

STANDARD OPERATING PROCEDURE
Bench-Scale Procedure for Measuring Residual Toxicity
Using the Freshwater Rotifer *Branchionus calyciflorus*

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RECORD OF AMENDMENTS:

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BACKGROUND

The Great Ships Initiative (GSI) is a collaborative effort to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System through independent research and demonstration of environmental technology, financial incentives and consistent basin-wide harbor monitoring. To that end, the GSI has established a shore-based high-flow Research, Development and Technology Evaluation (RDTE) facility in Superior, Wisconsin to provide intensive testing services to vendors of ballast treatment prospects suitable to Seaway-sized vessels. Laboratory space within the University of Wisconsin-Superior (UW-S) and University of Minnesota-Duluth is utilized to meet GSI bench-scale test objectives, as well as for non-time sensitive analysis of samples from the shore-based and shipboard scale tests. The UW-S has space in several of their research labs dedicated to the GSI project. Bench-scale experiments are conducted in the university's Aquatic Toxicity Laboratory which maintains active cultures of zooplankton, phytoplankton, and aquatic invertebrates. The laboratory contains a series of mini-diluters for water-only acute and chronic toxicity tests and is equipped to run static, intermittent renewal, and flow-through tests. A variety of meters are available for monitoring water quality including conductivity, salinity, pH, dissolved oxygen, temperature, and select ions.

INTRODUCTION

This bench-scale procedure measures the residual toxicity of water treated by a ballast water treatment method to organisms in receiving systems using the freshwater rotifer, *Branchionus calyciflorus*, during a 24 hour static test. During the test, organisms are continuously exposed to selected concentrations of water treated by a ballast water treatment method, with survival recorded daily for the duration of the test.

EQUIPMENT LIST

- Environmental chamber
- Test substance
- Specific conductance meter
- Hardness/alkalinity reagents
- Fire-polished pipette
- Dilution water
- Dissolved oxygen meter
- Controlled photoperiod lighting
- pH meter
- Partial immersion thermometer

- *B. calyciflorus* cysts
- Tissue culture plate (1.0 mL)

PROCEDURE

1. Conduct procedure in a vented work area, taking appropriate health and safety measures.
2. Prepare exposure solutions in the appropriate water type (harbor water or filtered harbor water), with the highest exposure concentration equal to the lowest concentration that resulted in 100 % mortality based on dose effectiveness testing (GSI/SOP/BS/DE/3). Make additional solutions using a 0.5 dilution scheme.
3. Age solutions in the dark at 25.0° C for 24 hours before beginning residual toxicity exposures. For any exposure solutions where survival is significantly different ($\alpha = 0.05$) from the controls at 24 hours, start a new set of exposures with solutions that have been aged 24 hours in the light at approximately 1000 lumens/m², 150 μ W/cm² UVA and 10 μ W/cm² UVB.
4. If the exposure water has been treated with a chemical (i.e., hydrogen peroxide), measure the concentrations of the chemical if they are detectable. It is not necessary to chemically analyze the dilution series; however the additional information may be useful if further testing is needed.
5. Conduct exposures experiments using test animals obtained by hatching cysts. Initiate the cyst hatching procedure approximately 16 hours before the start of the test. Hatching should begin after approximately 15 hours, and by 20 hours approximately 50 % of the cysts should have hatched. A hatching percent of 50 % is common.
6. Carry out tests in a chamber where the temperature can be controlled. All tests should be conducted at a temperature of 25 + 1 °C. Incubation should be conducted in a 16 hours light/8 hours dark cycle at illumination of approximately 962 lumens/m², 151.9 μ W/cm² UVA and 8.4. μ W/cm² UVB.
7. Measure and record temperature, pH, alkalinity, hardness, and dissolved oxygen of the test solutions at the beginning of the test. Enough sample must be prepared to allow the above parameters to be measured.
8. Collect rotifers using a micropipette with a bore large enough to allow animals to enter and exit without injury. Rotifers should be first transferred to a rinsing well containing the appropriate concentration of toxicant. Rotifers can then be transferred to test wells, observing under a microscope their exit from the micropipette and entry into the test solutions. Count exactly five animals per well ensuring that the volume of medium carried over with the rotifers is minimized, and that the rotifers are randomly assigned to the test chambers.

9. Ensure that each test concentration has four replicates, for a total of 20 animals per concentration. Test chambers containing only dilution water and animals should be set up as controls. No more than 15 % mortality may occur in 24 hours among control rotifers for the test to be valid. When a solvent is used with a toxicant, a solvent control exposure should be included at a concentration equal to the highest concentration of solvent used in the definitive test. Cover chambers with the well plate cover to prevent solution volatilization. Cross contamination may be a concern if toxicant is volatile and tests are conducted in well plates. Do not feed animals during the test.
10. All data should be recorded on a data recording sheet. Data should include the date that cultures were started, renewed, counted, and harvested as well as information on preparation of medium, signs of contamination, survival, etc.
11. It is desirable to determine a 24 hour LC50 using a reference chemical at least once every 10-15 tests with *B. calyciflorus* in order to demonstrate test animal sensitivity and conformity of the experimental procedure.

QUALITY ASSURANCE/QUALITY CONTROL

Control survival must be at least 85 % for the test to be acceptable. Concurrent toxicity tests of the same type as described above with a reference toxicant (KCl) must be performed. This test will document organism sensitivity.

Lab performance is demonstrated by performing at least one reference toxicant test per month if a concurrent test is not conducted as described above.

A control chart is prepared for each combination of reference toxicants, test species, test conditions, and endpoints. The chart consists of a running plot for the 20 most recent values (LC50). End points are determined to see if they are within acceptable limits. The control chart depicts the central tendency of the mean value and the upper and lower control values are set as two standard deviations from the mean.

REFERENCES

ASTM. 2004. Standard Guide For Acute Toxicity Test With The Rotifer *Brachionus*. E 1440 – 91 (Reapproved 2004).

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Fleming, K. 2004. State of Wisconsin Aquatic Life Toxicity Testing Methods Manual, 2nd edition, Wisconsin Department of Natural Resources, Bureau of Watershed Management. P.O. Box 7921, Madison, WI. 53707.

Great Ships Initiative Standard Operating Protocols: <http://www.nemw.org/GSI/protocols.htm>.

Great Ships Initiative website: www.greatshipsinitiative.org.

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