended Conditions</i>
Relevant Media	Water
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

<sup>1</sup>Test 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.

<sup>2</sup>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.

**Table 5: Acute Freshwater Testing: 10-Day Survival *Hyalella azteca* Toxicity Test**

<b>Method Recommendation</b>	
EPA/821/R-02/012 or validated and SWAMP-approved alternative method	
<b>Data Acceptability Requirements</b>	
<i>Parameter</i>	<i>Criteria</i>
Test Acceptability Criteria <sup>1</sup>	≥80% survival in controls
<b>Data Qualification</b>	
<i>Test Conditions</i>	<i>Required</i>
Test Type	Static renewal
Age at Test Initiation	7 – 14 days old
Replication at Test Initiation	5 (minimum)
Organisms/Replicate	10 (minimum)
Food Source	YCT
Renewal Frequency	80% renewal every 48 hours
Test Duration	10 days
Endpoints	Survival
<i>Test Conditions</i>	<i>Recommended<sup>2</sup></i>
Temperature Range	23 ± 1.0 °C (±3 °C required)
Light Intensity	10 – 20 µE/m <sup>2</sup> /s or 50 – 100 ft-c
Photoperiod	16 hours of ambient laboratory light, 8 hours dark
Test Chamber Size	300 mL
Replicate Volume	100 mL water
Feeding Regime	1.5 mL YCT every other day
Laboratory Control Water	Moderately hard water prepared in accordance with EPA protocols
Minimum Sample Volume	1L for one time grab sample
<i>Sensitivity</i>	<i>Performance Criteria</i>
Reference Toxicant Testing	See Table 2
<b>Water Chemistry</b>	
<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
Renewal 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	2.5 mg/L - 100% saturation
Initial pH Range	6.0 - 9.0
Conductivity Controls	Include appropriate controls when sample conductivities are 0 – 100, or >10,000 µS/cm
<b>Sample Handling/Collection</b>	
<i>Test Parameter</i>	<i>Recommended Conditions</i>
Relevant Media	Water
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

<sup>1</sup>Test 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.

<sup>2</sup>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.