

Measurement Quality Objectives for Chronic Marine Water Toxicity Test Methods



The following Measurement Quality Objectives establish recommendations and requirements for chronic marine water toxicity testing conducted for State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP) projects. Non-SWAMP projects should meet the minimum requirements established in the first edition of the U.S. EPA guidance document *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (600/R-95/136).

Table 1. Laboratory Quality Control for Chronic Marine Water 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 Marine Water 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 Marine Water Toxicity Test Methods

| Field 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 Marine Water Toxicity Test Methods

| Field 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 Marine Water 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. 7-Day Chronic Marine Water *Americamysis bahia* Survival, Growth, and Fecundity Toxicity Test

| | |
|---------------------------------------|--|
| Test Acceptability Criteria | ≥80% mean survival in the controls, and an average weight of 0.20 mg (required); fecundity may be used if 50% or more of females in controls produce eggs (required if fecundity endpoint is used) |
| Test Type | Static renewal (required) |
| Age at Test Initiation | 7 days old (required) |
| Replication at Test Initiation | 8 (required minimum) |
| Organisms per Replicate | 5 (required minimum) |
| Food Source | Newly-hatched <i>Artemia</i> nauplii (<24 hours old; required) |
| Temperature Range | 26 °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); egg development (recommended) |
| Salinity | 20 – 30 ppt ± 2 ppt (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 | 400 mL (recommended) |
| Replicate Volume | 150 mL (recommended) |
| Feeding Regime | 150 nauplii per mysid daily; half after test solution renewal and half after 8 – 12 hours (recommended) |
| Minimum Sample Volume | 3 L per day (recommended) |
| Laboratory Control Water | 1 μm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater and reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Renewal Water Chemistry | 2 DO measurements (1 in old solution and 1 in new solution); 1 pH, salinity, and temperature measurement (required) |
| Final Water Chemistry | 1 DO, pH, salinity, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |

Table 7. 7-Day Chronic Marine Water *Atherinops affinis* Survival and Growth Toxicity Test

| | |
|---------------------------------------|---|
| Test Acceptability Criteria | ≥80% mean survival in the controls, and an average weight of 0.85 mg; survival MSD must achieve <25%, and growth MSD must achieve <50% (required) |
| Test Type | Static renewal (required) |
| Age at Test Initiation | 9 – 15 days old, post-hatch (required) |
| Replication at Test Initiation | 5 (required minimum) |
| Organisms per Replicate | 5 (required minimum) |
| Food Source | Newly-hatched <i>Artemia</i> nauplii (<24 hours old; required) |
| Temperature Range | 20 °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) |
| Salinity | 5 – 36 ppt ± 2 ppt (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 | 600 mL (recommended) |
| Replicate Volume | 200 mL (recommended) |
| Feeding Regime | 40 nauplii per larvae, twice daily (recommended) |
| Minimum Sample Volume | 8 L for one-time grab sample (recommended) |
| Laboratory Control Water | 1 μm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater and reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Renewal Water Chemistry | 2 DO measurements (1 in old solution and 1 in new solution); 1 pH, salinity, and temperature measurement (required) |
| Final Water Chemistry | 1 DO, pH, salinity, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |

Table 8. 48-Hour Chronic Marine Water *Haliotis rufescens* Larval Development Toxicity Test

| | |
|---------------------------------------|---|
| Test Acceptability Criteria | ≥80% mean normal shell development in the controls; must achieve an MSD of <20% (required) |
| Test Type | Static non-renewal (required) |
| Age at Test Initiation | Not applicable |
| Replication at Test Initiation | 5 (required minimum) |
| Organisms per Replicate | 5 – 10 larvae per mL (required) |
| Food Source | Do not feed (required) |
| Temperature Range | 15 °C ± 1°C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required) |
| Test Duration | 48 hours (required) |
| Endpoint | Normal shell development (required) |
| Salinity | 34 ppt ± 2 ppt (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 mL (recommended) |
| Replicate Volume | 10 mL (recommended) |
| Minimum Sample Volume | 1 L for one-time grab sample (recommended) |
| Laboratory Control Water | 1 µm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater plus reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Final Water Chemistry | 1 DO, pH, salinity, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |

Table 9. 7-Day Chronic Marine Water Survival and Growth *Holmesimysis costata* Toxicity Test

| | |
|---------------------------------------|--|
| Test Acceptability Criteria | ≥75% survival in the controls, and an average dry weight of ≥0.40 µg; survival MSD must achieve <40%, and growth MSD must achieve <50 µg (required) |
| Test Type | Static renewal (required) |
| Age at Test Initiation | 3 – 4 days old, post-hatch juveniles (required) |
| Replication at Test Initiation | 5 (required minimum) |
| Organisms per Replicate | 5 (required minimum) |
| Food Source | Newly-hatched <i>Artemia</i> nauplii (<24 hours old; required) |
| Temperature Range | 13 °C ± 1 °C (recommended for mysids collected north of Pt. Conception); 15 °C ± 1 °C (recommended for mysids collected south of Pt. Conception); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required) |
| Renewal Frequency | 75% renewal at 48 and 96 hours (required) |
| Test Duration | 7 days (required) |
| Endpoints | Survival and growth (required) |
| Salinity | 34 ppt ± 2 ppt (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 | 1,000 mL (recommended) |
| Replicate Volume | 200 mL (recommended) |
| Feeding Regime | 40 nauplii per larvae, twice daily (recommended) |
| Minimum Sample Volume | 3 L for one-time grab sample (recommended) |
| Laboratory Control Water | 1 µm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater and reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Renewal Water Chemistry | 2 DO measurements (1 in old solution and 1 in new solution); 1 pH, salinity, and temperature measurement (required) |
| Final Water Chemistry | 1 DO, pH, salinity, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |

Table 10. 48-Hour Chronic Marine Water *Macrocystis pyrifera* Germination and Germ Tube Length Toxicity Test

| | |
|---------------------------------------|--|
| Test Acceptability Criteria | ≥70% mean germination in the controls, and an average ≥10 µm germ tube length; must achieve an MSD of <20% for both endpoints (required) |
| Test Type | Static non-renewal (required) |
| Age at Test Initiation | Not applicable |
| Replication at Test Initiation | 5 (required minimum) |
| Organisms per Replicate | 7,500 spores per mL of test solution (required) |
| Food Source | Not applicable |
| Temperature Range | 15 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required) |
| Test Duration | 48 hours (required) |
| Endpoints | Germination and germ tube length (required) |
| Salinity | 34 ppt ± 2 ppt (recommended) |
| Light Intensity | 50 ± 10 µE/m ² /s (recommended) |
| Photoperiod | 16 hours of ambient laboratory light, 8 hours dark (recommended) |
| Test Chamber Size | 600 mL (recommended) |
| Replicate Volume | 200 mL (recommended) |
| Minimum Sample Volume | 2 L for one-time grab sample (recommended) |
| Laboratory Control Water | 1 µm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater plus reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Final Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |

Table 11. 48-Hour Chronic Marine Water *Mytilus galloprovincialis* and *M. spp.* Larval Development Toxicity Test

| | |
|---------------------------------------|---|
| Test Acceptability Criteria | ≥50% mean survival in the controls, and ≥90% mean normal shell development; must achieve an MSD of <25% for normal shell development (required) |
| Test Type | Static non-renewal (required) |
| Age at Test Initiation | Within 4 hours of fertilization (required) |
| Replication at Test Initiation | 4 (required minimum) |
| Organisms per Replicate | 15 – 20 larvae per mL (required) |
| Food Source | Do not feed (required) |
| Temperature Range | 15 °C or 18 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required) |
| Test Duration | 48 hours (required) |
| Endpoints | Survival of larvae with prodissoconch (required) |
| Salinity | 28 – 34 ppt ± 2 ppt (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 mL (recommended) |
| Replicate Volume | 10 mL (recommended) |
| Minimum Sample Volume | 1 L for one-time grab sample (recommended) |
| Laboratory Control Water | 1 μm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater plus reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Final Water Chemistry | 1 DO, pH, salinity, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |

Table 12. 72-Hour Chronic Marine Water *Strongylocentrotus purpuratus* and *Dendraster excentricus* Embryo Development Toxicity Test

| | |
|---------------------------------------|---|
| Test Acceptability Criteria | ≥80% mean normal development in the controls; must achieve an MSD of <25% (required) |
| Test Type | Static non-renewal (required) |
| Age at Test Initiation | <1 hour, post fertilization (required) |
| Replication at Test Initiation | 4 (required minimum) |
| Organisms per Replicate | 250 embryos (required) |
| Food Source | Do not feed (required) |
| Temperature Range | 15 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required) |
| Test Duration | 72 hours (required) |
| Endpoints | Normal development (required); survival can be included (recommended) |
| Salinity | 34 ppt ± 2 ppt (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 mL (recommended) |
| Replicate Volume | 10 mL (recommended) |
| Minimum Sample Volume | 1L for one-time grab sample (recommended) |
| Laboratory Control Water | 1 μm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater plus reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Final Water Chemistry | 1 DO, pH, salinity, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |

Table 13. 20-Minute Chronic Marine Water *Strongylocentrotus purpuratus* and *Dendraster excentricus* Fertilization Toxicity Test

| | |
|---------------------------------------|---|
| Test Acceptability Criteria | ≥70% mean egg fertilization and appropriate sperm counts in controls; must achieve an MSD of <25% (required) |
| Test Type | Static non-renewal (required) |
| Age at Test Initiation | <1 hour, post fertilization (required) |
| Replication at Test Initiation | 4 (required minimum) |
| Organisms per Replicate | ~1,120 eggs and not more than 3,360,000 sperm per test tube (required) |
| Food Source | Do not feed (required) |
| Temperature Range | 12 °C or 15 °C ± 1 °C (recommended); the maximum temperature must not deviate from the minimum temperature by more than 3 °C (required) |
| Test Duration | 40 minutes (20 minutes + 20 minutes; required) |
| Endpoint | Fertilization of eggs (required) |
| Salinity | 34 ppt ± 2 ppt (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 mL (recommended) |
| Replicate Volume | 5 mL (recommended) |
| Feeding Regime | Do not feed |
| Minimum Sample Volume | 1L for one-time grab sample (recommended) |
| Laboratory Control Water | 1 μm filtered natural seawater or hyper-saline brine prepared from uncontaminated natural seawater plus reagent water (recommended) |
| Initial Water Chemistry | 1 DO, pH, salinity, ammonia, and temperature measurement (required) |
| Initial DO Range | 4.0 mg/L – 100% saturation (recommended) |