

**STANDARD OPERATING PROCEDURE**  
**Bench-Scale Procedure for Assessing Dose-Effectiveness of a**  
**Ballast Water Treatment System Using the Freshwater**  
**Rotifer *Branchionus calyciflorus***

Compiled By:

Signed: \_\_\_\_\_

Title: \_\_\_\_\_

Date: \_\_\_\_\_

Approved By:

Signed: \_\_\_\_\_

Title: \_\_\_\_\_

Date: \_\_\_\_\_

Cleared For Issue By:

Signed: \_\_\_\_\_

Title: \_\_\_\_\_

Date: \_\_\_\_\_

**RECORD OF AMENDMENTS:**

<u>No.</u>	<u>Date</u>	<u>Type</u>	<u>No.</u>	<u>Date</u>	<u>Type</u>
1.	_____	_____	7.	_____	_____
2.	_____	_____	8.	_____	_____
3.	_____	_____	9.	_____	_____
4.	_____	_____	10.	_____	_____
5.	_____	_____	11.	_____	_____
6.	_____	_____	12.	_____	_____

**STANDARD OPERATING PROCEDURE**  
**Bench-Scale Procedure for Assessing Dose-Effectiveness of a**  
**Ballast Water Treatment System Using the Freshwater**  
**Rotifer *Branchionus calyciflorus***

## 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 evaluates the biological effectiveness of a ballast water treatment system by measuring the viability of the freshwater rotifer *Branchionus calyciflorus* exposed to treated water. Rotifer cysts are induced to hatch in 16 to 22 hours by incubating them at 25 °C in standard dilution water. The cysts are exposed immediately to two or more concentrations of test solution (e.g. treated ballast water) plus a control in covered dishes. After 24 hours, the percentage of dead animals in each dish are recorded. An appropriate statistical method is used to calculate the lowest-observed-effective-dose (LOED), lethal dose that effects 99 % of the experimental population (LD99), and the lethal dose that effects 100 % of the experimental population (LD100).

## EQUIPMENT LIST

- Environmental chamber
- Test solution
- Specific conductance meter
- Hardness/Alkalinity
- Fire-polished pipette

- Reagents
- 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. Use test animals obtained by hatching cysts purchased from Aquatic Eco-Systems, Inc. Rotifer cyst hatching should be initiated approximately 16 hours before the start of the toxicity test. Hatching should begin after approximately 15 hours, and by 20 hours approximately 50 % of the cysts should have hatched. A hatching percent of 50-60 % is common.
3. Conduct a range-finding toxicity test to determine the approximate effect concentration of the test substance. Begin by using concentrations of 0.1, 10, 100, and 1000 mg/L (use higher or lower concentrations if necessary). The test should run 24 hours and need not to be duplicated.
4. Based on the results of the range-finding test, set up a definitive test using five concentrations with a dilution factor of 0.5 or greater. The test solution volume required is 2.0 mL per replicate. The highest test concentration will be that concentration which produced total mortality in the range-finding test. The test should run 24 hours.
5. Conduct the static acute toxicity test in an environmental chamber, where the temperature can be controlled. All tests should be conducted at a temperature of  $25 \pm 1$  °C. Incubation should be conducted in darkness.
6. Check the nominal concentrations of the test chemical by analyzing an aliquot of each of the stock exposure solutions.
7. Record the temperature, pH and hardness of the test solutions at the beginning and end of the test. Also measure dissolved oxygen at the beginning of the test. Adjust the volume of solution required to accommodate the exposure and samples required for chemical characteristic analysis.
8. Collect rotifers using a micropipette with a bore large enough to allow animals to enter and exit without injury. The volume of medium carried over with the rotifers

- should be minimized. Rotifers must be randomly assigned to the test chambers. This procedure permits counting exactly five animals per well. Rotifers should be collected and 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.
9. Each test concentration should have four replicates, for a total of 20 animals per concentration. Test chambers containing only test water without the toxicant 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. Cross contamination may be a concern if toxicant is volatile and tests are conducted in well plates. Animals are not fed during the test.
  10. All data should be recorded on the 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.

## **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 reference test will document organism sensitivity.

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

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).

Cangelosi, A.A. 2006. RDTE Facility for the Great Ships Initiative (GSI) (OAR-SG-2006-20000364). Project Proposal to the National Oceanic and Atmospheric Administration/U.S. Fish and Wildlife Service.

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](http://www.greatshipsinitiative.org).

DRAFT