GREAT SHIPS INITIATIVE
BENCH-SCALE TEST FINDINGS
Technical Report - Public

Sodium Hydroxide (NaOH)

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ABSTRACT

The Great Ships Initiative (GSI), an innovative collaboration whose objective is to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System, conducts bench-scale research to aid developers of innovative technologies which could have application as ballast treatment systems. This report describes 2008 findings from bench-scale evaluations of a ballast treatment proposed by researchers from the U.S. Geological Survey's Leetown Science Center in Kearneysville, West Virginia. The treatment, sodium hydroxide (NaOH) in the formulation for lye or caustic soda, is intended as a contingency treatment for ships with no ballast onboard (NOBOB) which have not purged unpumpable residuals enroute. GSI tested the proposed formulation to assist range-finding for effective doses, determination of the rates of chemical degradation, and the potential for residual toxicity. Please see www.greatshipsinitiative.org for more information about GSI's bench-scale testing program.

In these tests, the pH levels of 11.5, 12.0, and 12.5 were effective at killing the broad range of aquatic organisms tested. They were most effective at inactivating rotifer adults, adult daphnia, copepods, and did so within 4 hours. NaOH-treatment resulting in pH levels of 12.0 and 12.5 reduced *E. coli* and *Enterococcus* to less than 1 MPN within 2 hours. While heterotrophic bacteria and green algae (*Selenastrum)*, were more resistant to treatment; bacteria were reduced by three logs, and there was complete *Selenastrum* mortality at 48 hours in the pH level of 12.5.

There was no substantial change in elevated pH levels in NaOH-treated water (Duluth-Superior Harbor water or laboratory water) over 96 hours except where sediments were present. Sediments led to a slight lowering of pH. These results indicate that treated water will remain toxic until it is actively neutralized or diluted. However, there was no acute residual toxicity observed at either dilution of 1:100 or 1:1000. While this method could not be employed effectively (unmodified) for treating discharge from fully ballasted tanks, these tests give preliminary support to the proposal to use this method for ships in the NOBOB condition.

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INTRODUCTION

This technical report presents quantitative and measured findings from GSI bench-scale evaluations of sodium hydroxide (NaOH), i.e., lye or caustic soda, as a potential treatment for ballast residuals. Researchers from the U.S. Geological Survey's Leetown Science Center in Kearneysville, West Virginia, proposed NaOH as a contingency treatment for ships with no ballast onboard (NOBOB) which have not purged unpumpable residuals enroute. GSI undertook these bench-scale tests during 2008 at the Lake Superior Research Institute of the University of Wisconsin-Superior in Superior, Wisconsin. Tests included range-finding evaluations of dose effectiveness, chemical degradation and residual toxicity. A summary of these findings for non-scientific audiences can be accessed at http://www.nemw.org/GSI/GSI-BS-P-FS-5.pdf. Please note that GSI's bench-scale tests do not by themselves provide adequate information to assess a prospective ballast treatment's ability to meet a particular discharge standard or to achieve environmental soundness under shipboard application. Instead, these tests provide initial insights into possible strengths and weaknesses of the proposed treatment—information that can be used by developers to better design a more effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

BACKGROUND

The Great Ships Initiative

The Great Ships Initiative (GSI)[1] is a collaborative effort whose objective is to end the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System. To that end, the GSI established sophisticated independent third party ballast treatment evaluation capabilities at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives:

- GSI Bench-Scale Tests
  - Range finding for effective doses under a range of ambient conditions;
  - Chemical degradation over time under a range of ambient conditions;
  - Detection of any residual toxicity under a range of ambient conditions; and
  - Confirmation of treatment process.

- GSI Land-Based Tests
  - Detection of scale-up, mechanical operation issues;
  - Effectiveness of a dose with respect to the full range of ambient organisms; and

1 www.greatshipsinitiative.org

  - Detection of any whole water effluent toxicity.

- GSI Shipboard Tests
  - Confirmation of biological and operational performance as expected in the ship environment; and
  - Confirmation of performance as expected under a broad range of ambient conditions.

Developers of ballast water treatment systems apply for GSI research services online, and awards are offered based on an objective review process, regardless of the state of development of the proposed treatment. GSI status testing will be performed at the scale appropriate to the treatment state of development, with the goal of helping meritorious ballast treatment systems to progress as rapidly as possible to an approval-ready and market-ready condition.

To assure relevancy of test output, GSI test protocols are as consistent with the International Maritime Organization (IMO) Convention and federal requirements as practicable. In particular, bench testing directly supports IMO G9 evaluations, and land-based testing directly supports IMO G8 evaluations.

GSI tests are third party assessments. They are completely independent of any vested interest in outcomes. The GSI tests are supported by general project funds which derive from federal and state agency grants and contributions, Great Lakes port contributions, and in-kind contributions by the local government and universities. None of these funds come to the GSI with any strings (other than public disclosure).

Ms. Allegra Cangelosi of the Northeast-Midwest Institute is the Principal Investigator and Manager of the GSI. Researchers from the University of Wisconsin-Superior's Lake Superior Research Institute, and the University of Minnesota-Duluth's Natural Resources Research Institute, among others, provide critical scientific and technical expertise and implementation services to GSI's biological research activities, and the GSI generally. A GSI Advisory Committee comprising top-level officials of key stakeholder groups helps steer the GSI providing crucial assistance in making GSI award decisions and fund-raising. The GSI Advisory Committee includes elected leadership, environmental organizations, port directors and federal officials from the United States and Canada, and industry representatives. The American Great Lakes Ports Association advises the project, assuring that the GSI is meeting the needs of the maritime industry; and coordinating maritime industry and supply chain outreach.

The largest contributor of GSI operating funds is the United States Department of Transportation, including its Maritime Administration, and the Saint Lawrence Seaway Development Organization. GSI also receives significant funds and in-kind contributions from the National Oceanic and Atmospheric Administration, the Canadian St. Lawrence Seaway Management Corporation, the City of Superior, Wisconsin, and approximately ten U.S. and Canadian ports in the Great Lakes.

GSI Bench Tests

GSI bench-scale tests take place year-round at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior in Superior, Wisconsin. The LSRI is amply equipped with staff expertise and resources to conduct the tests, and has a long history of successfully undertaking similar tests.

The overarching goals of GSI bench testing are to explore dose-effectiveness, chemical degradation, residual toxicity, and sensitivity to challenge conditions of a proposed treatment method about which little is known. To that end, the tests are "range-finding" missions, to determine the optimal treatment dose/intensity that would maximize effectiveness and minimize residual toxicity. Findings help treatment developers better design an effective system and/or to move to the next stage of treatment evaluation. The tests are also a form of trouble-shooting to encounter possible problems with the proposed treatment in advance of more extensive and larger scale tests.

GSI bench-scale dose effectiveness tests help determine the range of concentrations of an active substance that is harmful to a variety of robust freshwater zooplankton, algae and bacteria known to be relatively resilient to stressors. Dose effectiveness test results are expressed as percent survival, percent mortality, and percent hatch. They are also expressed in terms of a series of absolute quantifications: LC_{99}, i.e., the experimentally derived concentration of an active substance estimated to kill 99 percent of the test population following 24 or 48 hours of continuous exposure; No Observed Effect Concentration (NOEC), i.e., the highest concentration of an active substance shown to have no significantly adverse effect on the test population compared to controls; and Lowest Observed Effect Concentration (LOEC), i.e., the lowest concentration of an active substance known to have a significantly adverse effect on the test population compared to controls.

GSI bench-scale chemical degradation tests determine the effect that various water quality or environmental parameters may have on the rate of chemical degradation of a treatment system involving active substances. No organisms are used in association with these analytical assays; instead test solutions are analyzed for their concentration of active substance (or active component of the substance). Test results are expressed as the percent change in active ingredient concentration.

GSI bench-scale residual toxicity tests help estimate the effect that treated water (following neutralization of the active substance, a degradation period, or no treatment at all) may have on non-target organisms in the receiving system. These test results are expressed in a manner similar to those for dose effectiveness assays. The principal difference between these tests and dose effectiveness tests is that the concentration of the active substance is adjusted to be consistent with potential discharge levels, and the tests are performed on sensitive organisms rather than robust species.

ABOUT SODIUM HYDROXIDE (NaOH)

Researchers from the U.S. Geological Survey's Leetown Science Center in Kearneysville, West Virginia proposed sodium hydroxide (NaOH) for use as a contingency treatment for ships with no ballast onboard (NOBOB) which have not purged unpumpable residuals enroute. The researchers proposed to adjust the pH of the unpumpable residuals to a pH in the range of 11.5, 12.0, and 12.5. The pH would be maintained at this level for a minimum exposure period before it is decreased by dilution during subsequent re-ballasting operations to a pH of less than 9. The extent of the decrease is related to the volume and alkalinity of the treated residuals and the final ballast tank volume.

METHODS

Standard Operating Procedures and Quality Assurance

GSI bench-scale evaluations are in keeping with GSI standard operating procedures (SOPs). In general, GSI SOPs are grounded in published standard methods and modified to reflect ballast treatment circumstances. They are also consistent with international and domestic guidelines where they exist. All GSI SOPs were peer-reviewed prior to acceptance and are subject to periodic review and revision to assure that the most up to date approaches are employed. In addition, all GSI bench-scale research activities comply strictly with a detailed Quality Assurance Project Plan (QAPP) which is consistent in format, detail and stringency with United States Environmental Protection Agency requirements. Table 1 outlines the GSI bench-scale SOPs utilized for the NaOH tests.

General Methods

Testing Apparatus and Venue

The test apparatus consisted of 300 mL borosilicate high-form beakers housed within environmental chambers with controlled temperature and light regime. The environmental chambers are located within a laboratory equipped with adequate ventilation, electrical connections, and climate control located at the LSRI testing facility.

Table 1. GSI Bench-Scale Standard Operating Procedures (SOPs) Utilized for Sodium Hydroxide (NaOH) Tests.

SOP Category	Subcategory	SOP Title	SOP Code
General	Administration	Procedure for Record Keeping	GSI/SOP/G/A/RK/1
General	Sample Custody	Procedure for Custody of GSI Bench-Scale Samples	GSI/SOP/G/RA/SC/1
General	Sample Custody	Procedure for Labeling Bench-Scale Samples	GSI/SOP/G/RA/SC/3
Research Activities	Culturing	Procedure for Culturing the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/C/1
Research Activities	Culturing	Procedure for Culturing the Freshwater Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/C/2
Research Activities	Culturing	Procedure for Culturing the Cladocerans *Daphnia magna* and *Ceriodaphnia dubia*	GSI/SOP/BS/RA/C/3
Research Activities	Culturing	Procedure for Culturing *Selenastrum Capricornutum* as Food for Aquatic Organisms	GSI/SOP/BS/RA/C/4
Research Activities	Chemical Degradation	Procedure for Determining Chemical Degradation of a Primary Treatment or Its By-Products	GSI/SOP/BS/RA/CD/1
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the Copepod *Eucyclops spp*.	GSI/SOP/BS/RA/DE/1
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the Daphnid *Daphnia magna*	GSI/SOP/BS/RA/DE/2
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/3
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using Resting Eggs of the Freshwater Rotifer *Branchionus calyciflorus*	GSI/SOP/BS/RA/DE/4
Research Activities	Dose Effectiveness	Procedure for Assessing Dose-Effectiveness of a Ballast Water Treatment System Using the *Selenastrum capricornutum*	GSI/SOP/BS/RA/DE/5
Research Activities	Dose Effectiveness	Procedure for Exposing Test Organisms to an Active Substance	GSI/SOP/BS/RA/DE/7
Research Activities	Dose Effectiveness	Procedure For Quantifying Heterotrophic Plate Counts Using IDEXX's SimPlate for HPC Method	GSI/SOP/BS/RA/MA/1
Research Activities	Dose Effectiveness	Procedure for Assessing Antimicrobial Activity Using Time-Kill Method	GSI/SOP/BS/RA/MA/2
Research Activities	Dose Effectiveness	Procedure for the Detection and Enumeration of *Enterococcus* using Enterolert™	GSI/SOP/BS/RA/MA/3
Research Activities	Dose Effectiveness	Procedure for the Detection and Enumeration of *E. coli* Using IDEXX'sColilert	GSI/SOP/BS/RA/MA/4
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using *Ceriodaphnia dubia*	GSI/SOP/BS/RA/RT/1
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using Fathead Minnows (*Pimephales promelas*)	GSI/SOP/BS/RA/RT/2
Research Activities	Residual Toxicity	Procedure for Measuring Residual Toxicity Using the Amphipod *Hyalella azteca*	GSI/SOP/BS/RA/RT/4
Research Activities	Chemistry	Procedure for Determining Total Residual Oxidants (TRO) in Water	GSI/SOP/BS/RA/C/2
Research Activities	Chemistry	Procedures for Measuring Organic Carbon in Aqueous Samples	GSI/SOP/BS/RA/C/3
Research Activities	Chemistry	Procedure for Determining Percent Transmittance (%T) of Light in Water at 254 nm	GSI/SOP/BS/RA/C/4

Method of Determination of pH

Measurement of pH was conducted using a Ross Ultra Combination pH electrode connected to an Orion Model 720A pH/ISE meter. The meter was calibrated daily prior to any pH measurements being made. Two pH buffers, pH 7 and 10, were used to calibrate the meter. Twenty mL samples were collected from the degradation and toxicity studies for measurement of pH. The samples were collected with a 1000-5000 μL pipettor set at 5000 μL. The samples were placed into 30 mL glass beakers, a stir bar was added, and the pH was measured while the sample was slowly stirred.

Mean pH values provided in the results tables were derived from actual experimental pH values that were used to determine the pOH values of the solutions. These values were used to calculate the average hydroxide ion (OH^-) concentrations. The average hydroxide ion concentrations were then used to calculate dose-response measures and the average pH values.

Method of Determination of Nonpurgeable Organic Carbon

Organic carbon analysis was conducted on a Shimadzu Model TOC-5050A Total Organic Carbon Analyzer. Before analysis, the samples were acidified with 0.2 % concentrated hydrochloric acid. Before being injected into the analyzer, samples were purged with high purity air to remove the inorganic carbon and purgeable organic carbon from the samples (see GSI/SOP/BS/RA/C/3).

An organic carbon stock solution was prepared by dissolving 0.2125 g of oven dried anhydrous potassium hydrogen phthalate (KHP) in deionized water, adding 125 μL of concentrated hydrochloric acid, and diluting to 1000 mL with deionized water. This organic carbon stock solution had a concentration of 1000 mg/L carbon. A series of working standards (1.0, 5.0, 10.0 and 100 mg/L C) were prepared from the organic carbon stock. Each standard was made to be 0.2 % in hydrochloric acid. The standards were used to generate a calibration curve which was used to determine the concentration of organic carbon in the samples.

Method of Preparing Sample Water

Experimental water was prepared in the laboratory in batches as follows:

- Duluth-Superior Harbor water (DHW) was collected from a depth of approximately 3 meters in the Duluth-Superior Harbor of Lake Superior. Alkalinity ranged from 65-69 mg/L as $CaCO_3$. Non-purgeable organic carbon ranged from 16-22 mg/L. The water is stained with tannins.

- Laboratory water (LW) was treated Lake Superior water from the City of Superior that is passed through an activated carbon column. Residual chlorine is removed by pumping a solution of sodium sulfite into the water. Alkalinity

ranged from 45-50 mg/L as $CaCO_3$. Non-purgeable dissolved organic carbon ranged from 0.5-2.0 mg/L. The water is transparent.

- Filtered harbor water (FHW) is the same as DHW (see above) but passed in sequence through a Whatman GF/B filter followed by a Millipore 0.45 µm membrane filter prior to use.

Method of Preparing Sediment Samples

Approximately 3 gallons of Duluth-Superior Harbor was collected using a hand held Ponar dredge and placed into a pre-cleaned polyethylene bucket. The sediment was then transported back to the testing laboratory, stored at 4.0 °C, with a half an inch of overlying Duluth-Superior Harbor water, and minimal head space until it was homogenized. Prior to testing, the sediment was homogenized for 15 minutes using a commercial drill equipped with a stainless steel mortar paddle. At 5 minute intervals, the sediment was briefly stirred manually to ensure further homogeneity. After homogenization, approximately 100 mL of sediment was added to a 300 mL high form beaker. Approximately 150 mL of test water was added slowly down the side of the beaker to minimize the agitation of the underlying sediment.

Method of Application of Sodium Hydroxide (NaOH) to Generate Treated Samples

Treatment solutions were prepared by adding a NaOH solution to either FHW or LW. Ten normal (10 N) NaOH solution was added slowly with stirring to the appropriate water type while the pH of the solution was being monitored. NaOH solution was added until the desired pH was obtained ± 0.03 pH units as determined with the pH meter.

Bench-Scale Dose Effectiveness Test Methods

Zooplankton and Algae

These GSI dose effectiveness tests measured the range of pH harmful to juvenile (less than 24 hours old) daphnids, adult copepods, newly hatched rotifers, rotifer resting eggs, and a green alga. The tests evaluated three pH levels (11.5, 12.0, and 12.5) and a control (no pH adjustment) at a temperature of 25.0 °C ± 1.0 °C. FHW and LW, which have contrasting physical/chemical properties, were used as test water. Test water was not renewed in the tests. The effects of sediment on treatment dose effectiveness were also investigated.

All exposures except for those involving rotifer adults and rotifer resting eggs took place in complete darkness over 48 hours, with 50 mL of exposure solution in a 300 mL beaker, and three replicates. Tests on rotifer adults and rotifer resting eggs involved four replicates and 2.0 mL of exposure solution. In addition, adult rotifers were exposed in complete darkness for 24 hours due to their sensitivity. The rotifer resting eggs were exposed to continuous light to stimulate hatching. In all cases, periodic observations were

made on mortality, and measured water quality parameters including temperature, dissolved oxygen, pH, conductivity, alkalinity and hardness. Table 2 describes the exposure conditions across organism type while table 3 arrays the types and numbers of organisms analyzed, the exposure concentrations, and the number of replicates per dose effectiveness test.

Sediment toxicity tests were performed with the following two freshwater species: the insect *Chironomus dilutus* and the oligochaete, *Lumbriculus variegatus*. The tests were initiated with juvenile life-stages in the case of *C. dilutus* (10-12 days old) and adult *L.variegatus* organisms. For these experiments, the sediment test organisms were added after the addition of the sediment and overlying test water.

Table 2. Exposure Conditions For GSI Dose-Effectiveness Tests on Zooplankton and Algae.

Organism Type	Exposure Volume per Replicate (mL)	Sediment Volume per Replicate (mL)	Exposure Duration (hr)	Light:Dark Cycle (hr)	Temperature (° C)
Cladoceran (D. magna)	50	0	48	0:24	25±1.0
Newly hatched rotifers, (*Brachionus calyciflorus*)	2	0	24	0:24	25±1.0
Rotifer resting eggs, (*Brachionus calyciflorus*)	2	0	48	24:0	25±1.0
Green alga (*Selenastrum* spp.)	50	0	48	0:24	25±1.0
Copepods (*Eucyclops* spp.)	50	0	48	0:24	25±1.0
Insect (*Chironomus dilutus*)	150	100	48	0:24	25±1.0
Oligochaete (*Lumbriculus variegates*)	150	100	48	0:24	25±1.0

Table 3. Numbers and Types of Organisms, and Initial pH Level Used For GSI Dose-Effectiveness Tests on Zooplankton and Algae.

Major Taxonomic Group	Type	Species	Test Water Types	Initial pH Level	No. of Organisms per Exposure /Control	No. of Replicates per Exposure /Control	Total Number of Organisms
Zooplankton	Juvenile daphnids	*Daphnia magna*	FHW	11.5, 12.0, and 12.5 and a control (no pH adjustment)	10	3	4 x 10 x 3 = 120
Zooplankton	Adult copepods	*Eucyclops* spp.	FHW	11.5, 12.0, and 12.5 and a control (no pH adjustment)	10	3	4 x 10 x 3 = 120
Zooplankton	Adult rotifers	*Brachionus calyciflorus*	FHW	11.5, 12.0, and 12.5 and a control (no pH adjustment)	5	4	4 x 5 x 4 = 80
Zooplankton	Rotifer resting eggs	*Brachionus calyciflorus*	FHW	11.5, 12.0, and 12.5 and a control (no pH adjustment)	20	4	4 x 20 x 4 = 320
Algae	Green alga	*Selenastrum spp.*	FHW	11.5, 12.0, and 12.5 and a control (no pH adjustment)	200,000 cells/mL	3	4 x 200,000 x 3 = 2,400,000

Bacteria

Dose effectiveness tests were also conducted to determine if the proposed pH values (11.5, 12.0, and 12.5) are harmful to bacteria. DHW experiments were conducted using ambient bacteria assemblages, and ambient bacteria assemblages spiked with test organisms (*Escherichia coli* and *Enterococcus faecalis*) to increase initial densities. The tests evaluated the three pH levels against a control in the spiked and unspiked condition at a temperature of 25.0 °C ± 1.0 °C. Samples were analyzed following 0 (control samples only), 2, 24 and 48 hours of exposure. *E. coli* and Enterococcus were enumerated using Colilert® and Enterolert™ assays using Quanti-Tray/2000 and the Quanti-Tray sealer from IDEXX laboratories. The Colilert® test has detection limits for total coliforms and *E. coli* of 1 colony forming unit (cfu) per 100 mL, and the Enterolert ™ test can detect Enterococcus at 1 cfu/100 mL. Both tests use Defined Substrate Technology® (DST) in which the bacteria metabolize the enzymes in the specific media causing the sample to fluoresce. Heterotrophic plate count (HPC) bacteria were analyzed using SimPlate for HPC medium which uses IDEXX's Multiple Enzyme Technology™ (MET™). Results are expressed as Most Probable Number (MPN) per 100 mL for the Colilert and Enterolert tests and MPN per 1 mL for the SimPlate tests. MPN is a common method of obtaining quantitative data on concentrations of discrete items from positive/negative (incidence) data and in this case is directly related to colony forming units (cfu).

Statistical Methods

At each time point (0, 4, 24, and 48 hours), treatment group means were compared using a one-way repeated measures analysis of variance (RM ANOVA). If the data passed the normality and equal variance tests, the Holm-Sidak method for pairwise multiple comparisons was used to determine if significant ($p < 0.05$) differences existed between the treatment group means at each time point. If the data did not pass the normality test, the data were compared using Kruskal-Wallis ANOVA on Ranks and Dunn's Method for pairwise comparisons to determine if significant ($p < 0.05$) differences existed between the treatment group ranks at each time point. A value of half the detection limit was used for replicates having a value below the detection limit. SigmaStat software (v. 3.5, Systat Software Inc., Chicago, IL) was employed to conduct the analysis.

Bench-Scale Chemical Degradation Test Methods

The GSI chemical degradation tests assessed the effect of various water quality or environmental parameters on the rate of NaOH degradation (measured as pH). The rate of degradation associated with two initial pH levels (11.5 and 12.5) were evaluated in five concentrations of DHW diluted with LW (resulting in five different levels of non-purgeable dissolved organic carbon). The exposure method was as follows: approximately 50 mL of pH adjusted test solution at each non-purgeable dissolved organic carbon dilution level was decanted into three replicate 300 mL high-form beakers made of glass. Three replicates of sample water at each dilution with no pH adjustment served as a control. Beakers were immediately covered with a glass plate to prevent evaporation and then placed in a completely dark environmental chamber set to 25 º C. The solutions were analyzed for pH at time periods 0, 2, 6, 24, 48, 72, and 96 hours using a Ross Ultra Combination pH electrode connected to an Orion Model 720A pH/ISE meter. Test results are expressed as pH over time. Table 4 arrays the test conditions and exposure concentrations used for this set of tests.

Table 4. Testing Scenarios for the Sodium Hydroxide (NaOH) Degradation Experiments.

Water Type	Light Regime and Temperature	pH Levels Tested	Approximate Concentrations of Nonprugeable Organic Carbon (mg/L)	No. of Replicates per Exposure /Control
LW	Dark / 25 º C	Unadjusted, 11.5, 12.5	2.0	3
12.5% DHW	Dark / 25 º C	Unadjusted, 11.5, 12.5	2.8	3
25% DHW	Dark / 25 º C	Unadjusted, 11.5, 12.5	5.5	3
50% DHW	Dark / 25 º C	Unadjusted, 11.5, 12.5	11.0	3
100% DHW	Dark / 25 º C	Unadjusted, 11.5, 12.5	22.0	3

Bench-Scale Residual Toxicity Test Methods

Residual toxicity tests were performed to measure the post-dilution pH and toxicity of NaOH-treated DHW after 1:100 or 1:1000 dilution with non-pH adjusted DHW. The test species were juvenile (less than 24 hours old) daphnids, juvenile (less than 24 hours old) fathead minnows and amphipods (7-8 days old). The pH adjusted test solutions were prepared as described above for the dose effectiveness tests in DHW.

All tests were performed at a temperature of 25.0 °C ± 1.0 °C and the test water was not renewed. Exposures were conducted in a 16 hour/8 hour light/dark cycle, and consisted of 50 mL of solution in a 300 mL beaker. There were three replicates per exposure solution and tests were 48 hours in length. Daily observations of mortality in control and treatment samples were compared with test results expressed as percent survival of organisms following the exposure period. Table 5 arrays the types and numbers of organisms analyzed, the exposure concentrations used (measured as total residual chlorine), and the number of replicates per test.

Table 5. Numbers and Types of Organisms, and pH Levels Analyzed in GSI Residual Toxicity Tests.

Major Taxonomic Group	Type	Species	Predilution pH Level s	No. of Organisms Per Replicate	No. of Replicates
Zooplankton	Juvenile daphnids	*Ceriodaphnia dubia*	11.5, 12.0, 12.5	10	3
Fish	Juvenile fathead minnows	*Pimephales promelas*	11.5, 12.0, 12.5	15	3
Zooplankton	Amphipods (7-8 days old)	*Hyalella azteca*	11.5, 12.0, 12.5	10	3

FINDINGS

Bench-Scale Dose Effectiveness Test Findings

Zooplankton and Algae

Results of GSI bench-scale dose effectiveness tests involving NaOH toxicity on robust species of freshwater zooplankton and an alga are presented in table 6. NaOH treatment within the pH range proposed (i.e. 11.5, 12.0 and 12.5) was significantly effective at reducing numbers of live organisms across taxonomic groups tested, but did not inactivate all individuals in FHW. There was 0 percent survival after four hours for the cladoceran *Daphnia magna*, the copepod *Eucyclops sp.*, and the adult rotifers

Branchionus calyciflorus in FHW at all pH levels tested. In contrast, only a 48 hour exposure to a pH level of 12.5 resulted in 0 percent survival for the green alga *Selenastrum sp*. and rotifer cysts in FHW.

Table 6. Percent Survival of Species (Standard Error) Following 24 and 48 Hours of Exposure to Sodium Hydroxide (NaOH) at pH Levels of 11.5, 12.0 and 12.5.

Major Taxonomic Group	Type	Species	Water Type	pH Level	Survival (%)	
					24 Hours	48 Hours
Algae	Green alga	*Selenastrum sp.*	FHW	Control	100 (0)	100 (0)
				11.5	99 (0.3)	95 (2)
				12.0	41 (1) *	9 (9) *
				12.5	41 (3) *	0 (0) *
Zooplankton	Cladoceran	§*Daphnia magna*	FHW	Control	100 (0)	N/A
				11.5	0 (0)	N/A
				12.0	0 (0)	N/A
				12.5	0 (0)	N/A
	Copepod	§*Eucyclops sp.*	FHW	Control	100 (0)	N/A
				11.5	0 (0)	N/A
				12.0	0 (0)	N/A
				12.5	0 (0)	N/A
	Rotifer	§*Branchionus calyciflorus*	FHW	Control	100 (0)	N/A
				11.5	0 (0)	N/A
				12.0	0 (0)	N/A
				12.5	0 (0)	N/A
		B. calyciflorus cysts	FHW	Control	Not Measured	23 (1)
				11.5	Not measured	5 (1)
				12.0	Not measured	5 (1)
				12.5	Not measured	0 (0) *

§ Endpoint was 4 hr. * The difference in the median values among the treatment groups are greater than would be expected by chance. Based on Dunn's Method there is a statistically significant difference ($p<0.05$).

Table 7 describes the results of the dose effectiveness experiments involving sediments. The table provides the average pH values and survival during the 48 hour exposure period of the midge larvae *C. dilutus* in sediment exposed to different concentrations of NaOH in the overlying water. In the exposures involving sediment, the pH levels dropped between 1-2 pH units during the course of the 48 hour exposure. There was no survival only in the highest pH (12.5) tested. At 48 hours the treatments of 12.0 and 12.5 had a significantly ($p < 0.05$) reduced survival when compared to the control.

Table 7. Average (n=5) Percent Survival (Standard Error) of *Chironomus dilutus* Exposed to Different Concentrations of Sodium Hydroxide (NaOH) in Sediment Collected from the Duluth-Superior Harbor When Exposed for 48 Hours

Sample	Average pH during 48 hr exposure	Survival (%)	
		0 hr	48 hr
Control	7.56	100 (0)	70 (8)
pH 11.5	9.11	100 (0)	36 (7)
pH 12.0	9.83	100 (0)	16 (14)*
pH 12.5	11.27	100 (0)	0 (0)*

* The differences in the median values among the treatment groups are greater than would be expected by chance; based on Dunn's Method there is a statistically significant (p<0.05) difference.

Table 8 describes the survival of *L. variegates* (oligachaete) in sediment exposed to different concentrations of NaOH. The measured pH values also dropped between 1-2 pH units during the 48 hour exposure period. All pH levels tested had zero percent survival by the 48 hour time period.

Table 8. Average (n=5) Percent Survival (Standard Error) of *Lumbriculus variegatus* Exposed to Different Concentrations of Sodium Hydroxide (NaOH) in Sediment Collected from the Duluth-Superior Harbor When Exposed for 48 Hours

Sample	Average pH during 48 hr exposure	% Survival	
		0 hr	48 hr
Control	7.65	100 (0)	100 (0)
11.5	8.69	100 (0)	0 (0)
12.0	9.65	100 (0)	0 (0)
12.5	11.16	100 (0)	0 (0)

Bacteria

Results from the tests showed a decrease in the number of total coliforms, *E. coli*, *Enterococci*, and heterotrophic bacteria in all pH levels during the 48 hour exposure period (tables 9-12). Within two hours *E. coli* specifically was reduced by 99.9 % in the pH 11.5 treatment and 100 % in the pH 12 and pH 12.5 treatments (table 9). Likewise, total coliforms were reduced 92 % in the pH 11.5 treatment, and 100 % in the pH 12.0 and pH 12.5 treatments (table 10).

Table 9. Average (n=2) Most Probable Number (MPN) of *Escherichia Coli* per 100 mL After 2 and 24 Hours of Exposure to Three Sodium Hydroxide (NaOH) Levels.

Sample	MPN of *E. coli* per 100 mL		
	0 hr	2 hr	24 hr
Harbor Water-Control	25	27	15
Spiked Harbor Water-Control	1430	1410	1550
pH 11.5		1	<1
pH 12.0		<1	<1
pH 12.5		<1	<1

Table 10. Average (n=2) Most Probable Number (MPN) of Total Coliforms per 100 mL After 2 and 24 Hours of Exposure to Sodium Hydroxide (NaOH) levels.

Sample	MPN of Total Coliforms per 100 mL		
	0 hr	2 hr	24 hr
Harbor Water-Control	579	613	328
Spiked Harbor Water-Control	1860	1990	1730
pH 11.5		142	<1
pH 12.0		1	<1
pH 12.5		<1	<1

Enterococci bacteria also declined in numbers in all three treatments and the control. Within two hours the spiked control was reduced by 52.8 %, the pH 11.5 treatment was reduced by 58.3 %, the pH 12 treatment by 94.3 %, and the pH 12.5 treatment by 99.9 % (table 11). At the 48 hour exposure period, the pH 11.5 treatment was reduced by 99.9 %, and no Enterococci were detected in the two samples of highest pH (table 11).

Heterotrophic bacteria increased in the control over the 48 hours, while all three of the treatments caused a slight reduction (table 12). The pH 12 treatment had the highest percentage of reduction at 98.8 %. The three blanks for the 48 hour analysis were

positive, averaging a MPN of 31.6 per mL indicating that the SimPlate media had been contaminated. The contamination of the blank at the 48 hour time period represents less than 1 % of the MPN of the spiked harbor water control and does not influence reduction observed from the treatment effect.

Table 11. Average (n=2) Most Probable Number (MPN) of Enterococcus per 100 mL after 2, 24 and 48 Hours of Exposure to Three Sodium Hydroxide (NaOH) Levels.

Sample	MPN of Enteroccus per 100 mL			
	0 hr	2 hr	24 hr	48 hr
Harbor Water-Control	20	22	6	2
Spiked Harbor Water-Control	2080	980	517	66
pH 11.5		866	2	3
pH 12.0		118	1	1
pH 12.5		2	<1	<1

Table 12. Average (n=2) Most Probable Number of Heterotrophic Bacteria per mL after 2, 24 and 48 Hours of Exposure to Three Sodium Hydroxide (NaOH) Levels.

Sample	MPN of Heterotrophic Bacteria per mL			
	0 hr	2 hr	24 hr	48 hr
Harbor Water-Control	285	540	2.1E+03	1.2E+03
Spiked Harbor Water-Control	400	555	9.2E+04	4.2E+04
pH 11.5		41	31	18
pH 12.0		25	27	5
pH 12.5		45	27	44

Bench-Scale Chemical Degradation Test Findings

Table 13 describes the results from the 96 hour pH degradation experiment using a series of DHW dilutions with LW. The controls (no pH adjustment) showed a slight increase in pH (table 13). All pH adjusted dilutions showed no appreciable change in pH over the 96 hour period.

Table 13. Average (n = 3) pH Values in Various Percentages of Duluth-Superior Harbor Water (DHW) Diluted with Laboratory Water (LW) Measured Over a 96 Hour Observation Period.

	Time (hr)						
Conditions	0	2	6	24	48	72	96
Lab water	7.39	7.55	7.72	7.96	8.02	8.08	8.14
Lab water - 11.5	11.50	11.48	11.46	11.46	11.41	11.37	11.24
Lab water - 12.5	12.50	12.51	12.50	12.52	12.51	12.50	12.55
12.5% Harbor water	7.68	7.70	7.78	7.99	8.03	8.09	8.14
12.5% Harbor water-11.5	11.51	11.57	11.56	11.55	11.51	11.48	11.50
12.5% Harbor water - 12.5	12.50	12.50	12.50	12.53	12.51	12.50	12.57
25% Harbor water	7.69	7.74	7.80	7.98	8.04	8.10	8.13
25% Harbor water-11.5	11.50	11.51	11.49	11.43	11.38	11.26	11.22
25% Harbor water - 12.5	12.50	12.51	12.50	12.53	12.52	12.52	12.58
50% Harbor water	7.77	7.75	7.79	7.96	8.06	8.08	8.09
50% Harbor water-11.5	11.51	11.51	11.49	11.50	11.45	11.37	11.31
50% Harbor water - 12.5	12.51	12.52	12.51	12.54	12.53	12.53	12.58
100% Harbor water	7.93	7.93	7.89	7.95	8.00	8.05	8.09
100% Harbor water-11.5	11.50	11.51	11.50	11.49	11.45	11.41	11.40
100% Harbor water - 12.5	12.50	12.52	12.52	12.54	12.52	12.53	12.56

Note: Non purgeable organic carbon was 22.0 mg/L in the 100 % DHW treatment.

Bench-Scale Residual Toxicity Test Findings

The results described in table 14 are the measured pH values from the residual toxicity dilution experiment. Immediately after the 1:100 dilutions of the initial pH values of the treatments 11.5 and12.0 were 8.27 and 8.87, respectively, while the pH level of 12.5 had a pH of 9.69 (table 14). After 24 hours the pH values ranged from 7.88-8.42 (table 14) in the 1:100 dilution across all pH levels tested. The dilution of 1:1000 produced pH values that ranged from 7.91 to 8.30 immediately after dilution with the 24 hour pH values ranging from 7.91-8.06 (table 14).

Survival results for each species tested in the residual toxicity experiment were high ranging from 97-100 % (table 15).

Table 14. Average (n=3) pH Results From Residual Toxicity Dilution Experiment Over 48 Hour Exposure.

Species	Initial pH	Dilution 1:100			Dilution 1:1000		
		0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
C. dubia	11.5	8.27	8.03	8.05	7.91	7.99	8.01
	12.0	8.87	8.11	8.08	7.93	7.99	8.04
	12.5	9.69	8.34	8.16	8.30	8.06	8.04
H. azteca	11.5	8.27	7.92	7.94	7.91	7.91	7.91
	12.0	8.87	8.02	8.00	7.93	7.89	7.91
	12.5	9.69	8.42	8.16	8.30	7.95	7.97
P. promelas	11.5	8.27	7.88	7.93	7.91	7.89	7.87
	12.0	8.87	7.96	7.97	7.93	7.92	7.93
	12.5	9.69	8.17	8.09	8.30	7.91	7.92

Table 15. Average (n=3) Percent Survival From Residual Toxicity Dilution Experiment Using Duluth-Superior Harbor Water (DHW) After 48 Hours Exposure.

Species	Initial pH	Dilution 1:100 % Survival	Dilution 1:1000 %Survival
C. dubia	11.5	97	100
	12.0	100	97
	12.5	97	100
H. azteca	11.5	97	100
	12.0	100	97
	12.5	100	100
P. promelas	11.5	100	100
	12.0	100	100
	12.5	100	100

Quality Assurance/Quality Control

Analytical

Quality control sample analysis consisted of collecting and analyzing samples in duplicate. Approximately 10 % of the samples analyzed were collected and analyzed in duplicate. The results of the duplicate analysis are provided in the following table (table 16).

Table 16. Duplicate analysis of pH samples.

	Duplicate Agreement (%)
Mean	99.9
Standard Deviation	0.2
Maximum	100
Minimum	99.3
Number of duplicate samples	19

Dose Effectiveness and Residual Toxicity

Toxicity tests were initiated with healthy, vigorous animals. Reference toxicant tests were performed with all test species prior to the start of the definitive test. Control charts are available upon request. Test conditions were monitored daily for parameters that might affect the outcome of the test (i.e., temperature, and dissolved oxygen). Daily and weekly calibration of test meters ensured optimal performance. The GSI Quality Assurance and Quality Control Officer performed inspections of logbooks, recorded measurements, and instrumentation used during the tests. Any deviations were discussed with the principal investigator and documented in the study logbook. Survival in the controls for the zooplankton was 100 % which is greater than the acceptable criteria of 80 % listed in the GSI QAPP and SOPs. It should be noted that there was an average of 70 % survival in the *C. dilutus* sediment test control. However, treatment effects were still greater.

Microbial

All Colilert® and Enterolert ™ blanks yielded no positive wells and positive controls were positive as appropriate for each test. All media blanks and sterile water blanks for the Colilert and Enterolert tests were negative. The three blanks for the 48 hour analysis were positive, averaging a MPN of 31.6 per mL indicating that the SimPlate media had been contaminated.

CONCLUSION

In conclusion, the pH levels of 11.5, 12.0, and 12.5 were effective at killing the broad range of aquatic organisms tested, though only the pH 12.5 level was effective at reducing live densities to below 1% for aquatic organisms tested. The elevated pH levels tested were most effective at inactivating rotifer adults, adult daphnia, and copepods, and did so within four hours. While heterotrophic bacteria and green algae (*Selenastrum),* were more resistant to treatment; bacteria were reduced by three logs, and there was complete *Selenastrum* mortality at 48 hours in the pH level of 12.5. NaOH-treatment resulting in pH levels of 12.0 and 12.5 reduced *E. coli* and *Enterococcus* to less than 1 MPN within two hours.

There was no substantial change in elevated pH levels in NaOH-treated water (DHW or LW) over 96 hours except where sediments were present. Sediments led to a slight lowering of pH. These results indicate that treated water will remain toxic until it is actively neutralized or diluted.

Our tests provide some insight into how much dilution and hold time may be necessary to meet discharge quality standards. Immediately after a 1:100 dilution, the pH level of 12.5 was 9.69 which is outside the U.S. EPA acceptable criteria of 6.5-9.0 for fresh water (U.S. EPA 1986). It was not until 24 hours after the dilution the pH fell to 8.34. However, the pH level of 12.5 fell within the acceptable range immediately after a dilution of 1:1000. There was no acute residual toxicity observed at either dilution of 1:100 or 1:1000.

In conclusion, a pH level of 12.5 was required to effectively kill all taxa tested, and a dilution of 1:1000 was required to lower that pH level to a safe level for immediate discharge. These tests give preliminary support to the proposal to use this method for ships in the NOBOB condition. These results also confirm the hypothesis that without modification, this method could not be employed effectively for treating discharge from fully ballasted tanks.