

STANDARD OPERATING PROCEDURE Procedure for Injecting Organisms and Solids into the GSI Land-Based RDTE Facility

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RECORD OF REVISIONS

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1	02/21/2011	Updated background; separated supplies from equipment; updated procedures; added figures.	7		
2	05/20/2011	Added definitions. Added text to "Introduction". Added Figures 1 and 3. Deleted incorrect SIS calculation and replaced with Figure 1. Added reference.	8		
3			9		
4			10		
5			11		
6			12		

STANDARD OPERATING PROCEDURE

Procedure for Injecting Organisms and Solids into the GSI Land-Based RDTE Facility

BACKGROUND

The Great Ships Initiative (GSI) is a regional effort devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. In support of that goal, the GSI has established superlative freshwater ballast treatment evaluation capabilities at three scales—bench, land-based, and on board ship. Each scale is dedicated to addressing specific evaluation objectives. These include:

GSI Bench-Scale Tests

- Range finding for effective treatment dose against diverse freshwater taxa and water quality conditions;
- Generation of freshwater relevant chemical degradation curves; and
- Estimation of residual toxicity given diverse freshwater taxa and water quality conditions.

GSI Land-Based Tests

- Pre-certification testing, i.e., operational and biological performance (including residual toxicity) status-testing given scale-up and a range of challenge conditions; and
- Certification/verification testing, i.e., formal assessment of performance against international and other discharge standards.

GSI Shipboard Tests

- Confirmation of biological and operational treatment performance as expected in the ship environment;
- U.S. Coast Guard Shipboard Technology Evaluation Program (STEP) testing;
- Shipboard type approval testing;
- Ship discharge monitoring; and
- Methods development.

GSI awards its independent status-testing services to candidate systems only if technical and programmatic criteria are met. Decisions are based on third party technical assessments as well as GSI Advisory Committee programmatic input. Testing services are currently offered at no cost to the developer with the exception of transportation and system installation/removal costs. Instead, tests are supported by general project funds which derive from federal and state agency grants, Great Lakes port contributions, and in-kind contributions by local governments and universities.

GSI has no involvement, intellectual or financial, in the mechanics, design or market success of the actual treatment systems it tests. To ensure GSI remains completely independent and is uncompromised by any real or perceived individual or project bias, GSI subjects itself to rigorous quality management policies and procedures. In addition, GSI test activities are subject to rigorous QAQC procedures and documentation. This attention to quality management and QAQC assures the high quality and credible evaluation of both GSI and its findings.

INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the procedure used to inject solids and organisms into the intake water of the GSI Land-Based Research, Development, Testing, and Evaluation (RDTE) Facility (Superior, WI) to achieve pre-determined physical, chemical, and biological challenge conditions. This SOP details how concentrations of total suspended solids (TSS), particulate organic carbon (POC), and mineral matter (MM) in ambient Duluth-Superior Harbor water can be augmented on intake using the solids injection system (SIS, see Figure 1). MM is defined as the difference between the TSS and POC concentrations; therefore, MM is augmented indirectly by the addition of solids to the intake water to increase TSS concentrations. In addition, this SOP describes the procedure used to collect and concentrate organisms, consisting largely of phytoplankton, from the Duluth-Superior Harbor, to increase the concentration of phytoplankton on intake using the organism pressure injection system (OPIS, see Figures 2 and 3). Phytoplankton are collected from the Duluth-Superior Harbor using 50 μm to 80 μm plankton nets, which facilitates the collection of an abundance of filamentous forms of phytoplankton that make up the majority of the $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ size class. Both the SIS and OPIS are kept separate to reduce the risk of phytoplankton mortality. The OPIS is used to inject surrogate organisms into the facility in a way that causes as little mortality of the organisms as possible. Air pressure is used to push organisms into the intake water, no pumps are used.

DEFINITIONS

Dissolved Organic Carbon (DOC): The fraction of total organic carbon (TOC) present in water that passes through a 0.45 μm pore diameter filter (Eaton *et al.*, 2005).

Mineral Matter (MM): Defined as the difference between the measured total suspended solids concentration and the particulate organic carbon concentration.

Nonpurgeable Organic Carbon (NPOC): The fraction of total organic carbon (TOC) not removed by gas stripping (Eaton *et al.*, 2005).

Particulate Organic Carbon (POC): Non-dissolved organic carbon, that fraction that would be retained on a 0.45 μm pore diameter filter (Eaton *et al.*, 2005). In this method it is defined as the difference between the NPOC and DOC. The POC concentration of intake water may be augmented through the addition of Micromate.

Soil Sterilization: According to the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service, sterilization of soil is defined as treating soil with dry heat at 250 °F (121 °C) for at least two hours (USDA, 2010).

Total Suspended Solids (TSS): Organic (e.g., algae) and inorganic (e.g., soil particles) material suspended in the water column. As TSS increase, the turbidity of the water and the absorption of light are also increased. The TSS concentration of intake water may be augmented through the addition of Fine Arizona Test Dust and Micromate.

EQUIPMENT

- Laboratory Oven (i.e., minimum temperature capability of 200 °C)
- Solids Diaphragm Pump
- 200 Gal. Cone Bottom Solids Tank
- Solids Injection Pitot
- Mechanical Agitator
- 100 Gal. Pressure Vessel
- Flow Meter
- Organism Injection Pitot
- Diaphragm Valve
- Sump Pump
- 6 foot diameter fiberglass aquaculture “ponds”
- Boat and outboard motor
- Computer with Microsoft® Excel (for calculation of mass/volume to be added to SIS and OPIS tanks)

SUPPLIES

- Sieve with 1/4 inch diameter mesh
- Plankton Nets (50-80 μm mesh)
- 5 gal. containers to transport phytoplankton
- wash bottles
- 50 mL Plastic Bottle
- Pipette
- Fine Arizona Test Dust (ISO 12103-1, A2, nominal 0-80 μm particle size; Powder Technology, Inc.; Burnsville, MN)
- Micromate (Micronized Humate Product for Liquid Suspension, average particle size is 25 μm ; Mesa Verde Resources; Placitas, NM)
- Clean Plastic Containers (i.e. 1 gal. capacity)
- Dust Mask
- Protective Glasses
- Laboratory Notebook(s)
- Balance
- Baking Dish

PROCEDURE

Preparation of Solids

1. Transfer the Fine Arizona Test Dust from the plastic jar that it was shipped in into a baking dish (either glass or metal). Sterilize the Fine Arizona Test Dust by baking in a laboratory oven at 130 °C for approximately four hours to ensure that no live foreign organisms are introduced into the Duluth-Superior Harbor (USDA, 2010). The timing should not start until the temperature of the Test Dust has reached approximately 130 °C.

Allow to cool and then transfer the dust back into a clean plastic container. Repeat this process for each jar that was received in the shipment (i.e., each jar is kept separate during the baking and cooling process).

2. Sterilize the Micromate by baking in a laboratory oven at 130 °C for approximately four hours to ensure that no live foreign organisms are introduced into the Duluth-Superior Harbor (USDA, 2010). The timing should not start until the temperature of the Micromate has reached approximately 130°C. The sterilization should be conducted very cautiously, as there is a risk that the dust may ignite if it reaches a temperature above 160 °C. The Micromate is received in plastic lined bags containing 30 pounds. Carefully transfer the material to glass baking pans in a fume hood to minimize exposure to the fine dust. After the Micromate has been sterilized and cooled to room temperature, transfer into plastic containers for storage.
3. Weigh (i.e., on a laboratory balance) and record the desired amount of sterilized Micromate to achieve the pre-determined particulate organic carbon (POC) concentration. Note: The physical/chemical challenge water conditions are dependent on the ballast water treatment technology being tested; therefore, the desired POC concentration will be specified in the Test Plan or Test Quality Assurance Project Plan (TQAP). The Micromate is weighed into clean, plastic bottles (1 gal. capacity) for use at the GSI Land-Based RDTE Facility.

Solids Injection System

1. Measure and record the TSS and POC concentrations of ambient Duluth-Superior Harbor water prior to the start of the trial. This may be done in two ways:
 - a. Using the ambient Duluth-Superior Harbor water concentrations from recent monitoring data, i.e., harbor water samples collected at the GSI Land-Based Test RDTE Facility throughout the land-based testing season to monitor the ambient physical/chemical conditions; or
 - b. Using the ambient Duluth-Superior Harbor water concentrations from a sample collected from the bay pump at the GSI Land-Based Test Facility on the day prior to the trial or on the morning of the trial.
2. Use the TSS and POC measurements of the ambient Duluth-Superior Harbor water (entered into the “Bay Pump/TSS” column in SIS calculator in Figure 1) to determine the mass of solids to add to the water in the SIS tank (entered into the “Fine Arizona Test Dust” row and “Micromate” row in Figure 1). This calculation is conducted using a Microsoft® Excel spreadsheet (SIS Calculator, Figure 1), which is saved and stored electronically. First, the mass of Micromate (in Kg) to be added to reach the goal POC is determined, then the mass of Fine Arizona Test Dust to add the remaining required TSS to meet the levels defined in the TQAP/test plan is determined. Note: TSS from Jars of Arizona Test Dust meeting ISO 12103 will be used when adding additional non-POC TSS. When partial jars of test dust are used they are not guaranteed to meet the strict grading requirements of ISO 12103, however, the particle size ranges will comply with

ISO12103 and 100% of the test dust added will contribute to TSS.

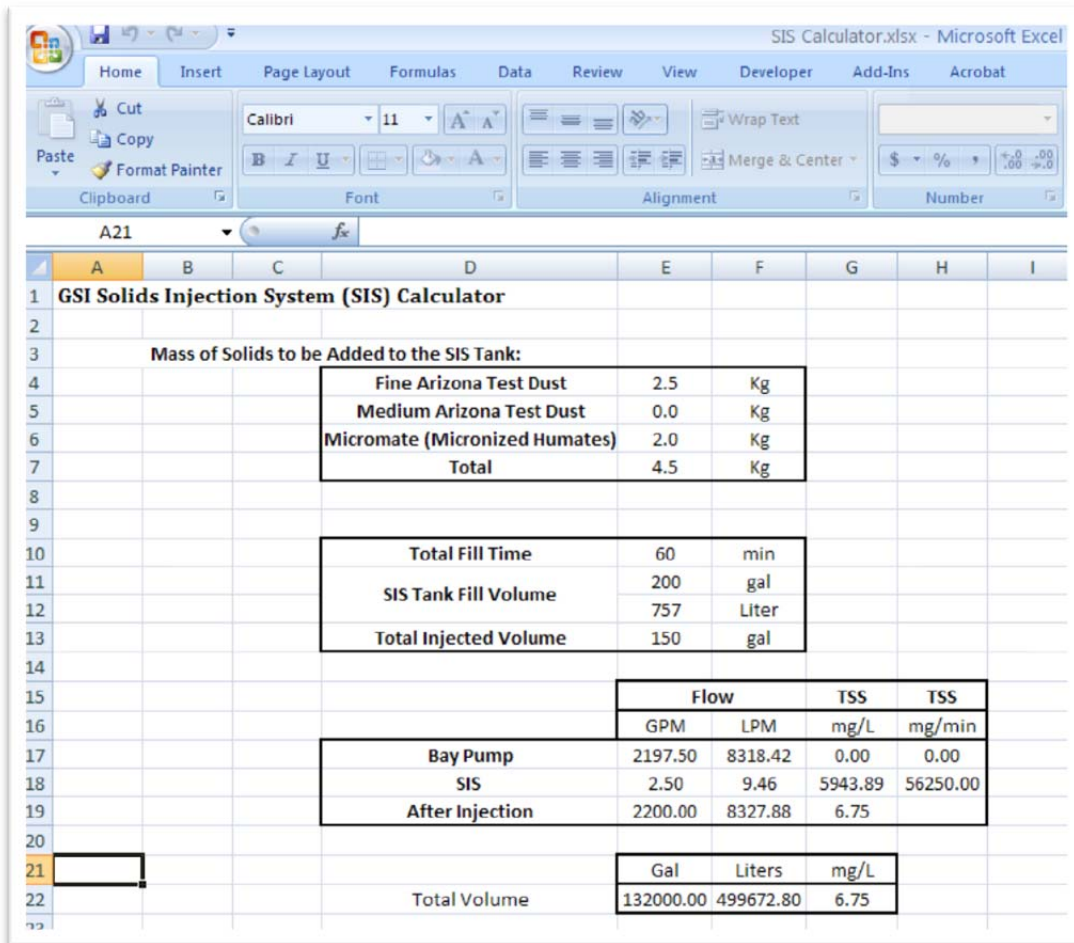


Figure 1. Screenshot of the Solids Injection System Calculator in Microsoft® Excel.

- Close SIS valves 1, 4, and 5 (Figure 2) prior to filling the tank; SIS valves 2 and 3 should be open.

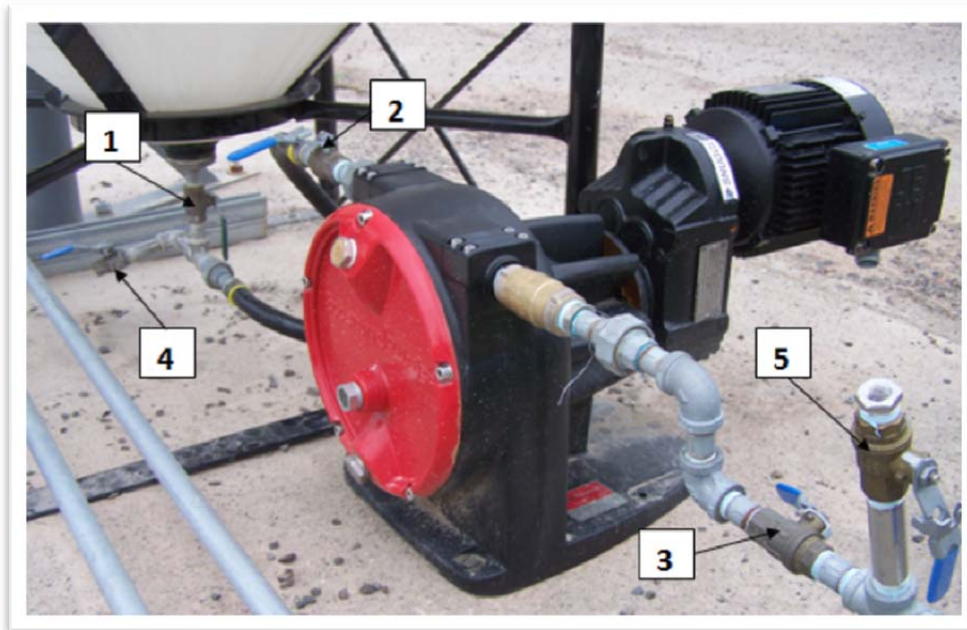


Figure 2. Location of the Solids Injection System (SIS) Valves.

4. Fill the SIS tank to a volume of 757 L (200 gal.) Once the tank is full of water, start the mechanical agitator and open the air control valve at the top of the tank leading to the bubbler in the base of the tank.
5. Measure out the mass (in Kg) of Micromate and Arizona Fine Test Dust to be added into jars, as determined from the SIS Calculator (Figure 1). Pour the contents one by one into the SIS tank. Do not add the next jar until there are no visible clumps of test particles from the previous jar. After all solids are added, mix the solids for a minimum of twenty minutes before the trial is started and the solids are injected into the facility.
6. Open SIS valve 1 (Figure 2) just prior to starting the ballasting simulation. Open valve 4 (Figure 2) briefly to let a small amount of the solid rich water to leak out and show that there is no clogging of the lines.
7. Turn the automatic solids injection “on” via the control panel and set the pump to the desired flow rate based on its flow capacity.
8. If the scenario start with flushing the injection will start thirty seconds after the flush has ended. If there is no flushing the injection will start thirty seconds after the bay pump starts, the diaphragm pump starts and solids are injected into the facility for the duration of the trial. At the end of the trial the diaphragm pump will automatically shut off.
9. Flush the remaining water and solids from the SIS tank and pump to the ground after the trial is complete.

Organism Collection

Before filling of the retention tanks can begin, it is critical to ensure that the intake density of organisms in the ≥ 10 and $< 50 \mu\text{m}$ size class is within the recommended concentration that is outlined in the Test Plan or TQAP, which is typically at least 1000 cells/mL. This is achieved by maintaining a mass culture of concentrated phytoplankton that can be injected during filling to supplement the ambient concentrations in the harbor water. The phytoplankton are collected from the Duluth-Superior Harbor one to two days prior to filling to minimize mortality.

1. Repeatedly collect phytoplankton by towing a $\frac{1}{2}$ m diameter plankton net with mesh size of 50 - 80 μm just below the water surface for 10 to 15 minutes or until the net clogs so badly that it is no longer filtering the water. Rinse the concentrated plankton from the net and cod end into collection containers and add harbor water to maintain temperature and minimize oxygen depletion. Several hours of collection may be needed to obtain enough phytoplankton for each test. Within 3 hours of collection, the 5 gal. containers of phytoplankton should be transported to the test site and placed in large fiberglass ponds (approximately 3 m^3) filled with harbor water. These ponds are open to the air and receive ambient illumination. Aeration is provided to keep the phytoplankton suspended.
2. Approximately one hour prior to injection, stir a pond using a canoe paddle, to ensure that the phytoplankton culture is fully mixed, including resuspension of any material that may have settled on the bottom of the pond. Use a 50 mL plastic bottle to collect a sample of the mixed phytoplankton from approximately 10 cm below the surface of the pond. Using a pipette, subsample 0.5 mL from the bottle and add it to a 25 mL sample bottle. Dilute the subsample to 20 mL (40X dilution) with filtered harbor water (see *GSI/SOP/LB/RA/SA/1* to derive filtrate). Assess the sample for viable cell density according to *GSI/SOP/LB/RA/SA/1*, ignoring taxonomic considerations (i.e., simply count living cells and do not assess entities or taxonomic information). Record the holding pond phytoplankton density on a laboratory notebook, and enter into the Microsoft® Excel OPIS Calculator (Figure 3).
3. Fill a sample tub with Duluth-Superior Harbor water by running the facility in a Sea-to-Control-to-Sea Scenario filling one tub so that approximately one half-hour prior to injection, a 1 L sample of harbor water can be collected from the sample collection tub. Assess the sample for viable cells according to *GSI/SOP/LB/RA/SA/1* without taxonomic consideration (i.e., simply count cells and do not assess entities or taxonomic information). Record the harbor water phytoplankton density on a laboratory notebook and enter into the Microsoft® Excel OPIS Calculator (Figure 3).
4. Based on the concentrations of cells in the pond culture and in the harbor, calculate (i.e., using the OPIS Calculator in MS Excel; Figure 3) the volume of pond culture needed for injection in order to derive a final, conservative concentration of 1500 cells/mL in the intake water, or other final concentration as specified in the TQAP or Test Plan (typically most or all of one pond is injected).

	A	B	C	D
1	harbor density		pond injection calculator	
2	dil'n from (ml)	745	dil'n from (ml)	0.5
3	to (ml)	15	to (ml)	20
4	transects	1.50	transects	1
5	count	957	count	491
6	density/ml	233.6	density/ml in pond	357,091
7		L>	harbor density/ml	234
8	Injection calculator. Rapid counts of ambient harbor and pond cell densities are performed prior to filling/injection. Calculations provide an estimate of concentrated pond water needed for injection.			desired density/ml
9			total tank vol (ml)	400,000,000
10			vol to spike (L)	1,419
11			vol to spike (gal)	374.8
12			draw down (inches)	21.82
13			liters per inch in pond:	65

Figure 3. Screenshot of Organism Pressure Injection System Calculator (in MS Excel).

Organism Pressure Injection System

1. Close OPIS valves 1, 2, 6 and 9 (Figure 4, valve 2 not pictured).

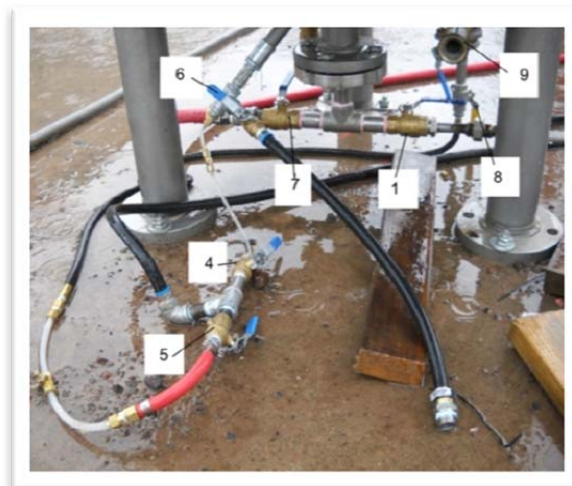


Figure 4. Location of the Organism Pressure Injection System Valves.

2. To ensure homogeneity of the culture pond prior to injection, stir the concentrated phytoplankton culture in the pond using a plastic paddle then add the culture to the

pressure vessel by either of two methods:

- a. Directly transfer the phytoplankton from one of the ponds to the pressure vessel by using a submersible sump pump. During filling, the water is passed through a coarse mesh sieve (approximately 1/4 inch mesh) to remove twigs, macrophytes and other large particles that could interfere with the injection equipment.
 - b. If the pond culture needs to be concentrated, pump (using a sump pump) the culture water from the tank through a 20 μm mesh plankton net and then transfer the concentrated organisms from the cod end of the net to the pressure vessel.
3. Once all the organisms have been added to the pressure vessel using either method, fill the vessel to the 100 gallon mark with bay water (filtration of the water is not required).
 4. Open the air release valve (OPIS valve 10, Figure 4) on the tank lid. Also open OPIS valves 4, 5 and 8 (Figure 4) to bubble water through the pressure vessel and keep the water agitated. Replace the tank lid and bolt down. Plan operations so that the tank sits in this state for as short a period of time as possible.

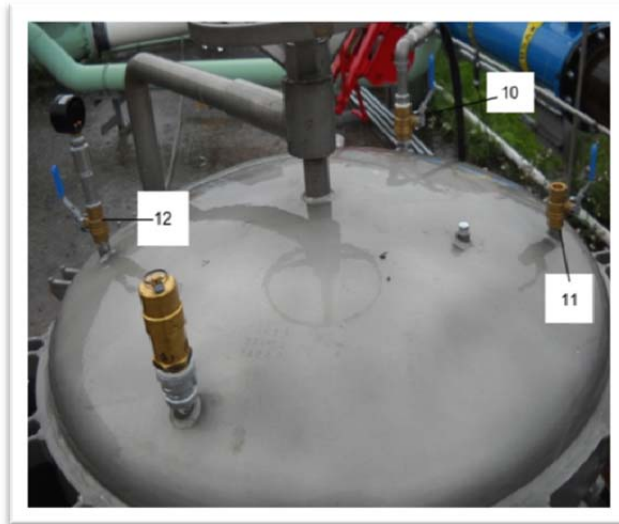


Figure 5. Location of the Valves on the Lid of the Organism Pressure Injection System.

5. Enter a flow rate on the control panel so that the entire OPIS tank can be discharged into the facility at a constant rate throughout the length of the fill. Enable automatic injection. Set valve FCV-500 to 0 % open. Open OPIS valves 1 and 3 (Figure 4, valve 1 not picture) between the pressure vessel and the main system.
6. Just before the trial is about to start close the release valve (OPIS valve 11, Figure 3) and open lid valve 10 (Figure 5). This will pressurize the pressure vessel. Adjust the regulator so that the pressure vessel maintains a pressure of 25 psi over the planned system pressure. Once the pressure differential is reached the OPIS is ready and the trial can begin.

7. If the scenario start with flushing the injection will start thirty seconds after the flush has ended. If there is no flushing the injection will stratr thirty seconds after the bay pump starts, Injection occurs when the diaphragm valve starts to open and organisms are pushed into the facility by the pressure differential. Note: On the SIS discharge line there is a diaphragm valve and flow meter. The flow meter reports and records the flow rates in the injection piping. From that flow rate the diaphragm valve will automatically adjust itself to reach the flow rate entered at the control panel.
8. Once the retention tanks have been filled and the bay pump shuts down the diaphragm valve FCV-500 also shuts.
9. Close OPIS valve 10 (Figure 5) and open lid valve 11 (Figure 5) to release the pressure inside the vessel. Check the pressure gauge on the top of the vessel to confirm that it reads 0 gauge pressure.
10. Unbolt and remove the top hatch of the OPIS pressure vessel. Drain the remaining water to the ground. Clean the vessel and flush the lines with potable water.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

1. Conduct all quality assurance/quality control procedures according to the *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Land-Based Tests (2011)*, as well as the Test Plan or TQAP specific to the ballast treatment technology being tested.
2. Follow all procedures outlined in this SOP. Any SOP amendments known ahead of time must be approved by the GSI Principal Investigator or one of the two Lead On-Site Investigators. Any deviations made during the experiment must be recorded and also approved by the GSI Principal Investigator or one of the two Lead On-Site Investigators as soon as practicable.
3. Record the measured weight of prepared Fine Arizona Test Dust and Micromate weighed into each plastic jar in a laboratory notebook. The jars must be labeled with the contents (i.e., Fine Arizona Test Dust or Micromate), the weight, the date, and the initials of the responsible individual.

DATA STORAGE AND ARCHIVING

1. Store and archive data according to *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Land-Based Tests (2011)*.
2. Archive all hard- and electronic-copies of data and records generated for a period of at least seven years.

REFERENCES AND RELATED DOCUMENTS

Great Ships Initiative website: www.greatshipsinitiative.org

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

GSI/QAQC/QAPP/LB/1 - Great Ships Initiative (GSI) Quality Assurance Project Plan (QAPP) for Land-Based Tests (2011).

GSI/SOP/LB/G/O/1 – Procedure for Operating the GSI Land-Based RDTE Facility.

GSI/SOP/LB/RA/SA/1 - Procedure for Algae/Small Protozoan Sample Analysis.

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (2010). How to Import Foreign Soil and How to Move Soil Within the United States. Circular Q-330.300-1. Plant Protection and Quarantine; 4700 River Road, Unit 133; Riverdale, Maryland 20737-1228.