

STANDARD OPERATING PROCEDURE
Bench-Scale Procedure for Measuring Residual Toxicity
Using the Amphipod *Hyaletta azteca*

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 Measuring Residual Toxicity
Using the Amphipod *Hyaella azteca*

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 amphipod, *Hyaella azteca*, during a 48 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

- Temperature controlled water bath
- Water/aeration sources
- Controlled photoperiod lighting
- 300-mL high-form lipless beakers
- Fire-polished pipette
- Dissolved oxygen, conductivity, and pH meters
- Alkalinity/hardness reagents
- Thermometer
- Examination pans
- Microscope

- Drying oven
- Desiccator
- Cleaning brush
- Labeling tape/permanent marker

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/2). 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 in 300 mL beakers containing 200.0 mL of exposure water and 10 test organisms. Each test concentration should have three replicates, for a total of 30 animals per concentration. Cover beakers with a glass plate to prevent solution volatilization and evaporation. Test chambers containing only control water and animals should be set up as performance controls. No more than 10 % mortality may occur in control organisms for the test to be valid.
6. Measure and record specific conductance, hardness, and alkalinity in the control, low and high test concentrations at the start of the test. Measure pH and dissolved oxygen in the control, low, median, and high test concentrations at beginning and end of test. Temperature must be measured in the control, low, median, and high test concentrations at 0, 24, 48 hours.
7. Record mortality data at least every 24 hours, after the beginning of the exposure. Note: Mortality can be used as a test endpoint if heartbeat cessation is determined by using a microscope. Use mortality data to estimate a median lethal concentration (LC50) with respective 95% confidence limits using the Trimmed Spearman-Kärber Method.

QUALITY ASSURANCE/QUALITY CONTROL

Control survival must be at least 90 % for the test to be acceptable. Concurrent toxicity tests of the same type as described above with a reference toxicant (KCI) 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

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.

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

Great Ships Initiative website: www.greatshipsinitiative.org.

U.S. EPA. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-99/064. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460.