

**STANDARD OPERATING PROCEDURE  
Bench-Scale Procedure for Assessing Dose-Effectiveness of a  
Ballast Water Treatment System Using a Copepod**

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## **STANDARD OPERATING PROCEDURE**

### **Bench-Scale Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using a Copepod**

#### **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 survival of a copepod exposed to treated water. The procedure estimates the acute toxicity of treated water to adult species during a 48 hr, static test. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and biological components that adversely affect the physiological and biochemical functions of the test organisms (EPA, 1994). Daily observations on mortality make it possible to also calculate acute toxicity for desired exposure periods less than 48 hours.

#### **EQUIPMENT LIST**

- Treated water
- Dilution water
- Thermometer
- Glass beakers
- Lighted magnifying lens
- Conductivity meter
- Temperature controlled chamber
- pH meter

- Dissolved oxygen meter

## PROCEDURE

1. Conduct procedure in a vented work area, taking appropriate health and safety measures.
2. Use only adult test animals purchased from a reputable supplier. Combine organisms from multiple shipping containers in to a single container to allow for complete randomization prior to the start of the test.
3. Prepare five exposure concentrations using a 0.5 or greater dilution scheme and one control (untreated water). Check the nominal concentrations of the test chemical (if relevant) by analyzing an aliquot of each the stock exposure solutions.
4. Add approximately 50 mL of an exposure solution to each of 3 x 300 mL glass beakers and label. Repeat this for each exposure concentration as well as the control solution.
5. Analyze an aliquot of each exposure solution for the following parameters: temperature, dissolved oxygen, pH, conductivity, alkalinity, and hardness and record. A range finding test may need to be conducted to determine concentrations used in the definitive test.
6. Add ten test organisms to each beaker. Cover beakers with a glass plate and place in a 25.0 °C temperature-controlled environment, in complete darkness. NOTE: The trays must be rotated at 24 hour due to the temperature differential in the chamber.
7. Count survival at a minimum of 24 hour intervals and record. Control survival must be at least 90 percent for the test to be acceptable. Death is defined as lack of any movement when viewing an organism with a microscope.
8. Measure temperature, dissolved oxygen, pH at the end of each 24 hour exposure period in control, low, middle, and high treatment and record. At the end of the test, temperature, dissolved oxygen, pH must be measured in at least one solution at each test concentration. Conductivity, alkalinity, and hardness should also be measured in at least one solution of each test concentration. It may be necessary to composite replicate samples to obtain enough sample volume for analysis. The toxicant concentrations of the test chemical should also be checked by analyzing an aliquot of each of the exposure solutions.
9. Use an appropriate statistical method to calculate the lowest-observed-effective-dose (LOED), lethal dose that effects 99 percent of the experimental population (LD99), and the lethal dose that effects 100% of the experimental population (LD100).

## QUALITY ASSURANCE/QUALITY CONTROL

Control survival must be at least 90 percent for the test to be acceptable. Concurrent toxicity tests of the same type as described above with a reference toxicant (KCl) must be performed to document organism sensitivity.

Lab performance is demonstrated by performing at least one reference toxicant test per month if the above concurrent test has not been conducted. A reference test will also be conducted when a new batch of organism is purchased from a supplier.

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 charts depict the central tendency of the mean value and the upper and lower control values are set as two standard deviations from the mean.

## REFERENCES

American Society for Testing and Materials. 2004. Water and environmental technology; biological effects and environmental fate; biotechnology and pesticides. E1192-97. In Annual Book of ASTM Standards, Vol 11.05. West Conshohocken, PA, pp 369 - 382.

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

Hamilton, M.A., R.C. Russo and R.V. Thurston. 1977. Trimmed Spearman-Kärber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714-719. Correction 12:417.

Weber, Cornelius (Ed). 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms, EPA 600/4-4-90/027F, August 1993, Office of Research and Development, US EPA, Cincinnati, OH 45268.