

STANDARD OPERATING PROCEDURE Procedures for Measuring Organic Carbon in Aqueous Samples

Compiled By -

Name: Tom Markee &
Kelsey Prihoda

Title: GSI Chemist &
GSI Assistant QAQC Officer

Date: May 28, 2009

Approved By -

Name: Nicole Mays

Title: GSI Senior QAQC Officer

Date: May 28, 2009

Cleared For Issue By -

Name: Allegra Cangelosi

Title: GSI Principal Investigator

Date: May 28, 2009

RECORD OF AMENDMENTS:

No.	Date	Type	No.	Date	Type
1			7		
2			8		
3			9		
4			10		
5			11		
6			12		

STANDARD OPERATING PROCEDURE

Procedures for Measuring Organic Carbon in Aqueous Samples

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, GSI has established research capabilities at three scales—bench, land-based, and shipboard. Each scale is dedicated to addressing specific evaluation objectives, with protocols as consistent with IMO and federal requirements as practicable. Developers of ballast treatment systems apply for GSI research services [online](#), and awards are offered based on an objective review process. GSI incubation/testing will allow meritorious ballast treatment systems to progress as rapidly as possible to an approval-ready and market-ready condition.

GSI bench-scale tests take place year-round at the University of Wisconsin-Superior's Lake Superior Research Institute (LSRI) 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-scale testing are to explore dose-effectiveness, chemical degradation, residual toxicity, and sensitivity to challenge conditions of a proposed ballast 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.

INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the method used to measure the organic carbon concentration in aqueous samples collected during testing of a prospective ballast treatment system (BTS). A variety of organic compounds in various oxidation states make up the organic carbon in water and wastewater. To measure the quantity of organically bound carbon, the organic molecules must be separated into single carbon units and converted to a single molecular form that can be measured quantitatively. The Total Organic Carbon (TOC) Analyzer converts organic carbon to carbon dioxide by combustion. The carbon dioxide is then detected by the non-dispersive infrared gas analyzer (NDIR). The NDIR generates a detection signal which is converted to a peak, whose area is calculated by a data processor.

Inorganic carbon (IC) interference is eliminated by acidifying samples to a pH < 2 which converts IC species to carbon dioxide. Purging the sample with purified air prior to analysis

removes the carbon dioxide (generated from IC) by volatilization. The difference between total carbon (TC) and IC is TOC.

DEFINITIONS

Brackish Water (BW): Synthetic water created from laboratory water (LW) with the addition of commercially prepared salts, such as Instant Ocean, to obtain a salinity of 16 parts per thousand (as measured by a refractometer).

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).

High Organic Content Laboratory Water (HOC-LW): Synthetic water created from laboratory water (LW) with the addition of tannic and humic acid. Used as a surrogate in place of Duluth-Superior Harbor water.

Inorganic Carbon (IC): The carbonate (CO_3), bicarbonate (HCO_3), and dissolved carbon dioxide (CO_2) present in water (Eaton *et al.*, 2005).

Laboratory Water (LW): City of Superior, Wisconsin municipal water that has been dechlorinated by passage through an activated carbon filter. Note: Based on data from previous testing, background levels of chlorine from 5-10 $\mu\text{g/L}$ are expected in dechlorinated laboratory water.

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

Prospective Ballast Treatment System (BTS): A system containing an active substance and/or component that mechanically, physically, chemically, or biologically serves to remove, render harmless, or avoid the uptake or discharge of potentially invasive organisms within ballast water (IMO, 2005).

Total Carbon (TC): The combination of inorganic carbon (IC), total organic carbon (TOC), dissolved organic carbon (DOC), and nonpurgeable organic carbon (NPOC) present in water (Eaton *et al.*, 2005).

Total Organic Carbon (TOC): All carbon atoms present in water that are covalently bonded in organic molecules (Eaton *et al.*, 2005).

Salt Water (SW): Synthetic water created from laboratory water (LW) with the addition of commercially prepared salts, such as Instant Ocean, to obtain a salinity of 32 parts per thousand (as measured by a refractometer).

EQUIPMENT LIST

- Personal protective equipment (i.e., protective eyewear, laboratory coat, anti-heat gloves, etc.).
- Shimadzu Total Organic Carbon Analyzer (Model TOC-5050A).
- Carrier and purge gas (high purity air, hydrocarbon free).
- Magnetic stirrer/TFE-coated stirring bars.
- Filtering apparatus.
- 0.45 μm filters.
- Deionized water.
- Anhydrous Potassium Hydrogen Phthalate or 1000 mg/L TOC Standard.
- Concentrated hydrochloric acid.
- Volumetric flasks (100 and 200 mL).
- Volumetric pipets (1, 2, 5 and 10 mL) and pipet bulb.
- Micropipetter and tips.
- Desiccator.
- Sample vials/tubes.
- Disposable glass pipettes.
- Micro cleaning solution bath.

PROCEDURE

Note: All glassware to be used in this experiment should be soaked for a minimum of 30 minutes in Micro cleaning solution (40 mL Micro cleaning solution: 2 L deionized water), rinsed with hot tap water until no cleaning solution residue remains and rinsed thoroughly with deionized water before use.

As described in the introduction, NPOC values are determined on non-filtered samples. If a dissolved organic carbon (DOC) concentration is desired, the sample should first be filtered through a 0.45 μm filter. A filter blank should be analyzed to indicate whether the filtering process is adding organic carbon to the sample. The filter blank is a deionized water sample filtered through the same filter type as used for the sample. The difference between the NPOC and DOC values can be reported as particulate organic carbon (POC). Samples must be acidified to 0.2 % with hydrochloric acid before being analyzed.

1. Conduct procedure in a vented work area, taking appropriate health and safety measures.
2. Prepare TOC Stock Solution and Standards:
 - a. An organic carbon stock solution can be purchased or prepared in the laboratory. The concentration of the stock solution should be 1000 mg/L TOC. When not in use the stock solution should be refrigerated. If a purchased stock is used, be sure to check the expiration date before using it.
 - b. A total organic carbon stock solution can be prepared by dissolving 0.2125 g of oven-dried anhydrous potassium hydrogen phthalate (KHP) in deionized water.

The chemical formula for KHP is $C_8H_5KO_4$. Add 200 μL concentrated hydrochloric acid to a 100 mL volumetric flask to preserve the stock and dilute to volume with deionized water. Be sure the KHP is dissolved completely before moving on to the next step. This organic carbon stock is 1000 mg/L TOC. A new 1000 mg/L stock should be prepared every 6 months. Refrigerate when not in use.

3. Prepare a series of working standards (see table 1) by pipetting the appropriate amount of the 1000 mg/L organic carbon stock into 200 mL volumetric flasks, adding 400 μL concentrated hydrochloric acid and diluting to 200 mL with deionized water. Working standards must be remade every 3 months or when evidence of a change in concentration occurs. Refrigerate standards when not in use.

Table 1. Working Standards.

TOC Conc. (mg/L)	Volume Pipetted (mL)	Volume Conc. Acid (μL)	Final Volume (mL)	Final Conc. (mg/L)
1000	10.0	400	200	50
1000	5.0	400	200	25
1000	2.0	400	200	10
1000	1.0	400	200	5.0
1000	0.5	400	200	2.5

4. Analyze Standards and Samples using the Shimadzu Total Organic Carbon Analyzer:
 - a. Open the main valve on the air cylinder. Adjust the valve on the regulator to provide a pressure of 85 psi. The Carrier Gas flow rate should be 150 mL/min. and the Sparge Gas flow should be 100 mL/min. The Carrier Gas flow should start as soon as the instrument is turned on. Note: The Sparge Gas flow can only be checked when a sample is being sparged.
 - b. Turn the TOC analyzer on using the switch on the left hand side of the instrument.
 - c. Use the **F1** key to get to the **Main Menu**. Press **3 Enter** to get to **General Conditions**, then use the **Down Arrow** key to get to the **Furnace On/Off** position. Press **1 Enter**. Return to the **Main Menu** by pressing **F2**.
 - d. Go to **Monitor** by pressing **6 Enter** to monitor the progress of the warm-up. When each of the conditions on the right of the screen reads OK, the instrument is ready to begin analysis. This will take approximately 30 minutes.
 - e. Prepare a blank by acidifying a volume of deionized water to 0.2 % with concentrated hydrochloric acid.
 - f. Return to the **Main Menu** when the instrument warm-up is complete by pressing the **F2** key and then **Sample Measurement**. Do not alter anything on the screen that follows.
 - g. Fill a sample tube about two-thirds full with blank solution. Place the instrument's sampling tube into the blank solution and press **F1** to move on to the next screen. The **Cycle Mode** and **Non-stop Mode** should read **2 (off)**. Press **Start** to analyze the blank. This procedure cleans the instrument. After the sample has run, repeat

the procedure using the same sample. If the area of the peak is greater than 5000, consult the GSI Chemist or Laboratory Manager.

- h. Develop a calibration curve or use one that has been stored in the instrument. If a stored curve is used, a blank and at least one standard should be checked before any samples are analyzed. The check standard (e.g., 10.0 mg/L) result should fall within 10 % of the actual concentration. If not, re-run the standard. If this second analysis also fails, the standard should be remade and reanalyzed. If the newly prepared standard is found to have a reported value within 10 % of the actual concentration, analysis of samples can begin. If not, a new set of standards should be prepared and a new calibration curve generated before proceeding to analysis of samples
- i. Prepare a new calibration curve by returning to the **Main Menu** by pressing the **F2** key and entering **1** for **Calibration**. Enter the exact concentration of the standards prepared in place of the 0.0, 2.5, 5.0 and 10.0 mg/L. Table 2 provides an example of the data to be entered.

Table 2. Data for Calibration Curve.

Type	1st Cal. Curve	2nd Cal.Curve
1st Std. Conc.	0.0 mg/L	0.0 mg/L
2nd Std. Conc.	2.5 mg/L	10.0 mg/L
3rd Std. Conc.	5.0 mg/L	25.0 mg/L
4th Std. Conc.	10.0 mg/L	50.0 mg/L
Range	X 1	X 5
Inj. Volume	53 μ L	26 μ L
No. Injects	3	2
Max. No. Injects	3	2
Spurge Time	3 min	3 min

Note: If you make changes to the conditions, you have to press **Enter** for the instrument to accept the changes.

- j. Press **F1** for **Next** after entering the calibration curve conditions. The instrument will prompt you to **Set 1st Std Vessel, Insert Sampling Tube**. Put the sampling tube into the blank sample vial and press **Start**. The instrument will analyze that standard and prompt you to analyze the subsequent standards. When all the standards in the 1st curve have been analyzed; develop a 2nd calibration curve (suggested concentrations 0.0, 10.0, 25.0 and 50.0 mg/L). The screen will automatically adjust the range and inject volume for the TOC concentrations. Do not change the range value and the inject volume value if they are different from those shown here. Go through the analysis for the 2nd standard curve.
- k. Return to the **Main Menu** and go to **Sample Measurement** by pressing **2 Enter**. Enter the # of the 0-10 mg/L calibration curve to be used as the 1st calibration curve under the **NPOC** column. Press **Enter**. Enter the # of the 0-50 mg/L calibration curve to be used as the 2nd calibration curve under the **NPOC** column.

Press **Enter**. This allows the sample to be analyzed on the 0-10 mg/L curve initially. If the concentration is higher than 0-10 mg/L, it will be rerun on the higher standard curve. Press **F1** to move on to the next screen.

- l. The **Cycle Mode** and **Non-stop Mode** should read **2** (off). Place the sampling tube in the sample container and press **Start**. After the sample has been analyzed, press **F1 (Next)**. The sample data will be printed as a hardcopy. Write the identity of the sample next to the print-out that it corresponds to. Continue the sample analysis until concentrations have been determined for all samples.
- m. To end, go to the **Main Menu**, enter **General Conditions** and turn the furnace off by entering **2** next to the **Furnace On/Off** line. Return to the **Main Menu**. Press **7 Enter** to enter the **Standby** options screen. Enter **1 (Next)** to **Finish/Running**. Press **F1** for **Standby**. The screen will inform you of how much time remains before the instrument can be shut off.
- n. Turn the power switch for the instrument off when the instrument display indicates it can be shut off. **Do not shut the instrument off prematurely**. This wait is necessary to allow the instrument to cool down.
- o. Close the main valve on the air tank. Turn the secondary valve on the air tank counterclockwise until it is loose.

QUALITY ASSURANCE/QUALITY CONTROL

1. Conduct all quality assurance/quality control procedures according to the GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009). Analyze data to ensure that all applicable data quality criteria are met.
2. Collect and analyze in duplicate at least 10 % of the samples to document sampling and analytical variability. Whenever possible, spike at least 10 % of the samples with a TOC spiking solution. A certified reference standard may be purchased and analyzed to document performance of analytical instrumentation.
3. Follow all procedures outlined in this SOP. Any deviations known ahead of time must be approved by the GSI Lead Investigator for Bench-Scale Studies. Any deviations made during the experiment must be recorded and also approved by the GSI Lead Investigator for Bench-Scale Studies as soon as practicable.
4. Record data on data collection forms or in specific laboratory notebooks. All instrument data output (e.g., chromatograms, absorbance scans, and/or measurements) and data forms must be stored in a project-specific three-ring binder. Ensure hard copies of instrument data output and data collection forms are scanned and stored electronically.

DATA STORAGE AND ARCHIVING

1. Store and archive data according to GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009).

2. Archive all hard- and electronic-copies of data and records generated for a period of five years.

REFERENCES AND RELATED DOCUMENTS

Cangelosi AA (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.

Eaton, AD, Clesceri, LS, Rice, EW, and AE Greenberg, Eds. (2005). Standard Methods for the Examination of Water and Wastewater, 21st Edition. American Public Health Association, Washington, DC.

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

Great Ships Initiative website: www.greatshipsinitiative.org.

GSI/QAPP/1 - Quality Assurance Project Plan (QAPP) for Great Ships Initiative Bench-Scale and Land-Based Biological Tests (2009).

International Maritime Organization (IMO) (2005). Guidelines for Approval of Ballast Water Management Systems (G8) Adopted by Resolution MEPC.125 (53). London, England.