Test Plan for the Performance Evaluation of the Siemens SiCURE Ballast Water Management System

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1. MERC and GSI Background and Objectives

The Maritime Environmental Resource Center (MERC) is a State of Maryland initiative that provides test facilities, information, and decision tools to address key environmental issues facing the international maritime industry. The primary focus is to evaluate the mechanical and biological efficacy, costs, and logistical aspects of ballast water treatment systems and to assess the economic impacts of ballast water regulations and management approaches. A full description of MERC structure, products, and services can be found at www.maritime-enviro.org.

To address the need for effective, safe, and reliable ballast water treatment systems to prevent the introduction of non-native species, MERC has developed as a partnership between the Maryland Port Administration (MPA), Chesapeake Biological Laboratory/ University of Maryland Center for Environmental Science (CBL/UMCES), U.S. Maritime Administration (MARAD), National Oceanic and Atmospheric Administration (NOAA), Smithsonian Environmental Research Center (SERC), and University of Maryland (UM) to provide independent performance testing and to help facilitate the transition of new treatments to operations. Treatment evaluation efforts will also take advantage of expertise and the rigorous technology evaluation format/process developed by the Alliance for Coastal Technologies (ACT, www.act-us.info). ACT is NOAA-funded distributed testbed, headquartered at CBL/UMCES, dedicated to fostering the development and adoption of effective and reliable sensors for studying and monitoring coastal environments.

The Great Ships Initiative (GSI) is a collaborative not-for-profit endeavor to resolve barriers to effective and efficient ballast water treatment by ships. To that end, GSI evaluates the performance characteristics of proposed ballast water treatment systems at the bench-, land-based and shipboard scales. GSI land-based and shipboard testing is reserved for operationally feasible treatment systems likely to meet prevailing performance standards and environmental soundness requirements. The goal of GSI ballast treatment research services at the land-based and shipboard scales is to provide shipping lines, treatment developers and regulators with an independent and credible assessment of treatment performance under realistic freshwater challenge conditions. To that end, the fundamental approach of GSI is to conduct independent, scientifically-sound, rigorous, and quality assured evaluations of ballast water treatment systems under challenging ambient freshwater conditions based on the International Maritime Organization (IMO) G8 guidelines and the U.S. Coast Guard supported ETV protocols under development.

The following protocols describe how MERC and GSI will evaluate the performance characteristics of the Siemens Water Technologies SiCURE^{TM} Ballast Water Management Systems through objective and quality assured land-based testing (dockside at a flow rate of 200m^3/hr). The goal of this specific evaluation is to provide shipping lines, regulators, and flag states with an independent and credible assessment of treatment performance under realistic conditions. Therefore, the data and information on performance characteristics will cover legitimate information that users need and will compare performance against the International Maritime Organization (IMO) D2 regulatory discharge standards.

It is important to note that MERC and GSI themselves do not certify technologies or guarantee that a treatment will always, or under circumstances other than those used in testing, operate at the levels verified. Treatment systems are not labeled or listed as acceptable or unacceptable but tests and presented results are in a format consistent with that requested by

specific regulations (e.g., IMO D2, G8 and G9) so that can be used to determine regulatory compliance by appropriate agencies of certification societies. Final reports on technology performance will be reviewed by the MERC and GSI Advisory Board/Committee and provided to Siemens and the MERC/GSI funding agencies prior to public release. All specific terms of a testing program associated with a particular treatment system, including management of test findings, are outlined in a Participation Agreement executed between the treatment developer and MERC and the Northeast Midwest Institute, the GSI managing entity.

2. Treatment to be Evaluated

Siemens Water Technologies has developed the SiCURE™ Ballast Water Management Systems (BWMS) based on the maritime industry proven Chloropac® Electrochlorination system for ship's cooling water piping. This system was first developed in the early 1970s and has been in operation onboard over 2,000 vessels.

SiCURE has several unique features designed to provide effective treatment of ballast water while minimizing risk to the environment, the ship, and its crew. SiCURE is based on electrolysis of seawater and use of hypochlorite as an Active Substance at a viable, "meet the demand" dose. The system injects only as much Active Substance into ballast water as required to achieve the necessary level of disinfection. This approach is aimed at eliminating over chlorination and associated risks of corrosion and generation of disinfection by-products.

3. Overview of Test Facilities

Basic Approach:

The specific protocols described below are based on the IMO G8 guidelines and the US Coast Guard supported ETV protocols under development. The fundamental approach of MERC and GSI is to conduct independent, scientifically-sound, rigorous, and quality assured evaluations of ballast water treatment systems. Therefore, MERC and GSI rely on challenging ambient conditions found in the Chesapeake Bay and Duluth-Superior Harbor, and do not artificially augment test waters in most evaluations, to avoid artifacts and the potential to overestimation of system performance (see Table 1). For example, rapid changes in physical conditions (such as salinity or total suspended solids) as ambient organisms are being brought in with ballast water may cause significant mortality, independent of treatment. Similarly, concentrating natural assemblages of plankton on nets, and introducing them into ballast water being pumped into tanks, can often result in significant handling associated mortality. Given the unpredictable physical and biological conditions found in all natural waters, IMO G8 MEPC 58/23 ANNEX 4, Part 2, Section 2.3.36 is used by MERC and GSI as the standard for a valid test trial: "If in any test cycle the average discharge results from the control water is a concentration less than or equal to 10 times the values in regulation D-2.1, the test cycle is invalid". While a goal of MERC and GSI is provide independent G8/ETV data on the performance of ballast water treatment systems, it is ultimately up to an Administration to decide if the system meets their requirements for Type Approval Certification.

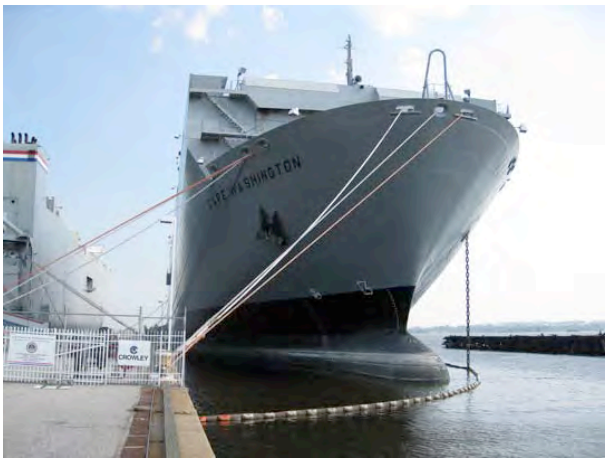
Table 1. Ranges of various physical and biological parameters in ambient water during the testing season (March/April – October/November) in the Port of Baltimore in comparison to ETV/USCG and IMO G8 recommended challenge conditions. Port of Baltimore data collected by MERC and various academic and agency studies or monitoring efforts in the general location of the *Cape Washington* (Patapsco River). Ranges in various physical and biological parameters of ambient water during the testing season (July – September) in the Duluth/Superior Harbor collected by GSI.

Parameter	Proposed ETV/USCG†	Recommended IMO G8‡	Historic Ranges* Port of Baltimore	Historic Ranges◊ Duluth/Superior Harbor
Temperature (^{o}C)	10 - 35	–	4 - 28	9 - 22
Salinity (psu)	0 - 31	Two salinities, >10 psu difference	5 - 15	0 - 1
Total Suspended Solids (mg/l)	> 15	> 50	1 - 60	2 - 21
Particulate Organic Carbon (mg/l)	> 1	> 5	0.5 - 6.0	TBD
Dissolved Organic Carbon (mg/l)	> 3	> 5	2 - 10	6 - 22
Zooplankton (> 50 μm) / m^3	> 10,000	> 100,000	10,000 - 300,000	10,000 - 3,000,000
Phytoplankton (10 - 50 μm) / ml	> 100	> 1,000	500 - 15,000	25 - 500
Heterotrophic Bacteria cfu / ml	> 1,000	> 10,000	10,000 - 10,000,000	5,000 - 15,000

† Generic Protocol for the Verification of Ballast Water Treatment Technologies: Draft v4 2008, US EPA Environmental Technology Verification (ETV) program under contract to US Coast Guard.
‡ IMO Guidelines for the Approval of Ballast Water Management Systems (G8), October 2008, Annex 4 Resolution MEPC.174(58).
* TSS, POC and DOC (2004-2007) MD DNR Chesapeake Bay Water Quality database: www.chesapeakebay.net/data_waterquality.aspx. Zooplankton (1998 – 2002) and phytoplankton (2004-2007) Chesapeake Bay Program: www.chesapeakebay.net/data_plankton.aspx. Bacteria (1998 – present) Cowell and Huq, University of Maryland; Louis et al. 2003, AEM 69:2773-2785.
◊ Values collected by GSI in 2007 and 2008 as part of facility validations and treatment systems testing www.nemw.org/GSI/.

For this specific evaluation of the SiCURE treatment system, Siemens Water Technologies has made a special request to MERC and GSI to augment intake water to more consistently approach the initial challenge water conditions described in the G8 guidelines during the test trials. While MERC and GSI do not make any guarantees on the precise conditions of challenge water, humic acid and Arizona Test Dust (as proposed by ETV and NRL Key West) will be injected inline during initial filling of control and test tanks at MERC and GSI to increase TSS, POC and DOC levels, and algae will be added inline to water during filling of control and test tanks at GSI. Details on these processes are available upon request and will be provided in the final report.

Summary of MERC Land-Based Facility and Sampling Design:



MERC will evaluate the biological efficacy of the SiCURE ballast water management system onboard the MARAD vessel M/V *Cape Washington* while docked in Baltimore Harbor, Maryland (right). The ballast system of the *Cape Washington* has been modified to allow for water at a flow rate of 400m^3/hr to be split equally, and delivered simultaneously, to a "control" (untreated) ballast tank and a "test" (passing first through the SiCURE system) ballast tank, each at 200m^3/hr. The ship's ballast tanks to be used for the required holding time of five days are essentially identical in size (~ 650 m^3) and structure. Each tank will be filled to approximately 250 m^3 for test trials. A detailed drawing of the modified ship ballast system can be found on page 24.

Care was taken in the design of the MERC *Cape Washington* test systems so that water entering the control and test tanks is handled (e.g., passing through same pump and similar piping) as close to identical as possible, aside from passing through the SiCURE system for treatment. Three test system performance runs have been conducted to assure that water in both control and test tanks have near identical physical and biological conditions. While initial physical and biological conditions are subject to natural variability, the MERC test system itself is not a source of mortality (data available upon request). The test ballast tank will also drained and manually rinsed/cleaned prior to conducting the first evaluation trial, and rinsed/flushed with 20 – 30 m^3 of potable water and drained completely between trials, to avoid the possibility of residual live organisms in the bottom of the empty test tank influencing results.

Five sequential samples will be taken for each of the following: (A) initial/intake conditions, just prior to the split of control and treated water, (B) initial conditions just downstream of the SiCURE system during filling of test tank, (C) control water upon discharge after a five-day holding time, and (D) treated water upon discharge after a five-day holing time. Sample volumes and details of the physical, chemical, and biological analyses for each sample are described below. A detailed drawing of the MERC *Cape Washington* test setup and sampling design is available on page 25.

All samples collected to quantify live organisms or water quality will be taken by inline sampling of ballast water during the initial filling or during discharge of water from the ship's tanks by sample ports place in appropriate filling or discharge pipes. All sample ports include a valve and sample tube with a 90^o bend towards the direction of flow, placed in the center of the piping system (based on the design developed and validated by the US Naval Research Laboratory, Key West Florida).

A total of 10 identical conical bottom mesocosms (shown below) have been installed on the *Cape Washington* to allow for precise and controlled sampling during each test trial. Five replicate mesocosms are used to sample initial, challenge conditions at the start of each trial, prior to the split in water to control and test tanks. The second five mesocosm are used to sample after water has passed through the SiCURE treatment during the initial filling of the test tank. At the end of each trial (after five-days), five mesocosms are used for sampling water from the

control tank, and the second five mesocosm for water from the test tank. At each sampling time (initial and after holding time), the designated five mesocosms will be filled to approximately 1.05 m^3 in sequence over 75 to 80 minutes of the 90 minutes required to fill or drain the ship's ballast tanks (i.e., sampling takes place > 80% of the time during filling or draining of tanks). Immediately after filling of each mesocosm (< 5 minutes), physical parameters of the water will be measured (see below), and then the precise samples volumes described below will be collected for each biological and water quality categories by gravity draining through a bottom valve and tubing. A table (Table 2) of samples to be collected, with corresponding volumes and purpose can be found on page 27.

Each mesocosm has been calibrated (by filling with potable water and a flow meter) and marked with known volumes to assure accurate sample collection. Each mesocosm will also be rinsed thoroughly with potable water for a minimum of three times after each use and kept clean and dry between uses.

MERC test and sampling system on the *Cape Washington*.

Summary of GSI Land-Based Facility and Sampling Design:

GSI evaluates the biological efficacy ballast water treatments at a purpose-built, land-based ballast treatment test facility located in the Duluth-Superior Harbor of Lake Superior (right). The facility draws raw intake water from Duluth-Superior Harbor at up to 680 m^3/hr. A Y-split in the intake piping simultaneously channels one half of the flow (up to 340 m^3/hr) to a treatment track and one half (also up to 340 m3/hr) to a matched control track. The treatment track directs water through the experimental treatment system and into a 200 m^3 cylindrical retention tank. The control track by-passes the treatment system and channels water directly into a matched control retention tank. After storage, water is discharged sequentially from the treatment and control tanks at m^3/hr to the harbor or wastewater treatment facility. A detailed design can be found on page 26 and information on GSI Facility Validation can be found at www.nemw.org/GSI/.

Water is sampled continuously throughout ballasting functions (intake or discharge) through in-line sample points. There are 14 in-line sample points at the GSI land-based facility in total, though not all are in use at this time. Each sample point is made up of three identical sample ports with a center-located elbow-shaped pitot tube (90^o) bent towards the direction of water flow used to sample the water. This pitot design is based on a design developed and validated by the U.S. Naval Research Laboratory in Key West Florida, and empirically at GSI. Intake sampling uses sample ports (page 26) at the paired intake sample points of SP#2 and SP#3 on the control and treatment tracks for concurrent sample water collection. Discharge sampling uses sample ports at the discharge sampling points of SP#9 and SP#10 (page 26), with sequential collection of control and treatment water.

Sample water at a given sampling location (i.e., intake line of the control track, intake line of the treatment track, or the discharge line for the control or treatment tracks) is transferred simultaneously and continuously throughout ballasting operations (intake or discharge) from at least two of three replicate in-line sample ports to at least two of three replicate 3.8 m^3 sample collection tubs via a 3.8 cm ID PVC transfer pipe and an automated pressure-controlled diaphragm valve.

Well-mixed time-integrated 1 l whole water phytoplankton, microbial and water quality samples are immediately extracted from the replicate sample collection tubs, with the remainder of the collection tub sample concentrated through 35 µm plankton nets to retain all zooplankton. Each replicate intake sample (control and treatment tracks) is time-integrated over the 45 minute fill period and at least 1 m^3 in volume. Each replicate discharge sample is time-integrated over the 45 minute discharge period and at least is 1 m^3 in volume for control track samples and up to 3 m^3 in volume for treatment track samples. On intake, control and treatment track samples are collected simultaneously. On discharge, they are collected sequentially, but within two hours of

each other. A table (Table 2) of samples to be collected, with corresponding volumes and purpose can be found on page 27.

Live analysis of zooplankton occurs on-site within one hour of filtration through the plankton net. Live analysis of phytoplankton samples occurs on-site within 1.5 hours of sample collection. All filtered or whole water samples are stored in coolers until they can be analyzed. Microbial samples, including spiked MS2 bacteriophage samples, are transported immediately following collection in an insulated container for analysis at the University of Wisconsin-Superior laboratory, located within 10 minutes drive of the land-based facility.

An on-site mobile field laboratory provides bench-scale facilities to support time sensitive assays associated with the GSI land-based tests, including live analysis of phytoplankton and zooplankton (right). The laboratory is climate-controlled, and has enough desk and counter space to allow for simultaneous microscopic and analytical analysis of samples.

4. Test Trials

Each facility (MERC and GSI) will conduct a maximum of six test trials (12 total) of the SiCURE system to assess its ability to meet IMO D2 ballast water discharge standards in land-based testing during the spring/summer 2009. As noted above, a valid test is regarded as one for which discharge densities of live organisms are at least 10 times the IMO D2 standard, consistent with IMO G8 MEPC 58/23 ANNEX 4, Part 2, Section 2.3.36.

Two treatment calibration test runs for the SiCURE system will also be allowed just prior to the formal evaluation at each facility. For any test that is considered valid (and for which the facility testing system functioned properly), an inability to: (a) successfully treat ballast water without interruption, (b) to meet D2 discharge standards after a five-days holding time, and/or (c) to discharge water environmentally benign (i.e., no residual toxicity) water (see page 12), will be considered a "failure". Results of tests regarded as failures will be noted and included in the final report. Two failures on the part of the SiCURE system may result in the termination of testing prior to the maximum of six test trials depending on the nature of the failures. MERC and GSI Senior Management will make a final decision on early termination of the tests, in consultation with Siemens staff.

This evaluation will be based on physical and biological characterization of water upon ballasting (uptake of water) and comparisons of organisms in control versus treated water after a five-day, in-tank holding time for the different D2 biological categories. Results will also be presented as concentration of viable organisms per biological category in treated water upon discharge versus IMO D2 standards.

MERC and GSI have worked to standardize methods and approached (described below) to evaluate the performance of ballast water treatment systems. Some subtle difference may exist as a result of specific facility design and estuarine versus fresh water and associated organism. However, these do not compromise the scientific rigor or comparability of results

between the two sets of tests. The following sections describe how each parameter and variable is sampled/analyzed and additional details can be found in Appendices and at www.nemw.org/GSI/SOPS.htm.

5. Methods

Quantifying Physical Conditions:

Temperature, salinity, dissolved oxygen, chlorophyll fluorescence, turbidity and pH will be measured every 15 minutes during the test trials by two identical multi-parameter probes (calibrated according to manufactures specification) placed, one each, into the control and test tanks. A third hand-held instrument will be used to measure temperature, salinity, and dissolved oxygen of water in each replicate sample (described above) as it is collected.

Initial inline samples (three replicates, 500 ml - 2 l each) of ballast water during the filling of the control and test tanks will also be collected, filtered, and analyzed for the water quality parameters of particulate organic carbon (POC), dissolved organic carbon (DOC), and total suspended solids (TSS). See Appendices A, B and C for details.

Quantifying Viable Organism > 50 µm in size:

As described above, MERC uses five 1 m^3 mesocosms (a 5 m^3 integrated sample) and GSI use three 1 m^3 mesocosms (a 3 m^3 integrated sample) to sample each time point and treatment type (Table 2, page 27). Sampling occurs during initial uptake of water, just downstream of the treatment systems during filling of the test tank, and upon discharge of control and treated water (after 5 days). Immediately after filling, each mesocosm will be drained through a 35 µm (50 µm diagonal dimension) plankton net to concentrate the zooplankton for examination under a dissecting microscope. The proportion and total concentration of live versus dead organisms will be determined using standard movement and response to stimuli techniques and this live/dead analysis will take place within one hours of collecting the individual samples. Depending on concentrations, quantification of zooplankton in initial samples (upon ballasting) and control samples may require analysis of sub-samples and extrapolation to the entire 1 m^3. Zooplankton samples will then also be fixed with buffered, 10% formalin in 125ml Nalgene bottles and shipped to the SERC for additional taxonomic evaluations. Total counts and general taxonomic classification will be conducted under a dissecting microscope at 25X, except for some taxa, which will be removed and identified using a compound microscope. Larval forms of invertebrates will be identified to higher taxonomic levels such as order (e.g., Decapoda) suborder (e.g., Balanomorpha) or class (e.g., Bivalvia). Adults will be identified to species in most cases.

Quantifying Viable Organism 10 - 50 µm in size:

MERC - Two liters of unfiltered water for each mesocosm (a 10 l integrated sample) will be collected immediately after filling, to determine concentrations of organisms in this size class using four distinct methods (A – D below, Table 2 page 27). All samples will be held in amber Nalgene bottles and transported on ice to laboratories where analyses occur within 3 hours of collection. (A) One sub-sample from the initial 2 l will be fixed with standard Lugol's solution, and placed in a 250 ml amber Nalgene bottles to determine total cell abundances under an inverted compound microscope using grid settlement columns and phase contrast lighting. (B) A

second 250 ml sub-sample will be stained using CMFDA (5-chloromethylfluorescein diacetate) as a selective live/viable indicator. Samples stained with CMFDA, are incubated and observed on a Sedgewick Rafter slide using a Leitz Laborlux S modified for epifluorescence. Cells are scored as live when showing strong fluorescence signature under excitation (some cells also showed motility). However, it is also widely accepted that these direct count and staining techniques have limitations (Lugol's does not selectively stain live or dead, various algal species take up CMFDA differently, and other particles in a sample can fluoresce). Therefore, analyses of chlorophyll are also conducted as supporting information. (C) A third sub-samples is filtered (Whatman GF/F 0.7 μm pore, 2.5 cm diameter membrane) and frozen (-80^{o}C) until analysis of total active chlorophyll-a by the CBL/UMCES Nutrient Analytical Services Laboratory using US EPA Methods 445.0 for extractive/fluorometric techniques (see Appendix D). (D) Finally a fourth sub-sample is used to determine chlorophyll levels after allowed to regrow under favorable conditions. Algae specific vitamins, minerals, and nutrients (Guillard 1975, F/2 formulation) are added to a sub-sample from each mesocosm and are placed in a standard algal culture light-dark regimen for six days, prior to extractive chlorophyll-a analysis. An increase in chlorophyll, or positive regrowth, indicates that viable phytoplankton were in the samples, whereas chlorophyll levels at or below detection limits of the laboratory analytical method suggests that there was no viable phytoplankton. Although precise abundances of cells/ml cannot be determined for diverse communities of phytoplankton using these types of regrowth experiments, this is a conservative method used to determine the presence/absence of living organisms.

GSI - For live analysis of organisms 10 – 50 μm in size at least 1 l of unfiltered water is taken from each of the triplicate control and treatment sample collection mesocosm/tub (a 3 l integrated sample, Table 2 page 27). Analysis occurs on-site within 1.5 hours of sample collection, with samples stored in coolers during the interim. Prior to analysis, samples are concentrated through a 10 μm plankton net and stored in a 25 ml sample container. Next, a 1.5 ml subsample is transferred to a 2-ml sample container, with 4 μl of FDA stock solution added. The subsample is then allowed to incubate in the dark for 5 minutes. For analysis, the concentrated algae sample is mixed and immediately transferred to a Sedgwick-Rafter cell, covered and placed on the stage of microscope that is set for simultaneous observation using brightfield and epifluorescence. At least 100 entities are then counted and identified along the horizontal transects, aiming for at least 100 entities (i.e., unicellular organism, colony or filament). Single cell entities and cells comprising colonial and filamentous entities are characterized as follows: alive = cells showing obvious green fluorescence from cell contents; dead = cells showing no or very little evidence of green fluorescence from cell contents (note: for entities containing multiple cells, all cells must be confirmed as dead to fulfill this category); and ambiguous = entities that cannot be clearly identified as alive or dead (should be uncommon). Entities that are less than 10 μm in all visible dimensions or greater than 50 μm in minimum dimension are not counted. Records are kept of transect lengths and widths so that the total counted area may be calculated later. Counting and measurement of entities follows standard procedures for individuals (length and width), colonies (e.g., number of cells, cell length and width) and filaments (e.g., number of cells, cell length and width or total filament length if cells cannot be discerned). The remaining concentrated sample in the 25 ml bottle is archived using a preservative (formalin or Lugol's) for long-term storage.

Quantifying Viable Indicator Pathogens:

A 1 l sample of water for each mesocosm/tub (a 5 l integrated sample for MERC and a 3 l integrated sample for GSI) is collected to determine concentrations of total heterotrophic bacteria and three specific indicator pathogens, *E. coli*, intestinal *Enterococci*, and toxigenic *Vibrio cholerae* (Table 2 page 27). Total heterotrophic bacteria are enumerated by spread plate method using NWRI agar according to *Standards Methods for the Examination of Water and Wastewater* (21st edition, 2005). The presence and abundance of *E. coli* and intestinal *Enterococci* is determined using a commercially available chromogenic substrate method (IDEXX Laboratories, Inc.; Noble et al. 2003) and 10 ml and 100 ml water sample aliquots. Additionally, concentrations of culturable *E. coli* and intestinal *Enterococci* are determined using a standard USEPA method, namely, membrane filtration on mTEC agar (*E. coli*) (1 ml, 10 ml and 100 ml) and mEA agar (*Enterococcus*) (10 ml and 100 ml). Abundance of total and toxigenic *V. cholerae* are calculated by filtration and selection on TCBS agar and enumerated using species-specific RNA colony blot (500 µl to 1 ml) and *ctxA* DNA colony blot (1-10 ml). Viable toxigenic *V. cholerae* is assayed with a commercial DFA kit specific for serogroup O1 (New Horizons Diagnostics) using monoclonal antibodies tagged with fluorescein isothiocyanate (FITC) (Hasan et al. 1994).

Data Analysis:

Although multiple mesocosms, samples, and measures from each tank will be taken, to avoid pseudo-replication, the unit of replication for statistical analyses is each trial (n = 5 or 6). We assume that all measures for a single trial provide one estimate of treatment efficacy. Thus, treatment efficacy for any biological parameter is estimated as changes found before and after trial (percent reduction), and as the difference in concentration between treated water and IMO standards. This approach controls for variation due to temporal changes in environmental conditions.

6. Protocols for Evaluations of SiCURE System Discharge Toxicity

MERC - The MERC Testing Team members at the University of Maryland Wye Research and Education Center (WREC) will evaluate the aquatic toxicity of the ballast water discharge. The testing is designed to meet Section 5.2 of the Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9) as resolved by the Marine Environmental Protection Committee of the International Maritime Organization (IMO, 2008). Section 5.2 states that, "The advantage of conducting toxicity testing on the ballast water discharge is that it integrates and addresses the potential for interactions of the Active Substances and Preparations with the possible by-products." This section requires that, "these toxicity tests should include chronic test methods with multiple test species (a fish, an invertebrate and a plant) that address the sensitive life-stage. The preference is to include both a sub-lethal endpoint (growth) and a survival endpoint." The MERC approach to meet these IMO guidelines use test methods and species employed by the EPA for Whole Effluent Toxicity (WET) testing of effluents. These methods are approved by the EPA (2002) and the American Society for Testing and Materials (ASTM, 2006). Personnel at WREC are vary familiar with these test species and methods and conducted WET testing from 1986 to 2003 for the Maryland Department of the Environment in support of its NPDES WET bioassay-monitoring program.

Test Species:

A fish, an invertebrate and a plant (algae) will be used in all ballast discharge tests. Because the test site in Baltimore Harbor is a mesohaline aquatic environment with salinities ranging from 5 to 15 psu, estuarine organisms will be used in these tests. The algal species will be *Isochrysis galbana* or *Tetraselmis suecica* depending on which species performs best in preliminary testing. The algae will be purchased from the University of Texas Algal Culture, University of Texas, Austin, Texas. The growth media for these species will be those given in Appendix A3 of ASTM Designation E 1218-04 "*Standard Guide for Conducting Static Toxicity Tests with Microalgae*" (ASTM, 2006). The culture conditions will follow those given in this guide. The fish species used in the test will be the sheepshead minnow (*Cyprinodon variegatus*) while the invertebrate species will be the mysid (*Americamysis bahia*; formerly *Mysidopsis bahia*). These are estuarine test species suggested for use in EPA's Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms (EPA, 2002). Test organisms will be purchased from Aquatic BioSystems of Fort Collins, Colorado. This company is our regular supplier of test organisms. They provide excellent QA/QC, including reference toxicant testing and quality control charts for all of their test species. Upon receipt by WREC, holding of test organisms will be conducted in accordance to guidelines outlined in the above referenced EPA manual (2002).

Active Substance and Measurement:

The test solution will be ballast water discharged from the test tanks during each trial of the SiCURE system. The active substance involved in this treatment is chlorine. According to Section 5.2.8 of the IMO G9 resolution, information on Total Residual Oxidants (TRO) and Total Residual Chlorine (TRC) should be provided as part of the application for evaluation, for both the ballast treatment process and the ballast water discharge. The Standard Methods for the Examination of Water and Wastewater Low-Level Amperometric Titration methods 4500-Cl D and E will be used to measure TRO and TRC in the ballast water discharge and in the various test dilutions. A Fischer and Porter amperometric titrator (Model 17T2000) will be used for all measurements. By using the high-sensitivity mode, a forward titration, and a 200 ml sample, TRO quantification limits for method 4500-Cl D are 15 µg/L TRO. With this sample size, 1 ml phenylarsene oxide (PAO, 0.00564 N) titrant equals 1 mg/L chlorine equivalents. For lower levels of oxidant, method 4500-Cl E will be used. A fourfold-diluted PAO titrant (0.00141 N) and a strip-chart recorder for signal amplification from the Fischer and Porter amperometric titrator (Model 17T2000) will be used to measure TRO concentrations to 5 µg/L. Samples will be analyzed immediately upon collection onboard the *Cape Washington* to avoid loss of oxidant due to holding. In addition to the amperometric titration method we will use a YSI Mulitmeter (Model # 556) equipped with a probe to measure oxidation reduction potential (ORP). The probe uses a platinum button sensor giving the instrument a range of -999 to +999 mV, an accuracy of ± 20 mV and a Resolution of 0.1 mV.

Experimental Design and Test Conditions:

Toxicity tests will be conducted on the discharge from all test trials. As required by the IMO G9, the discharge water will be tested with three estuarine species as described in Section 2.1. Both acute and chronic data will be generated for each test. A dilution series, using Baltimore Harbor water, will be run for each species.

Test samples will be collected at the time of discharge from the MERC facility. Samples will be collected by the MERC staff for analysis of both the efficacy of treatment at eliminating organisms from the ballast water and to investigate residual toxicity at discharge as described above. For the suite of toxicity tests, a volume of 38 L (10 gallons) must be collected. This includes enough water to do all of the test renewals. Test water will be stored in large HDPE containers and held at 4°C in the dark to retain as much of the initial toxicity as possible. Portions of this sample will be used each day to serve as the renewal water for the bioassay. Sub-samples of each will also be sent to a certified chemistry laboratory (TBD) for analysis of disinfection byproducts. MERC Testing Team will collect/deliver all samples for chemical analysis and manage analytical results but costs of chemical analysis will be covered by Siemens.

Summaries of the proposed test methods are given in Tables 4 through 8 (page 28). All of the tests will be conducted at the WREC toxicology laboratory. Since chlorine degrades rapidly, all toxicity tests will be initiated within two hours of the completion of a specific trial. Pilot studies have demonstrated that there is no measurable difference in chloronated water held for five days and then either tested within 30 minutes of collection or after a two hour holding and transport time. Standard EPA (2002) and ASTM (2006) methods that have used in at WREC since 1987 to conduct Whole Effluent Toxicity tests and single compound toxicity tests, will be employed. The survival and growth end-points from these tests are those required by the G9 document in Section 5.2.4 (IMO, 2008). The algae test represents a true population growth test.

In addition to the ballast water discharge efficacy testing, sampling and toxicity testing of the water in the test tank will also be conducted on a minimum of one of the six test trials. Sampling will be done at the time of tank filling/treatment, and at one day and five days. The analytical and bioassay methods described above will be used to analyze these different time point samples.

Statistical Analyses:

Toxicity endpoints will include survival in acute fish and invertebrate tests, survival and growth in chronic fish and invertebrate tests, and population growth in chronic algal tests as required in Section 5.2.4 of the G9 (IMO, 2008). Tests are designed with a dilution series to allow calculation of daily LC50 (concentration yielding 50% lethality) values from acute and chronic mortality data. In addition, chronic tests will include sufficient treatment replication to allow calculation of NOEC (no observable effect concentration), LOEC (lowest observable effect concentration) and EC25 (percent concentration yielding a 25% effect) values for all toxicity endpoints as required in Section 5.2.5 of the G9 (IMO, 2008). Statistical analyses will be performed using ToxCalc statistical software (TSS, 2006) according to methods from USEPA (2002) and ASTM (2006) guidance documents. Briefly, LC50s at daily intervals will be calculated from survival data using the Probit Method if an adequate dose response is achieved. If an adequate dose response is not achieved (e.g., only one partial mortality between the concentration causing 100% mortality and that causing 0% mortality), the Trimmed Spearman-Karber Method will be used. Chronic data will be tested using a Probit Method (EC25) and by analysis of variance (ANOVA) with means testing (NOEC/LOEC). Prior to ANOVA testing chronic data will be tested for normality using the Shapiro-Wilk's Test and for homogeneity of variance using the Bartlett's Test. Survival data will be arcsine square root transformed prior to analysis. If normally distributed and homogeneous survival and growth data will be analyzed

using a one-tailed ANOVA followed by a Dunnet's means comparison test (equal number of replicates/treatment) or a T-Test with Bonferroni Adjustment (unequal replicates/treatment) to determine differences from control data. If data do not pass the assumptions of normality or homogeneity, a Steel's Many-One Rank Test (equal replicates) or Wilcoxon Rank Sum Test with Bonferroni Adjustment (unequal replicates) will be performed. A *p* value of 0.05 will be used for all hypothesis tests; a *p* value of 0.01 will be used for testing assumptions of normality and homogeneity of variance. Results from the chronic statistical analyses will provide NOECs, LOECs, and EC25s for each ballast water treatment run.

Definition of Test Failure on the Grounds of Toxicity:

Permissible residual toxicity will follow the guidelines outlined by the EPA National Pollutant Discharge Elimination System (NPDES) for issuance of a Vessel General Permit (VGP) (full text is available at www.epa.gov/npdes/vessels; relevant sections on ballast discharge toxicity are 5.8.1.2 and 15.2). Based on these criteria a test trial will be considered a failure on the grounds of residual toxicity upon discharge if acute lethality (as indicated by determination of an LC50 of less than 100%) occurs in any test species. Determination of test failure as a result of chronic toxicity will be based on EC25 analyses. An EC25 is a point estimate of the toxicant concentration (expressed as percent effluent) that causes an observable adverse effect in 25 percent of test organisms. Chronic test results will be calculated in TUc (chronic toxicity units), where TUc = 100/EC25 (e.g., an EC25 of 100% (i.e., undiluted effluent) would yield a TUc of 1.0). In order for a test trial to pass, chronic toxicity of discharged ballast must not exceed 1.6 TUc for any species tested (equivalent to an EC25 of 62.5%). Calculation of a TUc greater than 1.6 for any test species will constitute a test trial failure based on residual toxicity within discharged ballast water.

Toxicity Quality Assurance:

Toxicity test acceptability (i.e., performance) criteria are presented in Tables 4 through 8. The quality assurance procedures for the algae tests will follow those discussed in detail in Section 13 of ASTM Designation E 1218-04 "*Standard Guide for Conducting Static Toxicity Tests with Microalgae*" (ASTM, 2006). Any deviations from the quality assurance procedures will be given in the final report.

GSI - The GSI's toxicity testing is designed to meet Section 5.2 of the Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9) as resolved by the Marine Environmental Protection Committee of the International Maritime Organization (IMO, 2008). Section 5.2 states that, "The advantage of conducting toxicity testing on the ballast water discharge is that it integrates and addresses the potential for interactions of the Active Substances and Preparations with the possible by-products." This section requires that, "these toxicity tests should include chronic test methods with multiple test species (a plant, an invertebrate and a fish) that address the sensitive life-stage. The preference is to include both a sub-lethal endpoint (growth or reproduction) and a survival endpoint." The following work plan will outline proposed methods to meet these IMO guidelines, using standard operating procedures (Table 3) developed by GSI which are based on methods approved by the USEPA (2002) and the American Society for Testing and Materials (ASTM, 2005).

Table 3. Great Ships Initiative Standard Operating Procedures Relative to Toxicity Testing.

GSI SOP Code	Test Type	Test Species	Test Endpoint
GSI/SOP/BS/RA/RT/5	Acute	*Selenastrum capricornotum*	Survival
GSI/SOP/BS/RA/RT/1	Acute	*Ceriodaphnia dubia*	Survival
GSI/SOP/BS/RA/RT/2	Acute	*Pimephales promelas*	Survival
GSI/SOP/BS/RA/RT/8	Chronic	*Selenastrum capricornotum*	Growth
GSI/SOP/BS/RA/RT/6	Chronic	*Ceriodaphnia dubia*	Reproduction
GSI/SOP/BS/RA/RT/7	Chronic	*Pimephales promelas*	Growth

Analytical Methods of Chlorine Determination:

Samples for chlorine analysis will be collected from the discharged water. Twenty ml of sample will be transferred from the sample collection container into a 30 ml beaker. The samples will have 200 µl of potassium iodide reagent and 200 µl of acetate buffer reagent added to them. Samples will be analyzed for total residual chlorine concentration as soon as possible after having been collected. Analysis will be conducted with a Thermo Orion Model 97-70 Residual Chlorine Electrode connected to an Orion Model 290A pH/mV/ISE meter.

A 100 mg/L chlorine stock solution will be prepared daily. Analytical standards, ranging in concentration from 5 to 3000 µg/l, will be prepared in deoxygenated deionized water by making dilutions of the 100 mg/l chlorine stock. Potassium iodide reagent and acetate buffer are added to the chlorine containing analytical standards. Chlorine present in the standards/samples oxidizes iodide to iodine in an acidic solution. The iodine concentration after the reaction is equal to the chlorine concentration present before the reaction. A calibration curve plotting log of the chlorine concentration (x-axis) versus the mV response from the Residual Chlorine Electrode (y-axis) is used to determine total residual chlorine concentrations in the samples.

Quality control sample analysis consists of analyzing duplicate samples and samples spiked with known amounts of chlorine. Approximately 10% of the samples will be analyzed in duplicate. This is also true for spiked samples in dechlorinated laboratory water.

Experimental Design and Test Conditions:

Toxicity tests will be conducted on the discharge from all test trials. As required by the IMO, the discharges will be tested with three freshwater species as described in Section 2.1 (IMO, 2006). Both acute and chronic data will be generated for each test. A dilution series, using Duluth-Superior Harbor water, will be run for each species.

Test samples will be collected at the time of discharge from the GSI facility. Samples will be collected by the GSI staff for analysis of both the efficacy of treatment at eliminating organisms from the ballast water and to investigate residual toxicity at discharge as described earlier in this document. Test water will be stored in large HDPE containers and held at 4°C in the dark to retain as much of the initial toxicity as possible. Portions of this sample will be used each day to serve as the renewal water for the bioassay. Sub-samples of each will also be sent to a certified chemistry laboratory for analysis of disinfection byproducts. GSI Testing Team will collect/ship all samples for chemical analysis and manage analytical results but costs of chemical analysis will be covered by Siemens.

All of the tests will be conducted in temperature-controlled incubators at the University of Wisconsin-Superior's Lake Superior Research Institute, which is located less than 6 city blocks from the testing facility immediately following sample collection.

Cold Water Effects:

One additional laboratory assay will be conducted to evaluate the toxicity and degradation of discharge water to simulate cold water treatment in Duluth-Superior Harbor. A 50 l subsample of treated water will be collected just downstream of the treatment system during one of the test trials, upon initial filling of the test tank. This subsample will then be placed in a dark, temperature controlled room at 4°C for the required 5-day holding time and then analyzed as described above, with the only modification being all assays will be conducted at 4°C.

Statistical Analysis:

Data will be analyzed using the SigmaStat® program (Jandel Corporation, 1995) and final results will be verified by using the TOXCALC® 5.0 program. Data analyses will included: normality, homogeneity of variance, one-way analysis of variance (ANOVA), and suite of tests for comparison between treatment means. Non-normal survival data will be transformed using the natural log (EPA 2000) to normalize the data. The endpoints of the dose-effectiveness experiments were the lethal concentration that provided 50 percent mortality, the lowest observed-effect-concentration (LOEC), and no-observed-effect-concentration (NOEC) of the test species except for the microbes. The LOEC is the lowest concentration in a test with a statistically significant response that is different from the control response. The NOEC is the highest test concentration for which there was no statistically significant difference from the control response. These measures are extrapolations of statistical results to the experimental endpoints. Mean percent survival and mean dry weight values for the laboratory controls and treatments will be analyzed with a statistical significance level of 0.05.

Quality Assurance/Quality Control:

Toxicity tests will be initiated with healthy, vigorous animals. Reference toxicant tests will be performed with the test species prior to the start of the definitive test. In the toxicity tests, percent survival and dry weights of survivors in the controls will be compared to published test acceptability criteria (U.S. EPA 2000) to determine the overall performance of the animals and the test system. Class I standardized weights are used as a check for the organism drying process and the performance of the balance. Daily and weekly calibration of test meters ensures optimal performance. Reference standards and duplicate samples will be used in the analysis of chlorine. The QC/QA documentation will be noted on the raw data sheets and study logbooks.

7. Evaluation Schedule (planned dates based on current plan and may vary)

- Test Plan for SiCURE finalized and Evaluation Agreements signed by May 22, 2009
- SiCURE ballast water management system delivered to *Cape Washington* for testing at MERC May 19, 2009
- SiCURE system installed and operating on the *Cape Washington* by May 29, 2009
- Two SiCURE calibration run completed by June 1, 2009
- MERC evaluation of the SiCURE systems initiated by June 15, 2009
- MERC will complete sample analysis and compile data from the evolution by July 31 2009
- SiCURE ballast water management system delivered to Superior, WI for testing at GSI by August 7, 2009

- SiCURE system installed and operating at GSI by August 17, 2009
- Two SiCURE calibration run completed by August 28, 2009
- GSI evaluation of the SiCURE system initiated by August 31, 2009
- GSI evaluation of the SiCURE system complete by October 9, 2009
- Draft report on the performance of the SiCURE system for review by the Advisory Board/Committee and Siemens by December 31 2009
- Final report submitted and released to public by March 2010

8. Data Recording, Processing, and Storage

This section describes methods employed during data recording, processing, and storage to minimize errors and assure high quality analyses.

MERC -

Documentation and Records:

A variety of data will be acquired and recorded electronically and manually by MERC partners (CBL/UMCES, SERC, UM and WREC) during this evaluation. Operational information and results will generally be documented in field/laboratory record books and on the data sheet/chain-of-custody forms (see below). Copies of these raw data will be transferred to the MERC office, which will store it permanently along with the rest of the study data.

Data Review:

All data are to be recorded directly in the field/laboratory record book as soon as they are available. Records are to be written in water-proof ink and written legibly. Any corrections will be initialed by the person performing the correction, will be crossed out with a line (not blackened or white-out), and will be dated according to the date that the correction was made. These data will include electronic data, entries in field/laboratory record books, operating data from the MERC test facility, and equipment calibration records. Records will be spot-checked within two weeks of the measurement to ensure that the data are recorded correctly. The checker shall not be the individual who originally entered the data. Data entries shall be checked in general for obvious errors and a minimum of 10 percent of all records shall be checked in detail. Errors detected in this manner shall be corrected immediately. The person performing the review will add his/her initials and the date to a hard copy of the record being reviewed. The MERC staff member will place this hard copy in the files for this evaluation. In addition, data generated by each MERC staff will be provided to the MERC Program Coordinator and reviewed before they are used to calculate, evaluate, or report results.

GSI -

Data Recording:

Specific forms (i.e., by size class of organism, by scale of testing, etc.) are used to record sample collection and analysis data. All relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of sample collection and analysis. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QAQC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QAQC officers and

checked for compliance. Problems identified by spot-checks are documented and included in a training/response file.

Specific forms (i.e., one for the GSI RDTE facility and one for bench-scale research activities) are also used to record sample custody, handling and storage information. Relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of changes to sample custody, and sample handling and storage. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QAQC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QAQC officers. Problems identified by spotchecks are documented and included in a training/response file.

Data Processing and Storage:

A database designed using the Microsoft Access software suite is used to store, manage and process data produced by the GSI. Microsoft Excel is used in conjunction with the database to create various dataset formats for subsequent analysis. Database entry and maintenance is the responsibility of the GSI database management staff. Regular checks for data entry errors are conducted by comparing database records with the original paper data sheets. This is a manual inspection process and though rather time consuming, it is an essential procedure for discovering errors. After examination and QA analysis, the data distribution files from the Access database are posted to the Lake Superior Research Institute's (LSRI's) Local Area Network (LAN) in an organized hierarchical folder system such that those relevant GSI personnel are able to access the data. A backup of the database is also made regularly to avoid any loss of data following computer/electronic glitches. Files are also posted to the GSI's sharepoint website such that those GSI personnel outside of the LSRI network can access the data. Posting to sharepoint also acts as a secondary data backup/storage mechanism.

9. Quality Assurance/Quality Control

MERC -

Treatment performance evaluations are implemented according to the Test/QA plans and technical documents (e.g., Standard Operating Procedures) prepared during planning of the evaluation. Prescribed procedures and a sequence for the work are defined during the planning stages, and work performed shall follow those procedures and sequence. Technical procedures shall include methods to assure proper handling and care of test instruments. All implementation activities are documented and are traceable to the Test/QA plan and SOPs and to test personnel.

Analytical Laboratory Quality Control:

The analyses for Chlorophyll, TSS and POC shall have the following Quality Controls:

a. Blanks

Three times during the evaluation, analysis of blanks. These blanks will be collected weekly during sampling and should include Field Blanks (see Section 7.4.2).

b. Control Charts. Two types of control charts are used in laboratories: a mean chart for blanks and a range chart for replicate analyses.

Quality Control for Instrument Calibration:

The test instrumentation to be used in the evaluation will be calibrated by the MERC staff according to the SOPs for the instrumentation prior to use. A calibration log will be created for each instrument. The logs shall include at least the following information: name of instrument, serial number and/or identification number of instrument, date of calibration, and calibration results. These logs shall be provided to the MERC Program Coordinator and maintained in a master calibration file as part of the QA/QC records.

Laboratory Test Quality Control:

All analytical measurements are performed using materials and/or processes that are traceable to a Standard Reference Material. Standard Operating Procedures are utilized to trace all quantitative and qualitative determinations to certified reference materials. All metrology equipment (analytical balances, thermometers, etc.) is calibrated using materials traceable to the National Institute of Standards and Technology (NIST) and maintained on a schedule to ensure accuracy.

All volumetric glassware must be calibrated as conforming to Class A. A valid certificate of calibration or compliance must be available for each item. If the item has been calibrated in-house, the laboratory shall have a documented record of the calibration data showing traceability to national standards. Since the capacity of volumetric glassware may change with use, the calibration should be verified at regular intervals. Volumetric capacity is normally determined gravimetrically, using water conforming to the MERC glassware calibration Standard Operating Procedure (SOP). Before starting, care will be taken to ensure that the glassware is clean.

Field Logs:

Standard uniform field logs will be maintained for the evaluation. These logs should report name of staff conducting fieldwork, date (month, day, and year), operating status of all equipment, and manual readings of environmental conditions.

Field Quality Control Samples:

Field quality control samples provide information on the potential for bias due to contamination of analytical results by sample collection, processing, shipping, and analysis. To ensure that the field sample collection and analysis procedures are properly controlled, field blanks and replicate samples will be taken three times during the evaluation. These will be analyzed in the same manner as the collected samples for Chlorophyll, TSS, and POC. Field blanks are generated under actual field conditions and will account for all sources of contamination that might be introduced to a sample including incidental or accidental sample contamination during the entire process of sampling, transport, sample preparation, and processing. While field blanks mimic sample collection and processing, they do not come in contact with ambient water.

Sample Custody:

All samples will be accompanied by the sample collection sheet and a Chain-of-Custody (COC) form.

The COC specifies time, date, sample location, unique sample number, requested analyses, sampler name, required turnaround time, time and date of transaction between field and laboratory staff, and name of receiving party at the laboratory. Proper labeling of sample bottles

is critical. The COC is a mechanism by which a sample can be tracked through the various phases of the process: collection, shipping, receiving, logging, sample prep/extraction, analysis, and final data QA/QC review.

When transferring the possession of the samples, the transferee must sign and record the date and time on the chain-of-custody record. Custody transfers, if made to a sample custodian in the field, should account for each individual sample, although samples may be transferred as a group. Every person who takes custody must fill in the appropriate section of the chain-of-custody record. The MERC staff member is responsible for properly packaging and dispatching samples to the laboratory for analysis. This responsibility includes filling out, dating, and signing the appropriate portion of the chain-of-custody record. The original and one copy of the chain-of-custody record form should be placed in a plastic bag inside the secured shipping container with the samples. One copy of the chain-of-custody record form should be retained by the MERC staff member at each MERC partner institution. The transportation case should then be sealed and labeled. All records should be filled out legibly in waterproof pen.

Sample Handling:

All collected physical, chemical, and biological samples will be handled in the same manner. Each sample will be dated and coded according to the appropriate sample sequence. The actual sample container will be labeled with a number for identification. Samples stored for any period of time shall be routinely inspected by the MERC staff member to assure proper preservation and label integrity. The storage containers and storage devices (e.g., freezers and locker) must be inspected routinely for proper operation and integrity. Results of all inspections shall be included in the sample records. All logs shall be duplicated weekly. The original shall be retained at the MERC partner site and a copy shall be sent to the MERC Program Coordinator.

Audits:

MERC Program Coordinator will perform a technical systems audit twice during the evaluation. The purpose of this audit is to ensure that the tests are being performed in accordance with the MERC Protocols, published reference methods, and any SOPs used. In this audit, the MERC Program Coordinator may review the reference methods used, compare actual test procedures to those specified or referenced in the Protocols, and review data acquisition and handling procedures. A technical systems audit report will be prepared, including a statement of findings and the actions taken to address any adverse findings.

MERC Program Coordinator will also audit approximately 10% of the evaluation data acquired during the tests to determine if data have been collected in accordance to the Protocols with respect to compliance, correctness, consistency, and completeness. The MERC Program Coordinator will trace the data from initial acquisition to final reporting.

Finally, each assessment and audit will be documented, and assessment reports will include the following:

a. Identification of any adverse findings or potential problems,
b. Response to adverse findings or potential problems,
c. Possible recommendations for resolving problems,
d. Citation of any noteworthy practices that may be of use to others, and
e. Confirmation that solutions have been implemented and are effective.

Corrective Action:

The MERC Program Coordinator, during the course of any assessment or audit, will identify to the MERC staff performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the MERC Program Coordinator will consult with MERC Primary Investigators and is authorized to stop work. Once the assessment report has been prepared, the MERC Program Coordinator will ensure that a response is provided for each adverse finding or potential problem and will implement any necessary follow-up corrective action. The MERC Program Coordinator will ensure that follow-up corrective action has been taken.

QA/QC Document Control:

It is the responsibility of the MERC Program Coordinator to maintain QA/QC records, which shall include the following:

1) records of the disposition of samples and data.
2) records of calibration of instruments.
3) records of QA/QC activities, including audits and corrective actions.

GSI -

Quality Assurance Project Plan (QAPP):

The GSI's Quality Assurance Project Plan (QAPP) outlines the management activities the GSI undertakes to assure the credibility of its biological research activities. The plan covers QA/QC data quality indicators, evaluation processes, performance measures and acceptance criteria; instrument certification and calibration; personnel training requirements; QA/QC of documents and records; data management; and QA/QC assessments and response actions; etc. The plan is updated annually, with a specific process used for review, comment, approval, distribution and posting. It closely follows the format of the U.S. Environmental Protection Agency's (EPA's) "*EPA Guidance for Quality Assurance Plans*".

Quality Assurance/Quality Control Activities:

GSI QA/QC Officers regularly observe all aspects of the GSI's biological research activities and conduct technical reviews of these activities to ensure that all information is complete, that SOPs are correctly followed, and that QA/QC objectives are met. The results of these observations are documented on a QA/QC audit report form. It is the responsibility of the GSI QAQC Officer undertaking the audit to maintain the form on file, create an electronic copy, and post to the GSI Sharepoint website for storage. The GSI Senior QA/QC Officer also uses these audits to help prepare an annual report describing all GSI QA/QC activities, including results of QA/QC observations, corrective actions, etc. Corrective action reports help resolve any identified deficiencies and non-compliance issues that relate to on-going activities and problems of a systematic nature.

Standard Operating Procedures (SOPs):

GSI SOPs are developed by the relevant GSI senior personnel in conjunction with the PI and GSI Senior QA/QC Officer. The SOPs follow a common format and all include specific QA/QC procedures and metrics. The Senior QA/QC Officer is responsible for distributing the

SOPs to the relevant parties for approval. Draft and final copies of all SOPs are posted to the GSI Sharepoint website; the final version is also posted to the GSI public website. SOPs are updated on an as-needed basis.

Documents and Records:

The GSI Senior QA/QC Officer is responsible for maintaining all documents and records for a period of ten years unless custody is transferred using a chain of custody form. Electronic versions of all GSI documents and records are saved to the GSI Sharepoint website once complete. Hard copies of GSI documents and records are scanned and also saved to the GSI Sharepoint website. Due care and diligence will be taken to properly dispose of documents and records that are no longer required after a 10 year period has elapsed. Disposal procedures will involve electronic deletion of documents and records from the GSI Sharepoint website and the personal computers of GSI personnel, as well as manual shredding of hard copies.

Notebooks:

Bound field and laboratory notebooks are used to record observations, sampling details and on-site laboratory and field measurements. Notebooks are also used to record instrument and equipment calibration and maintenance information. All notebooks are examined periodically by the GSI QA/QC officers and checked for compliance with SOPs. Problems identified by the periodic QA/QC review will be documented and included in a training/response file.

Sample Collection and Analysis Records:

Specific forms (see individual SOPs) are used to record sample collection and analysis data. All relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of sample collection and analysis. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QA/QC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QA/QC officers. Problems identified by spot-checks are documented and included in a training/response file.

Sample Management Records:

Specific forms are used to record sample custody, handling and storage information. All relevant GSI Senior Personnel are responsible for ensuring that the forms are correctly filled out at the time of changes to sample custody, and sample handling and storage. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QA/QC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QA/QC officers. Problems identified by spot-checks are documented and included in a training/response file.

Safety, Operation and Maintenance Records:

All relevant GSI personnel particularly those involved with operating the GSI RDTE land-based facility are responsible for ensuring that all forms associated with safety, operation and maintenance (i.e., confined space entry permit forms) are correctly filled out. They are also responsible for maintaining the forms on file, creating electronic copies, and posting to the GSI Sharepoint website for storage. QA/QC spot-checks of these forms and the processes used to complete and maintain them are undertaken periodically by GSI QA/QC officers. Problems identified by spot-checks are documented and included in a training/response file.

10. Roles and Responsibilities

The evaluation is coordinated and supervised by the MERC and GSI Principal Investigator, Program Coordinator and MERC and GSI personnel. Staff participate in this test by installing, maintaining, and operating the respective technologies throughout the test; operating the reference equipment, collecting the water samples, downloading the data from the instrument package, and informing the MERC Program Coordinator staff of any problems encountered. Manufacturer representatives shall train MERC and GSI staff in the operation of their treatment system. However, the proper installation, calibration, maintenance, and operation of the systems is ultimately the responsibility of the manufacturer. QA oversight is provided by the MERC and GSI Program Coordinator. In addition to aiding the development of these protocols, the MERC and GSI Advisory Board/Committee will be consulted during the evaluation in the event problems occur, will assist in the analyses of results, and will review the final Treatment Performance Report prior to release. Specific responsibilities are detailed below.

The MERC and GSI Principal Investigators have the overall responsibility for ensuring that the technical goals and schedule established for the evaluation are met and the final authority on decisions regarding this evaluation. The Principal Investigators shall:

- Prepare the draft Test Protocols/QA Plan and Treatment Performance Evaluation.
- Revise the draft Test Protocols/QA Plan and Treatment Performance Evaluation in response to reviewers' comments.
- Finalize the Test Protocols/QA Plan and Agreement for this Treatment Performance Evaluation.
- Sign the Treatment Performance Evaluations Agreement on behalf of MERC and GSI.
- Aid in treatment system testing.
- Aid in the preparation of a final report on this Treatment Performance Evaluation.
- Provide final approval of the Treatment Performance Evaluation Report.

The Program Coordinators shall:

- Help prepare the draft Test Protocols/QA Plan and Treatment Performance Evaluations
- Help revise the draft Test Protocols/QA Plan and Treatment Performance Evaluations in response to reviewers' comments.
- Coordinate distribution of the final Test Protocols/QA Plan and Treatment Performance Evaluation.
- Coordinate testing, measurement parameters, and schedules.
- Ensure that all quality procedures specified in the test/QA plan are followed.
- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary.
- Serve as the primary point of contact for manufacturers and Testing Teams.
- Ensure that confidentiality of proprietary manufacturer technology and information is maintained.
- Review the draft Test Protocols/QA Plan and Treatment Performance Evaluations.
- Conduct a technical systems audit (TSA) once during the evaluation.
- Audit at least 10% of the verification data.
- Prepare and distribute an assessment report for each audit.
- Verify implementation of any necessary corrective action.

- Determine if a stop work order should be issued if audits indicate that data quality is being compromised or if proper safety practices are not followed.
- Provide a summary of the audit activities and results for the verification reports.
- Review the draft Evaluation reports.
- Have overall responsibility for ensuring that the test/QA plan, SOPs and QMP are followed.

Testing Teams* shall:

- Assist in developing the Test Protocols/QA Plan.
- Perform sample collections and analyses as detailed in the test procedures section of the test/QA plan.
- One member of the Testing Team will conduct 10% data audit as described in QA procedures. This will be done for all data logs and electronically entered data.
- Provide all test data to the Program Coordinator electronically, in mutually agreed upon format.
- Provide the Program Coordinator access to and /or copies of appropriate QA documentation of test equipment and procedures (e.g., SOPs, calibration data).
- Provide information regarding education and experience of each staff member involved in the evaluation.
- Assist in reporting of their respective test facility's QA/quality control results.
- Review portions of the draft Performance Evaluations to assure accurate descriptions of their respective test facility operations and to provide technical insight on evaluation results.

*MERC Testing Team includes researchers from the University of Maryland Center for Environmental Science, Smithsonian Environmental Research Center, University of Maryland at College Park, University of Maryland Wye Research and Education Center, and the crew of the *M/V Cape Washington*. A complete list, with qualifications, is available upon request.

* GSI Testing Team includes researchers from the University of Wisconsin Superior Lake Superior Research Center; University of Minnesota-Duluth Natural Resources Research Institute, and private consultants. A complete list, with qualifications, is available upon request.

Manufacturers shall:

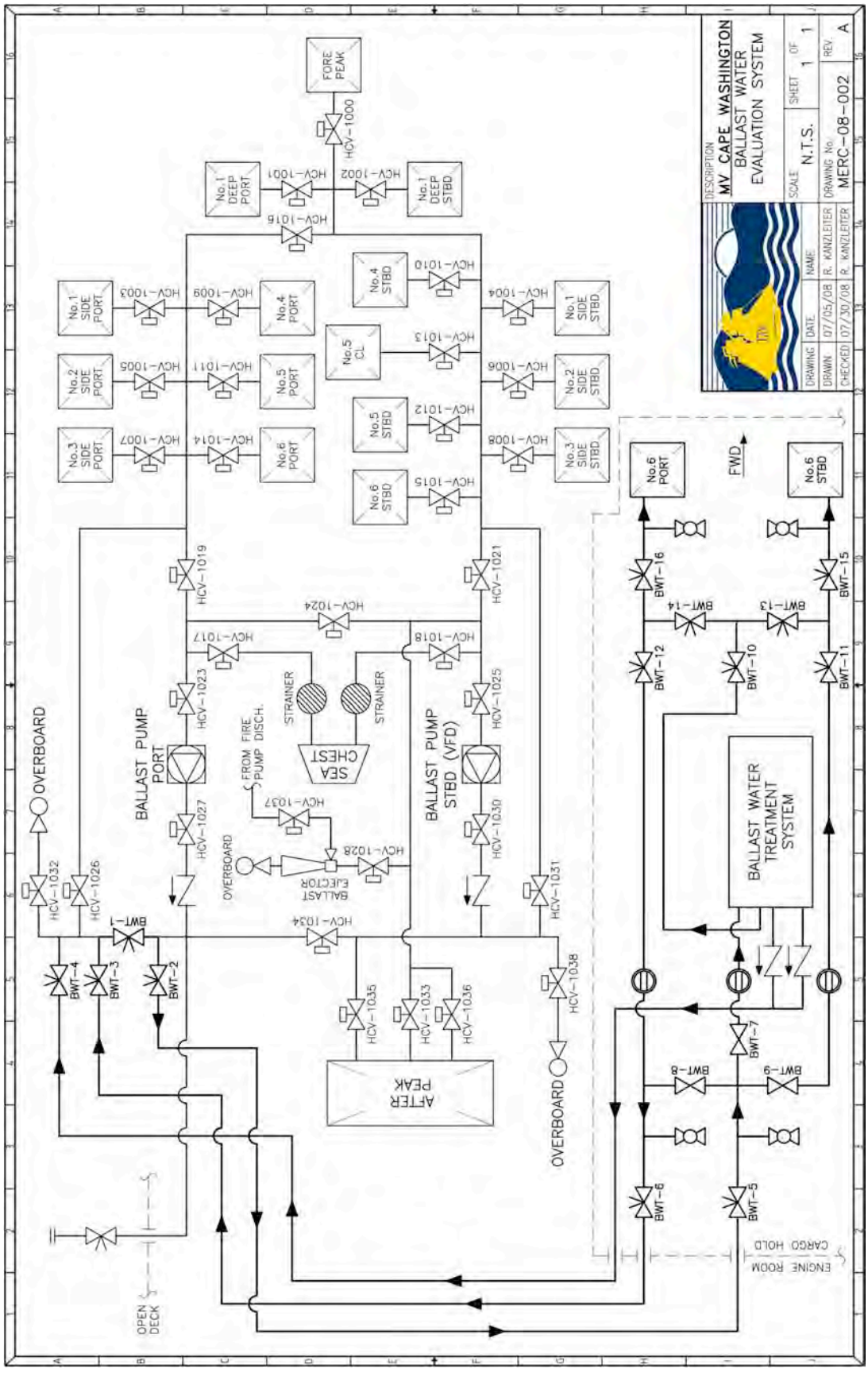
- Review the draft test/QA plan and provide comments and recommendations.
- Work with MERC to commit to a specific schedule for testing.
- Provide an operational treatment systems for the agreed upon test site.
- Aid in the installation, calibration and operation of treatment system for testing.
- Review and comment on draft Performance Report.

Advisory Board/Committee* shall:

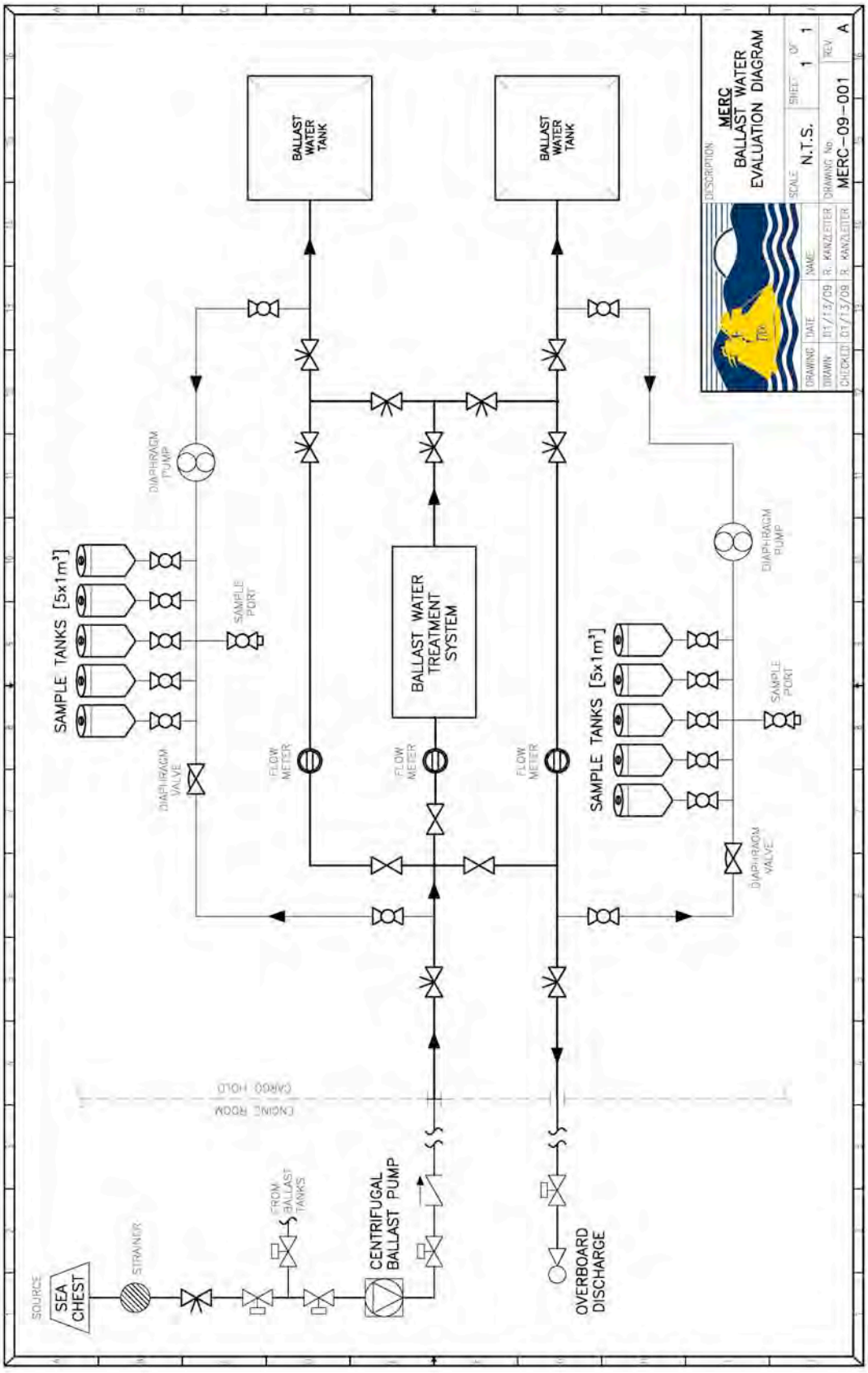
- Assist in developing the Test Protocols/QA Plan.
- Approve the final Test Protocols/QA Plan.
- Provide specific advice during testing.
- Review and comment upon draft Performance Report.

*A list of current MERC Advisory Board and GSI Advisory Committee members, and their affiliations, can be found at www.maritime-enviro.org and www.nemw.org/GSI/.

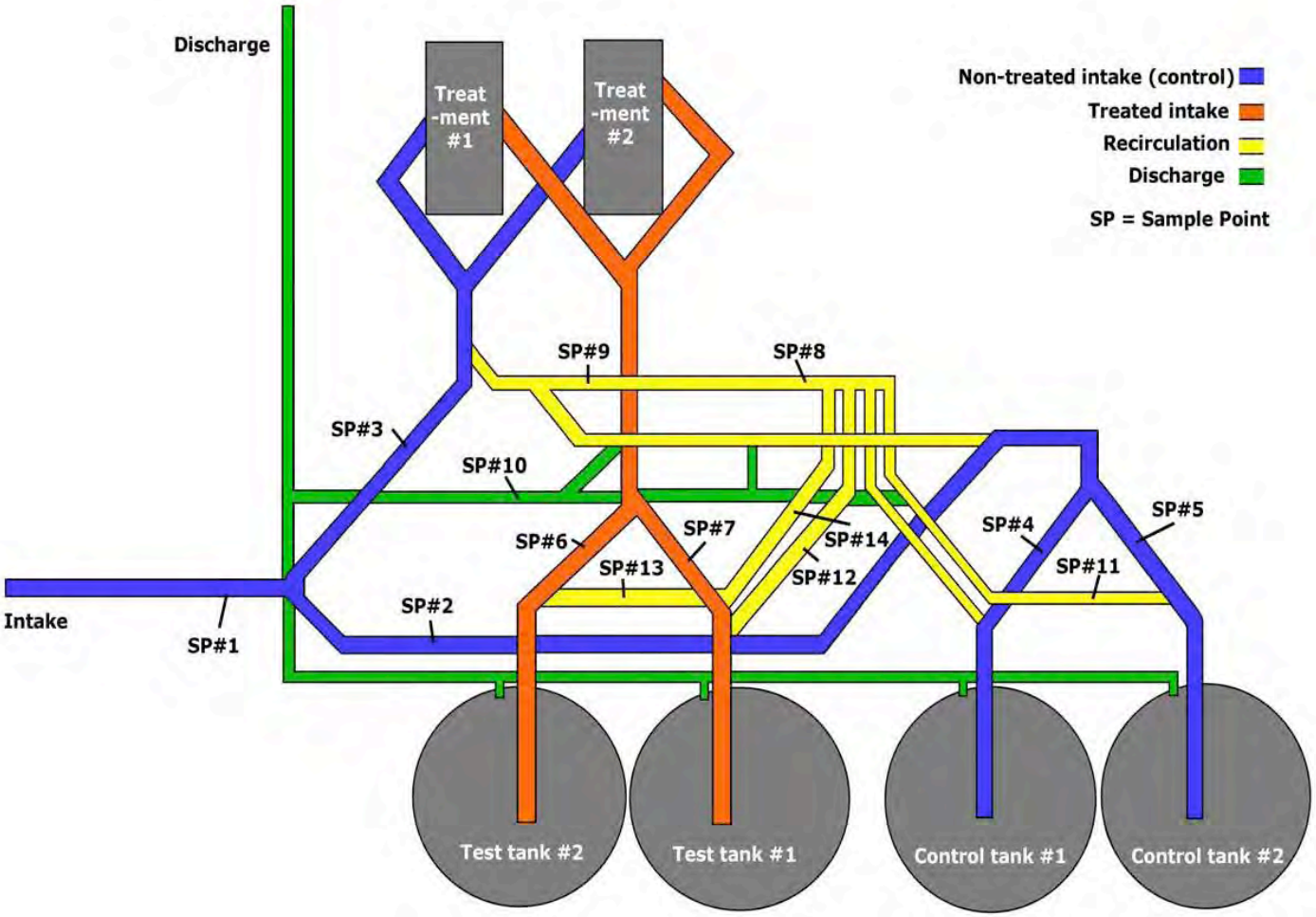
11. Modified *Cape Washington* ballast system to allow for treatment testing by MERC.



12. MERC *Cape Washington* test setup and sampling design.



13. Simplified schematic of the GSI land-based facility.
Detailed engineering drawings can be found at www.nemw.org/GSI/**.**



14. Table 2, Samples to be collected with corresponding volumes and purpose.

MERC and GSI will be collecting a variety of data on physical, chemical, biological, and toxicological parameters during this evaluation. In some case, values will be determined directly for the water using in situ sensors or instruments. Table 2 described the water sample that will be collected and analyzed for each time point and treatment of each test trial, excluding toxicity samples that are described above and in tables 3-8.

Parameter	Time Point / Treatment	Purpose	MERC volume	GSI volume
Total Suspended Solids (TSS)	Initial filling	Quantify challenge water	500 ml	500 ml
Particulate Organic Material (POC)	Initial filling	Quantify challenge water	500 ml	500 ml
Dissolved Organic Material (DOC)	Initial filling	Quantify challenge water	500 ml	500 ml
Zooplankton (> 50 μm) / m^3	a. Initial filling, b. After treatment, c. Control and treatment after 5 days	Quantify live organisms > 50 μm in size	5 m^3	3 m^3
Phytoplankton (10 - 50 μm) / ml	a. Initial filling, b. After treatment, c. Control and treatment after 5 days	Quantify live organisms 10 - 50 μm in size	10 l	3 l
Bacteria cfu / ml	a. Initial filling, b. After treatment, c. Control and treatment after 5 days	Quantify microbial communities	5 l	3 l

15. Tables 4 – 8, Summaries of MERC Toxicity Test Methods

Table 4. Summary of the Test Conditions and Test Acceptability Criteria for the Sheepshead Minnow *Cyprinodon variegatus* 96-Hour Acute Toxicity Test

Test type:	Static renewal
Test duration:	96 h
Temperature:	25 ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	250 ml
Test solution volume:	200 ml
Renewal of test solutions:	After 48-h
Age of test organisms:	1 to 14 days; 24-h range in age
No. organisms per test chamber:	10
No. replicate chambers per concentration:	2
No. organisms per concentration:	20
Feeding regime:	*Artemia* nauplii (<24 h old) during holding; Feed approximately 0.2 ml Artemia nauplii concentrate 2 h prior to renewal at 48 h.
Test chamber cleaning:	Cleaning prior to 48 h renewal
Test chamber aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test dilutions:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution series:	0.56 dilution series
Endpoint:	Mortality
Test acceptability criterion:	90% or greater survival in controls

Table 5. Summary of Test Conditions and Test Acceptability Criteria for the Sheepshead Minnow *Cyprinodon variegatus* Larval Survival and Growth Chronic Test

Test type:	Static renewal
Test Duration:	7 d
Temperature:	25 ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	500 ml
Test solution volume:	250 ml
Renewal of test solutions:	Daily
Age of test organisms:	Newly hatched larvae <24 hours old
No. larvae per test chamber:	10
No. replicate chambers per concentration:	4
No. larvae per test concentration:	40
Feeding regime:	*Artemia* nauplii (<24 h old). On days 0-2, feed 0.10g wet weight newly hatched (<24 hours old) brine shrimp nauplii daily. On days 3-6, feed 0.15g wet weight newly hatched (<24 hours old) brine shrimp nauplii daily.
Cleaning:	Siphon daily, immediately before test solution renewal
Aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution factor:	0.56
Endpoint:	Survival and growth (dry weight)
Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chamber equals or exceeds 0.60 mg

Table 6. Summary of the Test Conditions and Test Acceptability Criteria for the Mysid *Americamysis bahia* 96-Hour Acute Toxicity Tests

Test type:	Static renewal
Test duration:	96 h
Temperature:	25 ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	250 ml
Test solution volume:	200 ml
Renewal of test solutions:	After 48-h
Age of test organisms:	1 to 5 days; 24-h range in age
No. organisms per test chamber:	10
No. replicate chambers per concentration:	2
No. organisms per concentration:	20
Feeding regime:	*Artemia* nauplii (<24 h old) during holding; Feed approximately 0.2 ml Artemia nauplii daily.
Test chamber cleaning:	Cleaning prior to 48 h renewal
Test chamber aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution series:	0.56 dilution series
Endpoint:	Mortality
Test acceptability criterion:	90% or greater survival in controls

Table 7. Summary of Test Conditions and Test Acceptability Criteria for the Mysid *Americamysis bahia* Larval Survival and Growth Chronic Test

Test type:	Static renewal
Test Duration:	7 d
Temperature:	26ºC (± 1ºC)
Lighting:	Normal laboratory fluorescent
Photoperiod:	16 h light, 8 h dark
Test chamber size:	400 ml
Test solution volume:	150 ml
Renewal of test solutions:	Daily
Age of test organisms:	7 d
No. organisms per test chamber:	5
No. replicate chambers per concentration:	8
No. organisms per test concentration:	40
Feeding regime:	Feed 150 <24 h old *Artemia* nauplii daily, half after test solution renewal and half after 8-12 h.
Cleaning:	Siphon daily immediately before test solution renewal and feeding.
Aeration:	None, unless DO concentration falls below 4.0 mg/l. Rate should not exceed 100 bubbles/min
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution factor:	0.56 dilution series
Endpoint:	Survival and growth (dry weight)
Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chamber equals or exceeds 0.20 mg; fecundity may be used if 50% or more of females in controls produce eggs.

Table 8. Summary of Test Conditions and Test Acceptability Criteria for the Algae *Isochrysis galbana* and *Tetraselmiss uecica* Chronic Growth Test

Test type:	Static non-renewal (required)
Temperature:	20 ºC ± 1 ºC
Light quality	"Cool white" fluorescent lighting (recommended)
Light intensity:	360-440 foot candles
Photoperiod:	Continuous illumination
Test chamber size:	250 ml
Test solution volume:	100 ml
No. replicate chambers per concentration:	4
Renewal of test solutions:	None
Age of test organisms:	Log growth phase
Initial cell density in test chambers:	1-2 X 10^4 cells/ml
Shaking rate:	100 rpm continuous on a mechanical shaker or twice a day hand shaken
Aeration:	None
Nutrient solution	Algal assay culture medium nutrients added to each replicate (Appendix A3 of ASTM Designation E 1218-04; ASTM, 2006)
Dilution water:	Baltimore Harbor water collected at the same time as the initial untreated ballast water
Test concentrations:	100, 56, 32, 18, and 10 % ballast discharge or receiving water by volume plus a Baltimore Harbor and a Wye River control
Dilution factor:	0.56 dilution series
Test duration:	96 hours
Endpoint:	Growth (cell counts)
Test acceptability criterion:	Mean cell density of at least 1 x 10^6 cells/ml in the controls; and variability (CV%) among control replicates less than or equal to 20%

Chesapeake Biological Laboratory
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Determination of Total Suspended Solids (TSS) and Total Volatile Solids (TVS) in Waters of Fresh/Estuarine/Coastal Waters .

1. SCOPE and APPLICATION

1.1 Gravimetric analysis is used to determine total suspended solids (TSS) and total volatile solids (TVS), also known as volatile suspended solids (VSS) using a four place analytical balance.
1.2 A Method Detection Limit (MDL) of 2.4 mg/L TSS, and 0.9 mg/L TVS was determined using 3X the standard deviation of 7 replicates.
1.3 The quantitation limit for TSS was set at 0.0005 mg/L TSS.
1.4 This procedure should be used by analysts experienced in the theory and application of TSS. 1 month experience with an experienced analyst, certified in the analysis using the four place balance, is required.
1.5 This method can be used for all programs that require analysis of total suspended and volatile solids.
1.6 This procedure conforms to EPA Method 160.2 and Standard Methods 208 E.

2. SUMMARY

2.1 Measured aliquots of a water sample are filtered through a pre-weighed glass fiber filter pad. These pads are placed into a 105° C drying oven overnight to remove any remaining water. The pads are removed from the oven and placed into a desiccator to cool to room temperature. Once samples have reached room temperature, they are individually weighed on a four place balance and their respective weights are recorded in a spreadsheet and the concentration is reported as mg/L total suspended solids. If samples are to be used to determine total volatile solids they are placed into a numbered porcelain crucible and dried in a muffle furnace at 550° C for 1.5 hours. The samples are placed into a desiccator to cool to room temperature. Once they have cooled, they are weighed on the four place balance and their weights are recorded into the spreadsheet.

3. DEFINITIONS

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)
3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4 Analytical Range - 100 ppb - 4000 ppm using 250 µl syringe and 4 - 100 µl injection volume, using regular sensitivity catalyst.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of one to 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set if operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.10 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.11 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

- 3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.
- 3.11.2 Initial Calibration Verification (ICV) – An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.
- 3.11.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 10-15 field sample analysis.

Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.12 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.13 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.14 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.15 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)

3.16 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout filed and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.17 Field Reagent Blank (FRB) – A aliquot of reagent water or other blank matrix that is places in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.18 Furnace – Combusts samples at 550°C.

3.19 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid.(40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.20 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.21 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.22 Laboratory Reagent Blank (LRB) – A blank matrix (i.e., DI water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.

3.23 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing know and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.24 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.25 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.26 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.27 Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.28 May – Denotes permitted action, but not required action. (NELAC)

3.29 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.

3.30 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.31 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.32 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.33 Quality Control Sample (QCS) – A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.34 Run – One sample analysis from start to finish, including printout.

3.35 Run Cycle – Typically a day of operation – the entire analytical sequence of runs from the first run to the last run and including the transfer of run cycle data to the disc.

3.36 Sample Volume – Amount of volume filtered.

3.37 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.
3.38 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)
3.39 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)
3.40 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

4. INTERFERENCES

4.1 Excessive residue may form a water trapping crust. Sample size should be limited to yield < 200 mg of residue.
4.2 Samples from saline waters will not weigh to a constant weight. Therefore they must be rinsed with copious amounts of distilled water.

5. SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory.
5.2 The muffle furnace becomes extremely hot. Use care when removing crucibles from the furnace. Be sure they have cooled to the touch. Use gloves or tongs if necessary.

6. EQUIPMENT AND SUPPLIES

6.1 A four place analytical balance.
6.2 Desiccator with drying agents such anhydrous calcium sulfate or silica.
6.3 Muffle furnace capable of heating to 550° C.
6.4 Freezer, capable of maintaining -20° ± 5° C.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.
7.2 Blanks – ASTM D1193, Type I water is used for the LRB.

7.3 Quality Control Sample (QCS) – For this procedure, the QCS can be any certified dissolved sample which is obtained from an external source. If a certified sample is not available, then use the standard material.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for TSS and/or TVS should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.
8.2 Samples should be placed into an aluminum foil pouch and should be frozen at -20° C.
8.3 Frozen TSS/TVS samples may be stored longer than 28 days.

9. QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.
9.2 Initial Demonstration of Capability

9.2.1 The initial demonstration of capability (DOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.
9.2.2 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with analyses.
9.2.3 Method Detection Limits (MDLs) – MDLs should be established for TSS and TVS using a low level ambient water sample. To determine the MDL values, analyzed seven replicate aliquots of water. Perform all calculations defined in

the procedure (Section xx) and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = S x 3

Where, S = Standard Deviation of the replicate analyses.

9.2.4 MDLs should be determined yearly.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of Nanopure water treated the same as the samples. LRB data are used to assess contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – when using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3σ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these samples shall be used to determine batch acceptance.

9.3.3 The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards.

9.4 Data Assessment and Acceptance Criteria for Quality Control Measures

9.4.1 If a Total Volatile Solid (TVS) result is more than the Total suspended Solid (TSS) result, an error code 9 is assigned to the sample.

9.4.2 If duplicates have been provided for a sample, the results of the two numbers must be compared to each other. If the difference between the two numbers is equal to or more than 50% of the lower number then an error code 14 is assigned.

9.5 Corrective Actions for Out of Control Data

9.5.1 Out of control data is not reported. Generally portions of the pad are missing and therefore the measurement is considered useless. An error code is assigned.

10. CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily checks of calibration of balance using a certified weight must be performed before sample analysis may begin. The balance is professionally calibrated annually.

11. PROCEDURE

11.1 Total Suspended Solids

11.1.1 On a clean piece of paper lay out filter pads for numbering
11.1.2 Use a Sharpie permanent ultra fine or very fine point black marker, sequentially number outside edge of each pad.with a unique label.
11.1.3 After pads have been labeled, place in a Pyrex dish and dry overnight in a 105° C oven.
11.1.4 When ready to weigh,remove pads from oven and place into a desiccator to cool to room temperature.
11.1.5 Turn on analytical balance and computer.
11.1.6 Check calibration.
11.1.7 Click on Balancelink icon and be sure balance has been detected.
11.1.8 After pads have come to room temperature, weigh pads individually on balance and enter data into respective spread sheets and store in their labeled boxes for future use.
11.1.9 When ready to sample, place pad **numbered side down** onto filtering apparatus.
11.1.10 Filter a known volume of sample through the filter pad.
11.1.11 Rinse pad very well with deionized water to rinse down filter tower and remove any salts from the pad.
11.1.12 Fold pad in half ,sample side in and place pad into a labeled foil pouch and place in labeled storage bag and store in -20° C freezer.Place replicate pads side by side in pouch and not on top of each other.
11.1.13 When ready to analyze, place opened pouch with sample in 105° C drying oven overnight.
11.1.14 Repeat steps 11.1.4 – 11.1.7.
11.1.15 Calculate TSS value:

$$\text{mgTSS / L} = \frac{(\text{Wpost}_{(g)} - \text{Wpre}_{(g)}) \times 1000}{\text{V (L)}}$$

11.2 Total Volatile Solids

11.2.1 Place pads straight from box into a Pyrex dish and combust at 550° C in a muffle furnace for 1.5 hours.
11.2.2 Move pads to a 105° C oven for storage until ready to use.
11.2.3 Repeat steps 11.1.4 – 11.1.6.
11.2.4 After pads have come to room temperature, weigh pads individually on balance and enter data into respective spread sheets and store into individually labeled Petri dishes for future use.
11.2.5 When ready to sample, place pad onto filtering apparatus.
11.2.6 Repeat steps 11.1.9 – 11.1.13 to calculate TSS value.
11.2.7 Once TSS value has been determined place pad into a numbered porcelain crucible and record crucible number and sample id.
11.2.8 Combust samples at 550° C in a muffle furnace for 1.5 hours.
11.2.9 Repeat steps 11.1.4 – 11.1.7
11.2.10 Calculate TVS:

$$\text{mgTVS / L} = \frac{(W_{\text{combust(g)}} - W_{\text{post(g)}}) \text{ x } 1000}{V(L)}$$

12 References:

12.1 APHA. 1975. Method 208D. Total Nonfilterable Residue Dried at 103-105 °C (Total Suspended Matter) *in* Standard Methods for the Examination of Water and Wastewater, 14th Edition. American Public Health Association. Washington, D.C. 1193pp.

12.2 USEPA. 1979. Method No. 160.2 (with slight modification) in Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

12.3 APHA. 1975. Method 208E (with modification). Total volatile and fixed residue @ 550°C *in* Standard Methods for the Examination of Water and Wastewater, 14th Edition. American Public Health Association. Washington, D.C. 1193pp.

Chesapeake Biological Laboratory
University of Maryland Center for Environmental Science
Nutrient Analytical Services Laboratory

Determination of Carbon and Nitrogen in Particulates and Sediments of Fresh/Estuarine/Coastal Waters, Plant and Animal Tissue, and Soils Using Elemental Analysis

1. SCOPE and APPLICATION

1.1. Elemental analysis is used to determine particulate carbon (PC), and particulate nitrogen (PN) in fresh, estuarine and coastal waters and sediments as well as for plant and animal tissue and soils. The method measures the PC and PN irrespective of source (organic or inorganic.)

1.2. A Method Detection Limit (MDL) of 0.0759 mg C/l and 0.0123 mg N/l, for filtered samples, and 0.130 %C and 0.008% N for sediment samples, were determined using three times the standard deviation of seven replicates.

1.3. The quantitation limit for PC and PN has not been determined.

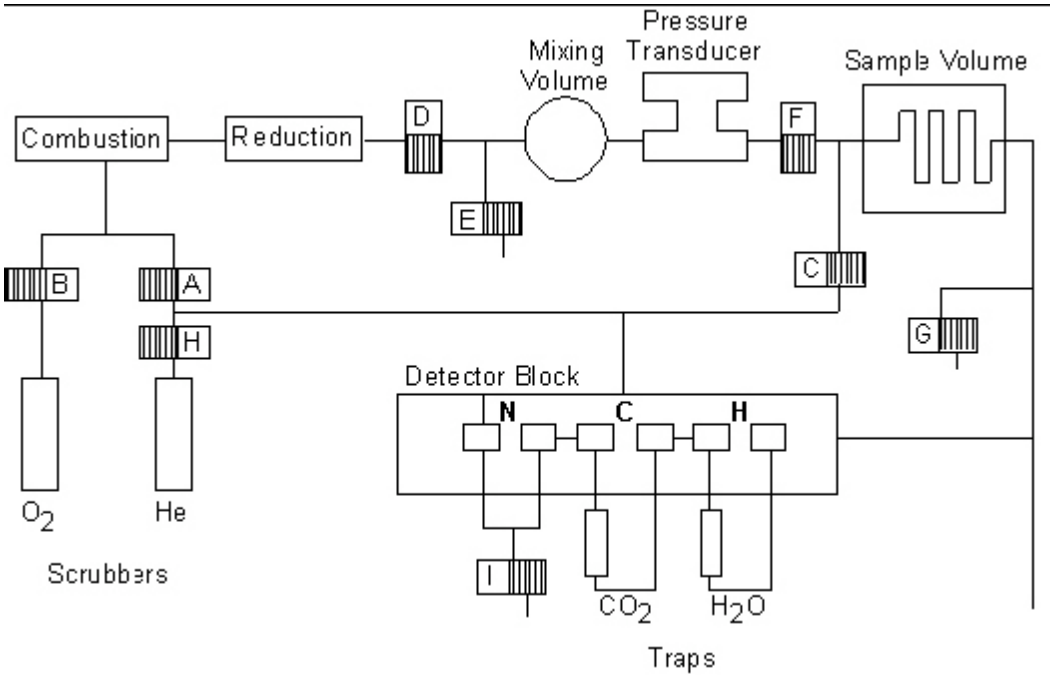
1.4. This procedure should be used by analysts experienced in the theory and application of elemental analysis. A minimum of 3 months experience with an elemental analyzer is recommended.

1.5. This method is for use by all programs that require analysis of particulate carbon and nitrogen in water and sediment, soils and tissues. The need to determine the organic fraction of the total particulate carbon and nitrogen in samples depends on the data-quality objectives of the study. Section 11.2.5 outlines the procedure used to ascertain the organic fraction.

2. SUMMARY

2.1. In the Exeter Analytical, Inc. Model CE-440 Elemental Analyzer, the carbon and nitrogen content in organic and inorganic compounds can be determined. Combustion of the sample occurs in pure oxygen under static conditions. The combustion train and analytical system are shown below in the CE-440 flow diagram. Helium is used to carry the combustion products through the analytical system to atmosphere, as well as for purging the instrument. Helium was selected for this purpose because it is chemically inert relative to tube packing chemicals, and it has a very high coefficient of thermal conductivity. The products of combustion are passed over suitable reagents in the combustion tube to assure complete oxidation and removal of undesirable by-products such as sulfur, phosphorus and halogen gases. In the reduction tube, oxides of nitrogen are converted to molecular nitrogen and residual oxygen is removed. In the mixing volume the sample gasses are thoroughly homogenized at precise volume, temperature, and pressure. This mixture is released through the sample volume into the thermal conductivity detector. Between the first of three pairs of thermal conductivity cells an absorption trap removes water from the sample gas. The differential signal read before and after the trap reflects the water concentration and, therefore, the amount of hydrogen in

the original sample. A similar measurement is made of the signal output of a second pair of thermal conductivity cells, between which a trap removes carbon dioxide, thus determining the carbon content. The remaining gas now consists only of helium and nitrogen. This gas passes through a thermal conductivity cell and the output signal is compared to a reference cell through which pure helium flows. This gives the nitrogen concentration.



Schematic diagram of the Exeter Analytical, Inc. (EAI) CE-440 Elemental Analyzer

3. DEFINITIONS

3.1. **Acceptance Criteria -** Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2. **Accuracy -** The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3. **Acetanilide** - Used as a standard or conditioner in the analyzer for calibration purposes. It has known percentages of C, H, and N.

3.4. **Aliquot -** A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.5. **Batch -** Environmental samples, which are prepared and/or analyzed together with the same process and the same personnel using the same lot(s) of reagents. A

preparation batch is composed of one to 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An analytical batch is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrixes and can exceed 20 samples. (NELAC/EPA)

3.6. **Blank -** A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7. **Blank** - Blank value = blank read minus blank zero. An indicator of the stability of the system. (Exeter)

3.8. **Bridge** - Electrical configuration of the thermal conductivity filaments.(Exeter)

3.9. **Calibrate -** To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.10. **Calibration -** The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.11. **Calibration Method -** A defined technical procedure for performing a calibration. (NELAC)

3.12. **Calibration Standard -** A substance or reference material used to calibrate an instrument. (QAMS)

3.12.1. **Initial Calibration Standards (STD) -** A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

3.12.2. **Initial Calibration Verification (ICV) -** An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.12.3. **Continuing Calibration Verification (CCV) -** An individual standard which is analyzed after every tenth field sample analysis.

3.13. **Capsule** - Aluminum container. Used for containing samples and standards with an accurate weight and maintains integrity prior to combustion.

3.14. **Calibration Standard (CAL)** - An accurately weighed amount of a certified chemical used to calibrate the instrument response with respect to analyte mass. For this procedure the calibration standard is acetanilide, 99.9%+ purity.

3.15. **Certified Reference Material -** A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)

3.16. **Combustion Time** - Time for sample to fully combust in an oxygen environment.

3.17. **Combustion Tube** - Quartz tube packed with reagents and used for sample combustion.

3.18. **Conditioner** - A standard chemical which is not necessarily accurately weighed that is used to coat the surfaces of the instrument with the analytes (water vapor, carbon dioxide, and nitrogen).

3.19. **Corrective Action -** Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.20. **Deficiency -** An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.21. **Demonstration of Capability -**A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.22. **Detection Limit -** The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.23. **Detector** - The heart of the analyzer consisting of three bridges. Determines the percentages of carbon, hydrogen, and nitrogen in the sample via thermal conductivity.

3.24. **Detector Oven** - Keeps the temperature of the detector, pressure transducer, mixing volume, and sample volume constant.

3.25. **Double Drop** - Two samples are dropped for one run - used for filter and inorganic applications. Sample requires a + prefix.

3.26. **Duplicate Analyses -** The analyses or measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory (EPA-QAD)

3.27. **External Standard (ES)** - A pure analyte (atropine) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.28. **Field Duplicates (FD1 and FD2)** - Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.29. **Fill Time** - Time required to build-up the pressure in the mixing volume to 1500 mm Hg.

3.30. **Filtered Sample –** An accurately measured amount of water from fresh, estuarine or coastal samples, filtered through a 25 mm Whatman GF/F filter or equivalent, which has been precombusted at 500^{o} C for 90 minutes.

3.31. **Furnace** - Heats the reduction and combustion tubes to operating temperature.

3.32. **Heated Line** - Connects the reduction tube outlet to the inlet of the mixing volume. Heated to prevent condensation of gases on tube walls.

3.33. **Holding Time -** The maximum time which samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.34. **Inject Solenoid** - Solenoid used on the automated injection system to actuate the rotation of the sample wheel.

3.35. **Injection** - Moving the ladle, containing a capsule with the sample into the combustion furnace.

3.36. **Injector Box** - The box assembly that houses the sample wheel.

3.37. **Instrument Detection Limit (IDL)** - The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.

3.38. **K-Factor** - Instrument sensitivity factor in microvolts per microgram, calibrated using an external standard.

3.39. **Laboratory Duplicates (LD1 and LD2)** - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.40. **Laboratory Reagent Blank (LRB)** - A matrix blank (i.e., a precombusted filter or sediment capsule) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.41. **Ladle** - Transports the capsule with the sample into a combustion furnace.

3.42. **Laboratory Control Sample (LCS) -** A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standards or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.43. **Limit of Detection (LOD)** - The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.44. **Limit of Quantitation (LOQ) -** The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.45. **Linear Dynamic Range (LDR)** - The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.46. **Material Safety Data Sheet (MSDS)** - Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.47. **May -** Denotes permitted action, but not required action. (NELAC)

3.48. **Method Detection Limit (MDL)** - The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.

3.49. **Mixing Volume** - Spherical bottle in which sample gases become homogenous.

3.50. **Mother Board** - The main printed circuit board. All CE 440 power supplies are located here.

3.51. **Must -** Denotes a requirement that must be met. (Random House College Dictionary)

3.52. **Precision -** The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.53. **Preservation –** Refrigeration, freezing and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.54. **Pressure Transducer** - Used to check for leaks in the system and to monitor pressure in the mixing volume.

3.55. **P Valve** - The valve on the injector box of the horizontal auto-injector (HA) used to automatically purge the box.

3.56. **Profile** - Generated by the bridge signal. Used to help determine if a leak or malfunction occurs in the system.

3.57. **Quality Control Sample (QCS)** - A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.58. **Reduction Tube** - Quartz tube with reduced copper that removes excess oxygen from the sample gas and reduces oxides of nitrogen to free nitrogen.

3.59. **Response Factor (RF)** - The ratio of the response of the instrument to a known amount of analyte.

3.60. **Run** - One sample analysis from start to finish, including printout.

3.61. **Run Cycle** - Typically a day or half day of operation - the entire analytical sequence of runs from the first run to the last run on the Sample Wheel.

3.62. **Sample Volume** - Tube where sample gas is exhausted from the mixing volume prior to entering the detector.

3.63. **Sample Wheel –** Sample holding device which contains up to 64 blanks, standards and samples. One wheel equals roughly 6 hours of run time, which is called the Run Cycle.

3.64. **Scrubber** - Removes water and CO_2 from the gas supplies.

3.65. **Sediment (or Soil) Sample** - A fluvial, sand, or humic sample matrix exposed to a marine, estuarine or fresh water environment.

3.66. **Sensitivity -** The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.67. **Shall -** Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.68. **Should -** Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.69. **Sleeve** - Nickel - to maintain integrity of the sample capsule and to protect the quartz ware from devitrification (to destroy the glassy qualities by prolonged heating).

3.70. **Standard Reference Material (SRM)** - Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

3.71. **Trap** - Used for removing water and CO_2 from the sample gas.

3.72. **Tissue sample** – Plant or animal tissue dried and ground ready for weighing.

3.73 **Zero Value** - Bridge signal with only pure helium flowing through the detector.

4. INTERFERENCES

4.1. There are no known interferences for fresh, estuarine or coastal water or sediment samples. The presence of C and N compounds on laboratory surfaces, on fingers, in detergents and in dust necessitates the utilization of careful techniques (i.e., the use of forceps and gloves) to avoid contamination in every portion of this procedure (EPA.)

5. SAFETY

5.1. Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats and safety glasses and enclosed shoes must always be worn. In certain situations it may also be necessary to use gloves and goggles. If solutions or chemicals come in contact with eyes, flush with water continuously for 15 minutes. If solutions or chemicals come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Business Manager of the incident. Contact the CBL Business Manager if additional treatment is required.

5.2. The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials and procedures.

5.3. High current and voltages are exposed near the furnaces, furnace control card, and mother board even while the 440 is OFF. If non-electrical trouble shooting is desired, remove the 440 line cord from the wall receptacle.

5.4. The combustion tube is brittle since it is fused quartz. Do not put any unnecessary stress on it.

5.5. The exterior of the furnace becomes extremely hot; do not touch it or the heat shield unless wearing appropriate gloves.

5.6. Do not wear any jewelry if electrically troubleshooting. Even the low voltage points are dangerous and can injure is allowed to short circuit.
5.7. The following hazard classifications are listed for the chemicals regularly used in this procedure.

Chemical	Health	Flammability	Reactivity	Contact	Storage
Acetanilide	1	1	0	2	Green
Magnesium Perchlorate	1	0	3	2	Yellow
Ascarite	3	0	2	4	White Stripe
Silver vanadate on Chromosorb	3	0	0	3	White
Silver oxide/Silver tungstate on Chromosorb	3	0	0	3	White
Silver tungstate/Magnesium oxide on Chromosorb	3	0	0	3	White
Copper wire	0	0	0	1	Green
On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)					
STORAGE Red - Flammability Hazard. Store in a flammable liquid storage area. Blue - Health Hazard. Store in a secure poison area. Yellow - Reactivity Hazard. Keep separate from flammable and combustible materials. White - Contact Hazard. Store in a corrosion-proof area. Green - Use general chemical storage (On older labels, this category was orange). Striped - Incompatible materials of the same color class have striped labels. These Products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.					

6. EQUIPMENT AND SUPPLIES

6.1. An elemental analyzer capable of maintaining a combustion temperature of 975°C and analyzing particulate and sediment samples for elemental carbon and nitrogen. The Exeter Model 440 is used in this laboratory.
6.2. A gravity convection drying oven, capable of maintaining 47^{o}C ± 2°C for extended periods of time.
6.3. Muffle furnace, capable of maintaining 875°C +/- 15^{o}C.
6.4. Ultra-micro balance that is capable of accurately weighing to 0.1 ug.
6.5. Vacuum pump or source capable of maintaining up to 10 in. Hg of vacuum.
6.6. Freezer, capable of maintaining -20^{o}C± 5°C.
6.7. 25-mm vacuum filter apparatus made up of a glass filter tower, fritted glass disk base and 2-L vacuum flask.
6.8. Flat blade forceps.
6.9. Labware - All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) must be sufficiently clean for the task objectives. Clean glassware by rinsing with deionized water; soaking for 4 hours or more in 10% (v/v) HCl and then rinsing with deionized water. Store clean. All traces of organic material must be removed to prevent carbon and nitrogen contamination.

7. REAGENTS AND STANDARDS

7.1. **Purity of Water** – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I.

7.2. **Purity of Reagents** – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.3. **Acetanilide, 99.9% + purity**, C_8H_9NO (CASRN 103-84-4) - Primary standard

7.4. **Blanks** – Three blanks are used for the analysis. Two blanks are instrument related. The instrument zero response (ZN) is the background response of the instrument without sample holding devices such as capsules and sleeves. The instrument blank response (BN) is the response of the instrument when the sample capsule, sleeve and ladle are inserted for analysis without standard or sample. The BN is also the laboratory reagent blank (LRB) for standards and sediment or other weighed samples. The LRB for water samples includes the sleeve, ladle and a precombusted filter without standard or sample. These blanks are subtracted from the uncorrected instrument response used to calculate concentration. The third blank is the laboratory fortified blank (LFB.) For sediment or other weighed sample analysis, a weighed amount of acetanilide or other standard is placed in an aluminum capsule and analyzed. For aqueous samples, a weighed amount of acetanilide or other standard is placed on a glass fiber filter the same size as used for sample filtration, and analyzed.

7.5. **Quality Control Sample (QCS)** – For this procedure, the QCS can be any assayed and certified sediment or particulate sample which is obtained from an external source. BCSS-1 from the National Research Council of Canada is used by this laboratory.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. **Water Sample Collection** – Samples collected for PNC analysis from fresh, estuarine and coastal waters are normally collected from a boat or pier using one of two methods; hydrocast or submersible pump systems. Follow the recommended sampling protocols associated with the method used. Whenever possible, immediately filter the samples as described in Section 11.1.1. Store the filtered sample in an aluminum foil pouch and freeze at -20°C or store in a low temperature (47°C) drying oven after drying at 47°C ± 2°C, until use. If storage of the unfiltered water sample is necessary, place the sample into a clean bottle and store at 4°C until filtration is performed. Dry samples in a low temperature (47°C+/-2°C) drying oven prior to analysis.

8.2. The volume of water sample collected will vary with the type of sample being analyzed. Table 1, see 8.3.2., provides a guide for a number of matrices of interest. If the matrix cannot be classified by this guide, collect 1 L of water from each site.

8.3. Sediment, Tissue, or Soil Sample Collection – Sediment samples are collected with benthic samplers. The type of sampler used will depend on the type of sample needed by the data-quality objectives. Tissue and soil samples are collected by a variety on methods. Store the wet sample in a clean jar and freeze at -20^{o}C until ready for analysis. Dry samples in a low temperature (47^{o}C+/-2^{o}C)) drying oven, and grind to a homogenous powder with a mortar and pestle, prior to analysis.

8.3.1. The amount of solid material collected will depend on the sample matrix. A minimum of 1 g is recommended.

8.3.2. Filtration Volume Selection Guide

Sample Matrix	25mm Filter
Open Ocean	500 – 1000 ml
Coastal	400 – 500 ml
Estuarine (Low particulate)	250 – 400 ml
Estuarine (High Particulate)	25 – 200 ml

9. QUALITY CONTROL

9.1. The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks field duplicates, and calibration standards analyzed as samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2. **Initial Demonstration of Capability**

9.2.1. **The initial demonstration of capability (DOC**) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2. **Quality Control Sample (QCS/SRM)** – When using this procedure, a quality control sample is required to be analyzed at the beginning or middle and end of the run, to verify data quality and acceptable

instrument performance. If the determined concentrations are not within ± 10% of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before proceeding with the initial determination of MDLs.

9.2.3. **Method Detection Limits (MDLs)** – MDLs should be established for PC and PN using a low level estuarine water sample, typically three to five times higher than the estimated MDL. The same procedure should be followed for sediments or other weighed samples. To determine the MDL values, analyze seven replicate aliquots of water or sediment and process through the entire analytical procedure. Perform all calculations defined in the procedure (Section 12) and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = 3 \times S$$

Where, S = Standard deviation of the replicate analyses.

9.2.4. MDLs should be determined annually, whenever there is a significant change in instrumental response, change of operator, or a new matrix is encountered.

9.3. **Assessing Laboratory Performance**

9.3.1. **Laboratory Reagent Blank (LRB)** – The laboratory must analyze at least one LRB (Section 3.40) with each batch of samples. For sediment samples the LRB consists of the ladle, sample sleeve and sample capsule, as there are no reagents involved in this procedure. For aqueous samples the LRB is a precombusted filter of the same type and size used for samples. LRB data are used to assess contamination from the laboratory environment. For sediment samples, the blank value for carbon should not exceed 150 uv and the blank value for nitrogen should not exceed 50 uv. For aqueous samples, the blank value for carbon should not exceed 375 uv and the blank value for nitrogen should not exceed 50 uv.

9.3.1.1. If the nitrogen blank during a BLANK analysis is in excess of 2000% the nitrogen blank in memory the "COPPER APPEARS SPENT" is printed. If the nitrogen blank increased over 100 uv over BN in memory and the first STANDARD KC/KN is more than any following STANDARD KC/KN by 0.2 uv/ug, then a "COPPER APPEARS SPENT" warning will be printed either during a BLANK analysis or a STANDARD analysis.

9.3.2. **Quality Control Sample (QCS)/ Standard Reference Material (SRM)** - When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3σ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses.

Corrective action documentation is required for all data outside ± 3σ. The sample weight of the SRM should mirror that of the unknown samples (~10 mg).

9.3.3. The laboratory must use QCS analyses data to assess laboratory performance against the required accuracy control limits of ± 3σ. The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards. The standard deviation data should be used to establish an on-going precision statement for the level of concentrations included in the QCS. This data must be kept on file and be available for review. Values for QCSs should be plotted with the other control data.

9.4. **Assessing Analyte Recovery**

9.4.1. Percent recoveries cannot be readily obtained from particulate samples. Consequently, accuracy can only be assessed by analyzing check standards as samples and quality control samples (QCS).

9.5. **Data Assessment and Acceptance Criteria for Quality Control Measures**

INDICATOR	ACCEPTANCE LIMITS	ACTION
K-factor	KC = 18 to 25 +/- 3σ 18 to 25 µv/µg is manufacturers recommended limits. KN = 7 to 10 µv/µg 7 to 10 µv/µ is manufactures recommended limits.	The k-factors must be within the specified limits or the standard must be reanalyzed. (see 10.3)
System Blank	BC < 150 µv BN < 50 µv	If the blank value is greater the acceptable value, replace the capsules and rerun the blanks.
External QC (QCS) start or middle and end of run cycle	± 3σ	Qualify data if not within acceptance limits. Rejection criteria for batch.
Standard Reference Material (SRM) (when required by data user)	± 3σ	If SRM is outside acceptance limits, qualify the data for all samples back to last acceptable SRM or QCS.
Duplicate analysis (when available)	± 50%	Duplicate sample data must be within ± 50% or be qualified. All duplicates for this procedure are field duplicates and are more a measure of field collection and filtration techniques.

9.6. **Corrective Actions for Out-Of-Control Data**

9.6.1. All samples must be qualified when external QC samples are out of control.

9.6.2. All samples between QCSs that are out of control must be qualified.

9.6.3. All problems with analytical runs must be documented on the bench sheet.

9.7. **General Operation**

9.7.1. To assure optimal operation and analytical results, it is advisable to track the stability of the instrument. Of primary importance is the precision and repeatability of standard and blank values during the course of a day of operation. Thus, a standard (as an unknown) should be inserted approximately every twenty runs. Try to use different standards for QA in order to assure the validity of the calibration values over the entire operating range of the instrument.

10. CALIBRATION, STANDARDIZATION and CALCULATIONS

10.1.1. Calibration - Daily calibration procedures must be performed and evaluated before sample analysis may begin. Single point calibration is used with the Exeter Model 440 Analyzer.

10.1.2. Establish single calibration factors (K) for each element (carbon, hydrogen, and nitrogen) by analyzing three weighed portions of calibration standard (acetanilide). The mass of the calibration standard should provide a response within 20% of the response expected for the samples being analyzed. Calculate the (K) for each element using the following formula:

$$K - factor(\mu v / \mu g) = \frac{RN - ZN - BN}{M(T)}$$

Where: RN = Instrument response to standard (μv)
ZN = Instrument zero response (μv)
BN = Instrument blank response (μv)
M = Mass of standard matter in μg
T = Theoretical % C, N, or H in the standard. For acetanilide %C = 71.09, %N = 10.36 and %H = 6.71.

10.2. The detector generates a signal directly proportional to the compound of interest in the sample. The following formula is used to calculate carbon, nitrogen and hydrogen concentrations in unknown samples.

$$\% = \frac{1}{K} X \frac{1}{W} X (R - Z - B) X\ 100$$

Where

K = calibration factor for the 440 instrument
W = sample weight
R = read signal of sample gas
Z = zero reading or base line of instrument
B = blank signal generated by instrument itself, including ladle and capsules

All the calculations are done by the PC computer but the operator decides which values to enter into the computer memory.

10.3. The K-factor is established by running samples of a known standard. The default value is for acetanilide, which we will use for our standard:

Acetanilide C = 71.09% H = 6.71% N = 10.36%

If another standard is used, the values will need to be entered into the computer using The Edit Standards function in the Customizing Menu.

10.3.1. Once the blank values have been established and entered into memory, proceed to run known standards to arrive at the calibration factors for carbon and nitrogen for the instrument.

10.3.2. Run a minimum of three standards, average the results, and enter into computer memory, or use the automatic enter mode. During the run, standards may be entered as samples to verify the K-factors and blanks.

10.3.3. Any time a STD1 is entered as sample ID the computer calculates and enters a new set of operating Ks based on a weighted formula using the last three sets of Ks in memory. This occurs only if all three Ks fall within the following windows:

New KC = KC in memory ± 1.0

KN = KN in memory ± 0.5

10.3.3.1. It is important that the Ks in memory be close to expected values or new Ks generated will not be within the window and therefore will not be accepted for automatic insertion.

10.3.3.2. The weighted formula for calculating the Ks:

$$K = k^1 + \left(0.5 \times k^2\right) + \frac{\left(0.25 \times k^3\right)}{1.75}$$

where:

k^1 = k found in this run

k^2 = Next k in memory

k^3 = Last k in memory

10.4. **Conditioner** - Before running any samples or blanks, it is necessary to run one or more conditioners. The purpose of the conditioner runs is to coat the walls of the system surfaces, especially the mixing and sample volume, with water vapor, carbon dioxide and nitrogen which simulates actual sample running conditions. To simulate this condition as closely as possible, it is advisable to use conditioners of approximately the same weight as the samples to the run.

10.5. **Blanks** - The blank values used in the calculation is the total signal generated by the system including the ladle and sample capsule. This blank should always be run immediately after a weighed conditioner to represent a true blank of the instrument. Never use the blank value after an empty run since the system dries up and the blank value would be lower than normal.

10.5.1. The blanks will only be accepted if they fall within the following:

New BC < 500

New BN < 250

10.6. **K-Factors** - Once the blank values have been established and entered into memory, proceed to run known standards in order to establish the calibration factors for carbon, hydrogen and nitrogen. Always run a conditioner before a standard. The computer will calculate K-factors as long as STD# has been entered as the sample ID. Run a minimum of three (3) standards, average the results, and enter into the

computer memory, or use the automatic enter mode. The instrument is now ready for running samples. Standards should be analyzed as unknowns during each run to verify the K-factors and blank values.

11. PROCEDURE

11.1. Aqueous Sample Preparation

11.1.1. Water Sample Filtration

Precombust 25-mm GF/F glass fiber filters at 500°C for 1.5 hours. Store filters covered, if not immediately used. Place a precombusted filter on a fritted filter base of the filtration apparatus and attach the filtration tower. Thoroughly shake the sample container to suspend the particulate matter. Measure and record the required sample volume using a graduated cylinder. Pour the measured sample into the filtration tower. Filter the sample using a vacuum no greater than 10 in. of Hg. Vacuum levels greater than 10 in. of Hg can cause filter rupture. Do not rinse the filter following filtration. It has been demonstrated that sample loss occurs when the filter is rinsed with an isotonic solution or the filtrate. Air dry the filter after the sample has passed through by continuing the vacuum for 30 sec. Using flat-tipped forceps, fold the filters in half while still on the fritted glass base of the filter apparatus. Store filters as described in Section 8.1.

11.1.2. If the sample has been stored frozen in foil pouches, place in a drying oven at $47^{o}C \pm 2°C$ for 24 hours before analysis. Slightly open the pouch to allow drying. When ready to analyze, fold, and insert the filter into a precombusted nickel sleeve using forceps. Tap the filter pad down into the nickel sleeve using a stainless steel rod. The sample is ready for analysis.

11.2. Sample Analysis

11.2.1. As the filters are packed into the nickel sleeves they are placed into the 64 position sample wheel. The calibration series must be placed at the beginning of the batch. The sample schedule consists of a conditioner, a blank, a conditioner and three standards. ACS grade acetanilide must be used to calibrate the instrument.

11.2.2. Set up the sample tray in the following manner (used for aqueous samples):

Position #	Contents	Notes	Schedule Entry	Weight
1	Capsule + sleeve	Blank	Blank	0
2	Conditioner	Acetanilide (1500-2500 µg)	Conditioner	Weight of Acetanilide
3	Capsule + sleeve	Blank	Blank	0
4	Conditioner	Acetanilide (1500-2500 µg)	Conditioner	Weight of Acetanilide
5	Standard	Acetanilide (1500-2500 µg)	STD1[a]	Weight of Acetanilide
6	Standard	Acetanilide (1500-2500 µg)	STD1	Weight of Acetanilide
7	Standard	Acetanilide (1500-2500 µg)	STD1	Weight of Acetanilide
8	Sleeve + filter pad	Filter Blank	LRB	0
9-31	Samples			Volume

				filtered/10
32	Sleeve + filter pad + standard	Atropine (1500-2500ug)	LFB	Weight of Atropine
33-61	Samples			Volume filtered/10
62	Capsule + Sleeve	Blank	Blank	0
63	Sleeve + capsule+ standard	Atropine (1500-2500ug)	LFB	Weight of Atropine
64	Capsule + Sleeve	Blank	Blank	0

[a] Always use STD1 in the Standard position. The system recognizes this as acetanilide and makes the appropriate calculations for the K factor.

11.2.3. By entering volume filtered/10 for the weight of the aqueous filtered samples, results are printed out which represent micrograms of carbon or nitrogen per liter. This corresponds directly to the known amount of liquid that has passed though the filter. The maximum sample capacity per run is approximately 4,000 to 5,000 micrograms of carbon on the filter pad. Filters containing more than that amount can be cut in half and analyzed separately and the results added.

11.2.4. Filter Preparation for Analysis

11.2.4.1. Work on a clean, non-contaminating surface.

11.2.4.2. Using two pairs of clean forceps, fold the filter in half so that the exposed surface is inside. Continue folding the filter in half until you have a compact package.

11.2.4.3. Place a pre-combusted 7 x 5 mm nickel sleeve into the filter loading die, which functions as a holding device. Use the clean 4 mm loading plunger to force the compressed filter through the clean loading funnel and into the nickel sleeve.

11.2.4.4. Make sure no excess filter protrudes above the lip of the sleeve.

11.2.4.5. Place loaded sleeve in the 64-sample wheel.

11.2.5. Determination of Particulate Organic and Inorganic Carbon

11.2.5.1. Thermal Partitioning is the method used. The difference found between replicate samples, one part of which has been analyzed for total PC and PN and the other which was muffled at 550^{o}C for three hours to drive off organic compounds, and analyzed is the particulate organic component of that sample. This method of thermally partitioning organic and inorganic PC may underestimate slightly the carbonate minerals' contribution in the inorganic fraction since some carbonate minerals decompose below 500^{o}C, although $CaCO_3$ does not. This method is used for filtered samples where at least two filters per sample must be supplied. For sediment samples at least 1 g of sample is required and at least 0.5g of sample is weighed into a crucible of known weight. The weight is recorded. The crucible is then muffled as above, and weighed again. The percent remaining of the ash is calculated and multiplied times the %C in the ash which is then determined by the 440.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Raw results for each run are printed by the dot matrix printer attached to the instrument. These data are then manually entered into a LOTUS 123 spreadsheet. Results are reported in mg/l for aqueous samples, and in % for sediment or other weighed samples, standards and SRMs or QCSs.

12.2. Recalculation of data (if necessary)

12.2.1. The software gives the analyst the opportunity to recalculate values generated by the run. This option can be useful for adjusting the values of the data due to explained or unexpected changes in the blank or calibration (K) factor during an analytical run cycle. Blanks can change due to sample handling, different capsules or sleeves, small leaks in the system and contamination. K factors should remain stable but can drift due to flow changes caused by variable pressure drops in the traps or helium scrubber, or by changing delivery pressure at the helium regulator.

12.2.2. Before the analyst can change calibration values and recalculate the results, there must be a valid reason. When data is recalculated, always document the incident.

12.3. Example of LOTUS spreadsheet of results:

	A	B	B	C	D	E
1	2/29,3/3/08					
2	Jane Doe					
3	DNR MAINSTEM SPLITS					
4	2/08					
5	SAMPLE	MG N/L	MG C/L			
6	56	0.1440	0.9520			
7	57	0.1510	0.9980			
8	58	0.1440	0.9460			
9	59	0.1430	0.9260			
10	BCSS1, 2/29	0.20	2.09	%		
11	BCSS1, 3/3	0.20	2.14	%		
12	LAB DUPS	PN		PC		
13	SAMPLE	DUP 1	DUP 2	DUP 1	DUP 2	
14	56	0.1430	0.1460	0.9360	0.9670	
15	58	0.1430	0.1450	0.9470	0.9450	
17	BLANKS N= 16		K VALUE N= 7.493			
18	C= 140		C= 20.771			
19	BLANKS N= 15		K VALUE N= 7.393			
20	C= 126		C= 20.352			

21 ATROPINE 2/29 N= 4.85 %
22 ATROPINE 2/29 C= 70.23 %
23 ATROPINE 3/3 N= 4.90 %
24 ATROPINE 3/3 C= 70.35 %

12.3.1. Cell 1A - Analysis date
12.3.2. Cell 2A – Analyst's name
12.3.3. Cell 3A – Sample source or client
12.3.4. Cell 4A – Sample date
12.3.5. Cell 5A – Column heading for Sample
12.3.6. Cell 5B – Column heading for N concentration
12.3.7. Cell 5C - Column heading for C concentration.
12.3.8. Cells 5A to 11D – Sample Results table.
12.3.9. Cells 10 D and 11 D - % to indicate that BCSS-1 is reported in %N or C
12.1.10. Cells 12A to 15E – QC table for field duplicates. The mean of these values is reported in the sample results table.
12.1.11. Cells 17A to 20F – Instrument values for the Blanks, and Ks.
12.1.12. Cells 21A to 24B- Values for LRB (atropine) for each day of analyses and middle and end of analytical run.

12.2. Sample data should be reported in units of mg/L as carbon or nitrogen for aqueous samples, and as percent carbon or nitrogen for sediment samples.

12.3. Report analyte concentrations to three significant figures for both aqueous and sediment samples.

12.4. For aqueous samples, calculate the sample concentration using the following formula:

$$Concentration\,(mg/L) = \frac{Corrected\ sample response(\mu g/L)}{1000 mls/L}$$

12.5. For sediment samples, % N or %C are already calculated by the instrument software.

13. METHOD PERFORMANCE

13.1. The procedure validation MDL, based on seven filtrations of a sample, was found to be 0.0633 mg/L for carbon and 0.0105 mg/L for nitrogen.

13.1. Twenty analyses of the BCSS-1 Marine Sediment QC, from 7/2007 to 3/2008, produced an average value of 2.13 +/- 0.4% C. The true value for the QC is 2.19 +/- 0.09% C. The true value for %N is not given, but the value obtained by our procedure was 0.194 +/- 0.008%N.

13.2. Forty analyses of the LRB (acetanilide), from 7/2007 to 3/2008, produced the following values for carbon and nitrogen: The true value for carbon in acetanilide is 71.09%. The average value over the time period was 70.35% ± 0.70%. The true value for nitrogen in acetanilide is 10.36%. The average value over the time period was 10.31% ± 0.10%.

13.3. Atropine became the standard used for LRB analyses as of 3/15/08. The true value for carbon is 70.56%. The average value form 3/15-4/7/08 was 70.14 ± 0.42%. The true value for nitrogen in atropine is 4.84%. The average value for the period was 4.92 ± 0.03%.

14. POLLUTION PREVENTION

14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity of toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the agency recommends recycling as the next best option.

14.2. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street N. W., Washington, D.C. 20036.

15. WASTE MANAGEMENT

15.1. The reagents used in this procedure are minimal and are not hazardous with the exception of the Ascarite and magnesium perchlorate. Due to the small quantity of Ascarite and magnesium perchlorate used, the spent reagent can be flushed down the drain with running water.

15.2. For further information on waste management consult The Waste Management Manual for Laboratory Personnel, available from the American Chemical Society.

16. REFERENCES

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16.8. Zimmermann, C. F., Keefe, C. W., and Bashe, J. 1997. Method 440.0. Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis. USEPA

17. DETAILED PROCEDURE

17.1. Exeter 440 Operation

17.1.1. The following sequence should be followed when initially starting up the system or when restarting after a shutdown.

17.1.1.1. Make sure the power switches on the computer and on the CEC- 490 (Interface) are off.

17.1.1.2. Remove the 440 cover from instrument.

17.1.1.3. Check that the helium regulator is set at 18 psig and oxygen at 20 psig and open the in-line gas valves.

17.1.1.4. If restarting, check that the combustion and reduction tubes, scrubber and traps are not exhausted.

17.1.1.5. Turn the selector switch to SYSTEM. Turn on the CEC-490 and the computer. The monitor will now display the Menu. If this is a cold re-start, set combustion and reduction furnace temperature controls to values previously established. Wait until the reduction furnace has reached operating temperature. DO-NOT PUSH DETECTOR RESET BUTTON AT THIS TIME!

17.1.1.6. With the combustion to reduction tube end connector removed, go to "Tube Replacement" in the Service Menu, then follow the directions under "Combustion Tube Replacement" to purge the helium and oxygen regulators twice. This will also serve the purpose of conditioning the reduction and combustion tubes. Then go to Main Menu and install the end connectors.

17.1.1.7. After allowing the 440 oven to reach operating temperature (about one hour) go to the Service Menu and select Calibrate CEC-490. Calibrate all and follow instructions.

17.1.1.8. Run 2 to 3 blank runs to establish a fill time of about 20 to 40 seconds. If the fill time has been exceeded, increase the helium pressure by ½ psig, and repeat running until fill time is achieved. If the system still aborts after the helium pressure has been increased to 22 psig, go through the leak test mode.

17.1.1.9. After the first complete run, push DET RESET. High concentrations of air or oxygen in the analytical system will damage the filaments in the detectors if power is applied. To protect the detectors, a detector safety circuit is provided which shuts off power when the helium carrier gas becomes

contaminated with air or oxygen at levels generating an imbalance of about 450 uv or higher. The safety circuit will activate should leaks develop or when the helium supply is depleted. The safety circuit monitors the gross imbalance between the two sides of the nitrogen bridge. If air or oxygen is present on both sides of the bridge, the safety circuit may not activate and damage to the detectors may occur.

Make certain that helium gas is flowing and that the instrument is purged before pressing the DETECTOR RESET button.

The safety circuit is also activated when accidental or deliberate power interruption occurs. If power has been interrupted for more than 5 minutes, do not push DETECTOR RESET until the system has been run as if to run a blank. Do not hold the DETECTOR RESET button in or more than one second. If the light stays on when the button is released, further running is necessary before pushing the button again. Go through one blank run before turning on the detector.

17.1.1.10. After the last run go to the Service Menu and monitor the bridge readings. Adjust the "zero" reading to approximately 2500 μv by turning the respective potentiometers on the Bridge Balance Card located in the left rear corner of the "Motherboard". Typically the bridges should be set well above negative or zero to approximately + 2500 μv. This is after the instrument has stabilized. Stability is based on furnace and oven temperatures being steady for a period of not less than 1 hour.

17.1.1.11. Check the furnace and oven temperatures. If these have reached operating levels, let the instrument go through another three sets of runs in order to purge the system and condition the reagents. This can be done through the CHN Run Mode (Run Menu).

17.1.1.12. Turn off the B-valve using the Parameters mode in the Customize pull-down menu. Continue running helium blanks until the base line (zero reading) is steady and/or until the blank for nitrogen and carbon is less than 200 μv, and for hydrogen less than 1500 μv.

17.1.1.13. Turn ON the B-valve and run oxygen blanks until consecutive runs agree within 10 μv for nitrogen and carbon, and 50 μv for hydrogen.

17.1.1.14. Go to the Service pull down Menu and calibrate all of the CEC-490 again.

17.1.1.15. The instrument is now ready for system calibration with known standards.

17.1.2. Standby Mode - To reduce helium consumption and minimize wear on the terminal screen, the overnight or short term standby mode is used.

17.1.2.1. Select the overnight standby mode (in the Run pull-down menu).

17.1.1.1. Return to normal operation.

17.1.1.1.1. Select Stop Overnight Standby in the Run pull-down menu

17.1.2. Powering Down - It is preferable for the system to remain powered up at all times since this will extend the life time of the glassware, reagents, and electronics. However, helium and power will be consumed during this standby and it might be necessary to power down the 440 instrument. To assure minimum disruption for a future start up after a power down, proceed as follows:

17.1.2.1. Turn the furnace temperature controllers to zero.

17.1.2.2. Allow several hours for the furnace temperatures to drop below 100°C.

17.1.2.3. Turn off the power to the instrument as well as gas valves between the instrument and the regulators.

17.1.2.4. Turn off the gas on the cylinder.

17.2. 440 Software Summary

17.2.1. Run Pull-Down Menu

17.2.1.1. Carbon, Hydrogen, Nitrogen Run

17.2.1.2. Oxygen

17.2.1.3. Sulfur

17.2.1.4. Overnight Standby (save carrier gas)

17.2.1.5. Change Blanks and Ks

17.2.1.6. Balance Interface Weight Entry

17.2.2. Service Pull-Down Menu

17.2.2.1. Datalog Signals

17.2.2.2. Leak Test

17.2.2.3. Profiles

17.2.2.4. Tube Replacement (Includes packing and installing)

17.2.2.5. Valve Rebuild

17.2.2.6. Maintenance Schedule

17.2.2.7. Maintenance Log

17.2.2.8. Bridges

17.2.2.9. Test Injector Drive

17.2.2.10. Calibrate CEC-490

17.2.2.11. Diagnostics

17.2.2.12. Balance Interface Test

17.2.3. Calculate Pull-Down Menu (Manipulating existing data)

17.2.3.1. Recalculate data and statistics

17.2.3.2. BTU/lb.

17.2.3.3. Dry, Dry Ash Free

17.2.3.4.H/C, N/C, C/H, C/N Ratio
17.2.3.5.C/C, H/H, N/N, O/O, S/S Ratio
17.2.3.6.Empirical Formula
17.2.4. Customize Pull-Down Menu (Customizing software)
17.2.4.1.Set parameters
17.2.4.2.Users
17.2.4.3.Edit Standards (names, weights, percents)
17.2.4.4.Create Report Format
17.2.4.5.Change Infinite Run Counter
17.2.4.6.Set Automation Type
17.2.5. Help
17.3. Run Pull-Down Menu
17.3.1. Select "Carbon, Hydrogen, Nitrogen Run"
17.3.2. Select "Yes" for a new run
17.3.3. Enter message for this run series
17.3.3.1.Check "Enter the Ks and Blanks automatically".
17.3.3.2.Enter date followed by AM or PM as appropriate
17.3.3.3.Press "Enter Data"
17.3.4. Sample Entry Screen
17.3.4.1.Enter Weight (µg)
17.3.4.1.1. When entering the weight of the sample press [ENTER] to use the present weight or enter a new weight. If a weight of zero [0] is entered then the ID is assumed to be a blank. If a weight of 100 has been entered the results will be reported in micrograms (µg). When analyzing aqueous samples, enter the volume filtered/10 as the weight. The results will be reported in ug/l. When analyzing sediment samples or weighed QC samples, enter the weight in ug. The result will be reported in %.
17.3.4.2.Enter Sample ID
17.3.4.2.1. Enter the sample ID as either STD1, blank, or any other text. If STD is entered as the first three letters, then Ks will be calculated on the result report. If blank is entered, then blanks will be calculated. If a weight of 100 has been entered, the results will be reported in micrograms (µg). If a "weight" of volume filtered/10 has been entered, the results will be in ug/l. If a weight of ug has been entered, the result will be reported in %.

17.3.4.3. Worksheet

Position #	Sample ID	Weight or volume/10	Comment, Sample Date or Client
1	Capsule+sleeve		
2	Conditioner		
3	Capsule+sleeve		
4	Conditioner		
5	STD1		
6	STD1		
7	STD1		
8	Capsule+Filter		
9	FD1		
10	FD2		
11			
12			
13			
14			
15			
16			
17			
18			
19			
20	FD1		
21	FD2		
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32	LFB Atropine		
33			
34			
35			
36			
37			
38			
39			
40			
41			
42	FD1		
43	FD2		
44			
45			
46			
47			
48			
49			

50			
51			
52			
53	FD1		
54	FD2		
55			
56			
57			
58			
59			
60			
61			
62	Capsule+sleeve		
63	LFB Atropine		
64	Capsule+sleeve		

17.3.4.4. Press “Start Run”

17.3.4.5. Loading the Sample Wheel into the Injector Box

This mode opens the ADF and C valves allowing helium to enter the injection box and minimize air in this area while installing the sample wheel for the 64 sample automatic injector. The pressure will build up and eventually equilibrate to the helium tank pressure if the instrument is left in this mode for a long period of time. This is not recommended, therefore, do not delay carrying out the following steps:

17.3.4.5.1. Open the manual purge valve on the injector box (right side, behind the P valve) to relieve the internal pressure. **NOTE: The injector housing should not be opened while pressurized. Vent the housing with the manual purge valve prior to opening the lid.**

17.3.4.5.2. Loosen the 4 cover screws and lift the lid. Remove the empty wheel from the sample chamber.

17.3.4.5.3. Vacuum out, or blow out with canned air, any material that might be in the box from the previous run (Loose material from the previous batch can contaminate samples, blanks and standards).

17.3.4.5.4. Insert the loaded sample wheel with the locking pin in place. Tilt the wheel slightly, line up the scribe mark on the wheel with the ratchet in the housing, lower the wheel and make sure that it is properly seated. Place the locking pin in the center hold. Check that the o-ring of the cover is clean and well seated in the groove before closing the cover.

17.3.4.5.5. Close the cover, and tighten equally on all four screws. This should be performed in an alternating sequence to achieve a uniform seal. Never over-tighten or use any tools on the screws.

17.3.4.5.6. Open and remove any spent capsules in the capsule receiver. Re-grease the gasket and re-install cover.

17.3.4.5.7. Close the purge valve, let pressure build up for about 30 seconds. Re-open the purge valve for about 5 seconds and then close again.

17.3.4.5.8. Select "OK" to continue operation.

17.3.4.6. The Sample Run

17.3.4.6.1. The sample is automatically injected into the combustion tube at the appropriate time. Upon completion of the fill time the ladle is retracted and allowed to cool. At the end of the run the results are printed and the soft key commands are followed if any has been selected. The screen returns to sample entry.

17.3.4.7. Run Display and Commands

Once the run begins, the screen displays the following information:

17.3.4.7.1. Run number, Sample Weight and ID., the operating K and B values, the preset combustion and purge times, valve status, and the elapsed time in minutes:seconds.

17.3.4.7.2. Temperatures and Pressure are also displayed near the bottom of the screen. These numbers may not be updated all of the time as time critical sections of a run occur. Run counters for the various tubes are displayed above the valve status diagram. The run counters will change from blue to red when they approach 10% within the thresholds set by the user.

17.3.4.7.3. During the run the analyst has various options available through the buttons at the top of the screen (accessed via simply selecting one). If a key is actuated, the button changes from grey to white. The buttons are for the following functions:

a. **Ks & Bs** - To access the Ks and Bs table at the end of the current run. This allows the operator to change the operating values.

b. **PARAMETERS -** Goes to parameters table at the end of the current run.
c. **LEAK TEST** - The leak test program is activated at the end of the run cycle.
d. **STANDBY -** At the end of the run cycle the instrument will go into overnight standby.
e. **DATALOG -**At the end of the run cycle a datalog is printed every half hour. A, D, and F valves are turned on, as in the overnight standby mode.
f. **SSI -** An HA function to activate the SSI (single sample inject) program after the completion of the current run. The HA program will automatically resume after the SSI run (unless SSI is pressed again).
g. **MENU NEXT -** Goes to the Analytical Menu at the end of the current run. The data will be stored on the data disc at that point.
h. **STOP -** Aborts the current operation and goes to the Analytical Menu. This is typically only used during emergency operations. If you exit an HA run cycle prematurely and you wish to start over or resume the HA run with the sample IDs and weights already in memory, then DO NOT exit the Analytical Menu. If you exit or reboot the Analytical Menu then the IDs and weights will be erased.
i. **NONE –** Nothing at end of run or run cycle.

17.4. Tube Replacement

17.4.1. This mode is used when one or more of the reagent tubes in the 440 need to be changed, as indicated by the maintenance schedule, poor analytical results or in the case of a cold restart.

17.4.2. Go to the Service Pull-down Menu. Select "Tube Replacement." "Select CHN Analysis." Another menu will be displayed that will contain options for tube packing information or for replacement of any tubes used for that analysis. If a new gas cylinder or regulator is to be replaced, select the appropriate tank changing from the menu.

17.4.2.1. Tube packing. By selecting the tube of interest the appropriate tube packing information is graphically displayed. In the individual tube replacement options,

follow the step by step instruction shown on the screen. If the procedure is followed correctly and to its conclusion, the Maintenance Schedule Information for that tube will be reset. You can return to the Service Menu at almost any point by pressing "End."

17.4.2.2. For the CHN Analysis there are instructions for:

17.4.2.2.1. Tube Packing Information
17.4.2.2.2. Helium Scrubber Replacement
17.4.2.2.3. Oxygen Scrubber Replacement
17.4.2.2.4. Carbon Dioxide Trap Replacement
17.4.2.2.5. Water Trap Replacement
17.4.2.2.6. Combustion Tube Replacement
17.4.2.2.7. Reduction Tube Replacement
17.4.2.2.8. Combustion & Reduction Tubes Replacement at the same time

17.4.3. Combustion Tube

17.4.3.1. Hold the tube vertically with the short end from the indentation up. Roll up a piece of platinum gauze so that it will fit snugly into the combustion tube. Slide the gauze plug into the tube and up against the indentation.

17.4.3.2. Add a small plug of quartz wool. (Quartz wool may be muffled for one hour at 850 °C to remove any residual carbon).

17.4.3.3. Add 1½" of silver tungstate/magnesium oxide on chromosorb. Gently tap the tube to prevent the reagent from channeling.

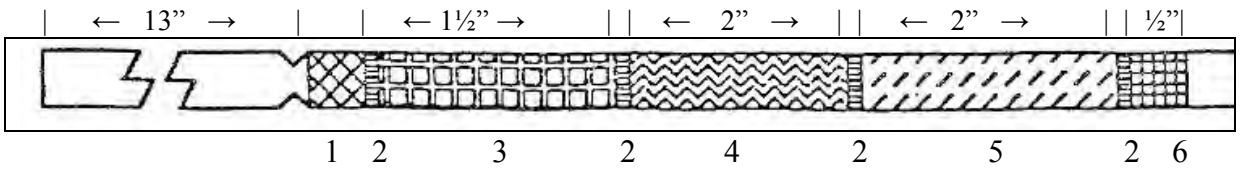
17.4.3.4. Add a small plug of quartz wool.

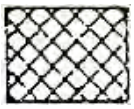
17.4.3.5. Add 2" of silver oxide/silver tungstate on Chromosorb tap the tube and add another small plug of quartz wool.

17.4.3.6. Slide a rolled-up piece of silver gauze into the tube and pack against the quartz wool. Make sure that there is no less than ½" of space between the end of the tube and the silver gauze since the silver gauze will conduct heat and damage the o-ring on the end connector.

17.4.3.7. The amount of each reagent used can be varied to suit the type of materials to be analyzed. For example, if predominantly fluoridated compounds are run proportionately more silver tungstate/magnesium oxide should be packed into the tube.

17.4.3.8. There is rarely such a thing as a "too tightly" packed combustion tube. Loosely packed combustion tubes can cause non-linearity.



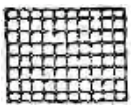
 #1 - Platinum gauze

 #2 - Quartz wool

 #3 - Silver tungstate / Magnesium oxide on Chromosorb

 #4 - Silver oxide / Silver tungstate on chromosorb

 #5 - Silver vanadate on Chromosorb

 #6 - Silver gauze

17.4.3.9.Function of Combustion Tube Packing Material

17.4.3.9.1. Silver Vanadate on Chromosorb

Reacts with and removes chlorine, bromine, iodine and sulfur contained in the combustion gases. When absorbing sulfur, it changes color from yellow to dark brown when saturated. In absorbing halogens, exhaustion of the silver vanadate is indicated by color changes on the surface of the silver gauze at the end of the combustion tube. Each element forms a distinctively colored salt deposit – silver chloride is gray, silver bromide is brown, and silver iodide is purple. The gauze can be rejuvenated by heating in the upper, reducing

portion of a Bunsen burner or muffling at 550°C for 90 minutes.

17.4.3.9.2. Silver Tungstate / Magnesium Oxide on chromosorb: Removes fluorine, phosphorus, and arsenic.

17.4.3.9.3. Silver Oxide / Silver Tungstate on chromosorb: Removes sulfur and halogens (except fluorine).

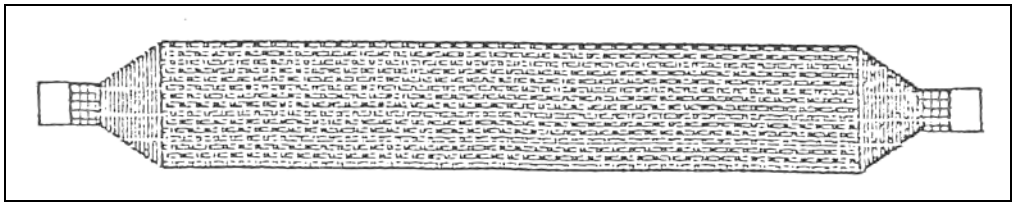
17.4.4. Reduction Tube

17.4.4.1. Pack about ¾" of quartz wool into the bottom of the tube from the opposite end.

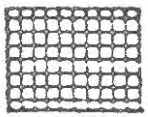
17.4.4.2. Fill the tube with copper wire while gently tapping to tightly settle the copper and avoid channeling.

17.4.4.3. Pack another plug of quartz wool into the tube against the copper.

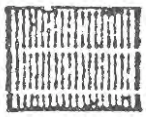
17.4.4.4. Insert a rolled-up piece of silver gauze into each small diameter tube end.



| 1| 2 | 3 | 2 | 1|



#1 - Silver gauze



#2 - Quartz wool

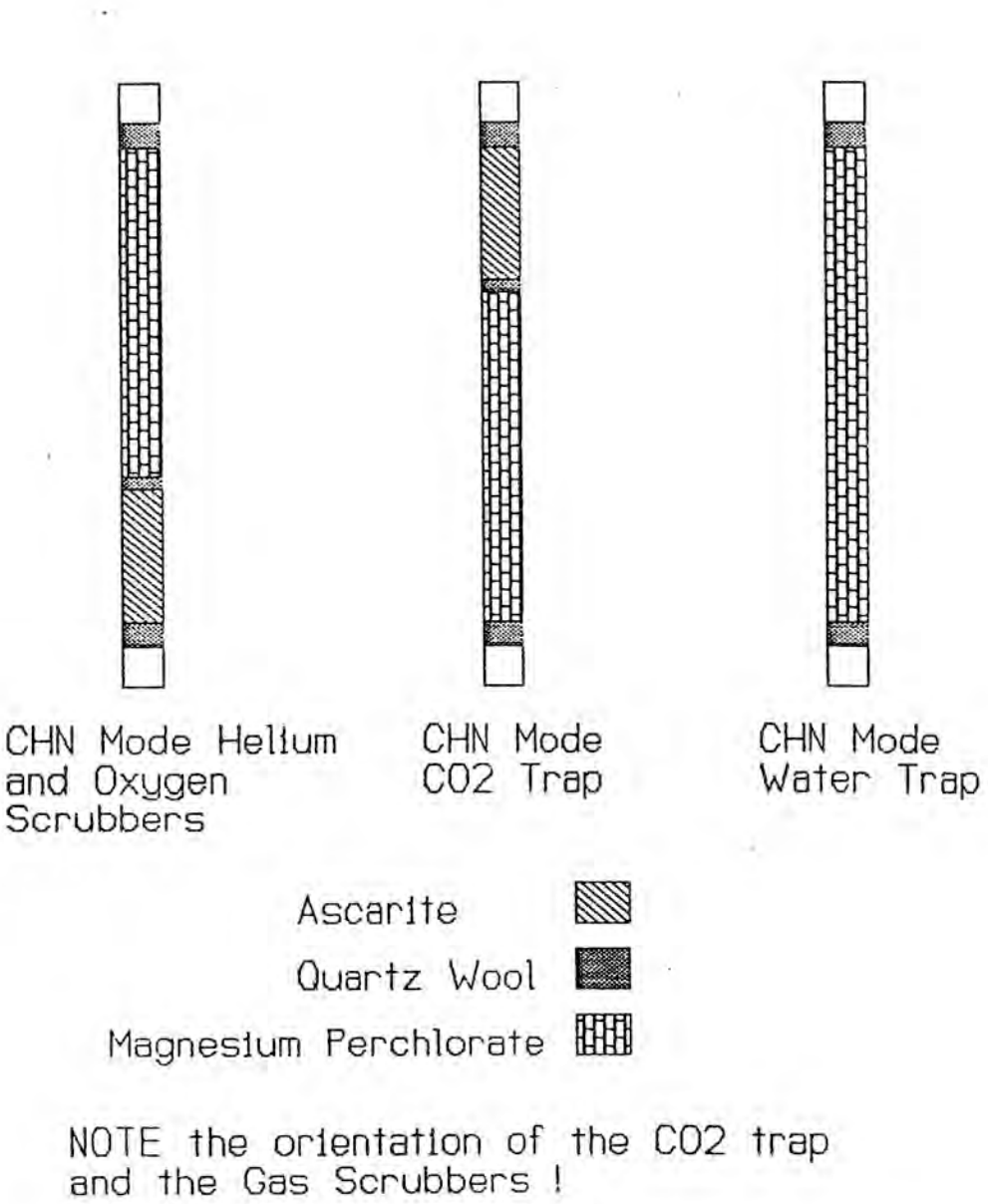
#3 - Copper wire

17.4.5. Carbon Dioxide Trap and Gas Scrubbers (3)

17.4.5.1. These three tubes are identically packed even though the Scrubbers are a larger diameter. Pack a ¼" plug of quartz wool into one end of the tube.

17.4.5.2. Add 3½" Ascarite (Colorcarb) while gently tapping the tube.

17.4.5.3. Add ¼" plug of quartz wool.
17.4.5.4. Add 1½" magnesium Perchlorate while gently tapping the tube.
17.4.5.5. Add ¼" plug of quartz wool.
17.4.5.6. There should be about ¼" of free space at each end of the tube.
17.4.5.7. Gas scrubbers should be loosely packed to allow for the high gas flows associated with the 440.
17.4.5.8. Note the orientation (in the instrument) of the helium and oxygen scrubbers versus the CO_2 scrubber. The orientation is reversed for the CO_2 scrubber.



17.4.6. Helium Scrubber Replacement

17.4.6.1. Close the inlet helium gas valve and back off the regulator valve. [HIT RETURN WHEN DONE]

17.4.6.2. At this point the helium tank can also be replaced by removing the regulator and installing a new tank.

17.4.6.3. To replace the helium scrubber carefully loosen the tube nut with a 440 tube nut wrench, loosen the wing nuts and lift the top assembly gently until the scrubber can be removed.

17.4.6.4. Repack the scrubber as described in 18.6.5.

17.4.6.5. Check the o-rings and effluent filters at this time. Make sure any quartz wool fibers, which could prevent a good seal, are removed from the outside of the scrubber before inserting.

17.4.6.6. Replace the tube, bring the top assembly down and tighten the wing nuts. Tighten the lower nut ONLY. Very carefully open the in-line valve and increase the helium gas pressure to 5 psig. [HIT RETURN WHEN DONE]

17.4.6.7. Wait one minute. A tone will sound. A clock on the screen counts down the time. When the tone sounds, the screen displays the message: "I'm finished purging the helium scrubber." [HIT RETURN TO ACKNOWLEDGE]

17.4.6.8. Tighten the top nut on the helium scrubber. Increase the pressure to normal. [HIT RETURN WHEN DONE]

17.4.6.9. Wait 5 minutes. This serves to purge the gas lines. Once the 5 minutes have passed, the Tube Replacement Menu for the chosen analysis mode will be displayed.

17.4.6.10. The instrument should be conditioned after replacing the halogen scrubber by running two blanks before proceeding to a sample run.

17.4.6.11. If the helium tank has been replaced, purge the regulator 5 times and run a helium blank profile to verify good gas.

17.4.7. Oxygen Scrubber Replacement

17.4.7.1. Close the inlet oxygen gas valve and back off the regulator valve. [HIT RETURN WHEN DONE]

17.4.7.2. At this point the oxygen tank can also be replaced by removing the regulator and installing a new tank.

17.4.7.3. To replace the oxygen scrubber carefully loosen the tube nut with a 440 tube nut wrench, loosen the wing nuts and lift the top assembly gently until the scrubber can be removed.

17.4.7.4. Repack the scrubber as described in 18.6.5.

17.4.7.5. Check the o-rings and effluent filters at this time. Make sure any quartz wool fibers, which could prevent a good seal, are removed from the outside of the scrubber before inserting.

17.4.7.6. Replace the tube, bring the top assembly down and tighten the wing nuts. Tighten the lower nut **ONLY**. Very carefully

open the in-line valve and increase the oxygen gas pressure to 5 psig. [HIT RETURN WHEN DONE].

17.4.7.7. Wait one minute. A tone will sound. A clock on the screen counts down the time. When the tone sounds, the screen displays the message "I'm finished purging the oxygen scrubber. [HIT RETURN TO ACKNOWLEDGE]

17.4.7.8. Tighten the top nut on the oxygen scrubber. Increase the pressure to normal. [HIT RETURN WHEN DONE]

17.4.7.9. The instrument should be conditioned after replacing the oxygen scrubber by running two blanks before proceeding to a sample run.

17.4.7.10. The procedure for replacing the oxygen scrubber is identical to that of the helium scrubber. The only difference is the omission of the 5 minute purge.

17.4.8. Carbon Dioxide Trap Replacement

17.4.8.1. Replace the carbon dioxide trap. Tighten the lower nut only. [HIT RETURN WHEN DONE]

17.4.8.2. Be sure to orient the trap correctly, with the Ascarite portion toward the top.

17.4.8.3. Check the o-rings and re-grease lightly, also check the effluent filters at this time.

17.4.8.4. Wait 1 minute. A tone will sound. A clock counts down the time on the screen and then displays "I'm finished purging the carbon dioxide trap". [HIT RETURN TO ACKNOWLEDGE]

17.4.8.5. Tighten the top nut on the carbon dioxide trap. Increase the pressure to normal. [HIT RETURN WHEN DONE] (Ignore the instructions regarding pressure).

17.4.8.6. When completed, the Tube Replacement Menu for the CHN analysis mode will be displayed.

17.5. Important Factors for Proper 440 Operation

17.5.1. Pack the scrubber tubes loosely.

17.5.2. Vibrate or tap down the combustion tube packing chemicals while packing to assure a fairly tight tube. DO NOT over-tighten.

17.5.3. Oxygen pressure should be at ≈ 20 psig.

17.5.4. Helium pressure should be at ≈ 18 psig and the fill time (FT) for a run should be between 20 and 40 seconds.

17.5.5. When greasing o-rings or gaskets, it is recommended to use Krytox (R) by Dupont.

17.5.6. The furnace temperatures reach set temperature very quickly. Do not set the furnaces to anything but the temperature for analysis.

17.5.7. Never set the combustion temperature above 1100 °C.

17.5.8. Never set the reduction temperature above 900 °C.

17.5.9. All valves are "Normally Closed" type.

Chesapeake Biological Laboratory
University of Maryland Center for Environmental Science
Nutrient Analytical Services Laboratory

Determination of Dissolved Organic Carbon (NPOC), Total Organic Carbon, and Dissolved Inorganic Carbon in waters of Fresh/Estuarine/Coastal Waters using High Temperature Combustion and Infrared Detection.

1. SCOPE and APPLICATION

1.1 High temperature combustion (680°C) is used to determine dissolved organic carbon (DOC), also known as non-purge able organic carbon (NPOC), total organic carbon (TOC), and total (TIC) or dissolved inorganic carbon (DIC), using a non-dispersive infrared detector (NDIR). The method is used to analyze all ranges of salinity.

1.2 A Method Detection Limit (MDL) of 0.18 mg/L DOC, and 0.03 mg/L DIC was determined using 3X the standard deviation of 7 replicates.

1.3 The quantitation limit for DOC and DIC was set at 0.05 mg/L C.

1.4 This procedure should be used by analysts experienced in the theory and application of organic carbon analysis. Three months experience with an experienced analyst, certified in the analysis using the organic carbon analyzer, is required.

1.5 This method can be used for all programs that require analysis of dissolved and total organic and inorganic carbon.

1.6 This procedure conforms to EPA Method 415.1.

2. SUMMARY

2.1 The Shimadzu TOC-5000/5000A uses a high temperature combustion method to analyze aqueous samples for TIC, TOC and non-purge-able organic carbon (NPOC).

2.2 NPOC samples are treated with hydrochloric acid and sparged with ultra pure carrier grade air to drive off inorganic carbon. TOC samples are injected directly onto the catalyst bed with no pretreatment. High temperature combustion (680☜C) on a catalyst bed of platinum-coated alumina balls breaks down all carbon compounds into carbon dioxide (CO_2). The CO_2 is carried by ultra pure air to a non-dispersive infrared detector (NDIR) where CO_2 is detected.

2.3 Samples for inorganic carbon (TIC) are injected directly into a receptacle of 25% phosphoric acid where the carbonates are reduced to CO_2 and detected by the NDIR.

3. DEFINITIONS

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)

3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)

3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)

3.4 Analytical Range - 100 ppb - 4000 ppm using 250 µl syringe and 4 - 100 µl injection volume, using regular sensitivity catalyst.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of one to 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set if operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.10 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.11 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.

3.11.2 Initial Calibration Verification (ICV) – An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.

3.11.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 10-15 field sample analysis.

3.12 Certified Reference Material – A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025)

3.13 Combustion tube – Quartz tube filled with platinum catalyst, heated to 680° C, into which the sample aliquot is injected.

3.14 Conditioning Blank – DI water run before the calibration curve to decrease the instrument blank and stabilize the column conditions.

3.15 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.16 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.17 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.18 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.19 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)

3.20 External Standard (ES) – A pure analyte (potassium hydrogen phthalate (KHP)) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.21 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout filed and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.22 Field Reagent Blank (FRB) – A aliquot of reagent water or other blank matrix that is places in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site,

exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.23 Furnace – Heats the combustion tube to the operating temperature of 680° C.

3.24 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.25 Injection – The sample aliquot that is drawn into the syringe and injected into the combustion tube.

3.26 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.27 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.28 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., DI water) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.

3.29 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.30 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.31 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.32 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.33 Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.34 May – Denotes permitted action, but not required action. (NELAC)
3.35 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.
3.36 Must – Denotes a requirement that must be met. (Random House College Dictionary)
3.37 Non-Dispersive Infrared Detector (NDIR) – The detector found in the Shimadzu 5000/5000A TOC analyzer. Carbon dioxide is detected.
3.38 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)
3.39 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.
3.40 Quality Control Sample (QCS) – A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.
3.41 Run – One sample analysis from start to finish, including printout.
3.42 Run Cycle – Typically a day of operation – the entire analytical sequence of runs from the first run to the last run and including the transfer of run cycle data to the disc.
3.43 Sample Volume – Amount of sample injected into the combustion tube.
3.44 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.
3.45 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)
3.46 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)
3.47 Sparge Time – The time required to aerate an acidified sample with ultra pure air to remove inorganic carbon to determine the concentration of organic carbon.
3.48 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

4. INTERFERENCES

4.1 Carbonates and bicarbonates may interfere with the determination of organic carbon by increasing the concentration of CO_2 detected. These are removed by adding enough 2N HCl to the sample to bring the pH to 2 or below, then sparging with ultra-pure air for a predetermined time.

5. SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Business Manager of the incident. Contact the CBL Business Manager if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Chemical	Health	Flammability	Reactivity	Contact	Storage
Potassium Hydrogen Phthalate	0	1	0	1	Green
Sodium Carbonate, Anhydrous	1	0	1	2	Green
Sodium Bicarbonate	1	1	1	1	Green
Phosphoric Acid	3	0	2	4	White
Hydrochloric Acid	3	0	2	4	White
Sodium Hydroxide	3	0	2	4	White Stripe
Platinum Catalyst on Alumina Beads	1	0	1	1	Green
Soda Lime	1	0	1	3	White

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)
STORAGE

Red – Flammability Hazard: Store in a flammable liquid storage area.
Blue – Health Hazard: Store in a secure poison area.
Yellow – Reactivity Hazard: Keep separate from flammable and combustible materials.
White – Contact Hazard: Store in a corrosion-proof area.
Green – Use general chemical storage (On older labels, this category was orange).
Striped – Incompatible materials of the same color class have striped labels. These products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.

6. EQUIPMENT AND SUPPLIES

6.1 A Total Organic Carbon Analyzer capable of maintaining a combustion temperature of 680° C and analyzing for organic and inorganic carbon. The Shimadzu TOC5000 and the Shimadzu TOC5000A are used in this laboratory.
6.2 Freezer, capable of maintaining -20 ± 5° C.
6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives. This laboratory soaks all lab ware related to this method in a 10% HCl (v/v) acid bath overnight.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.
7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.
7.3 Potassium Hydrogen Phthalate (KHP) C_6H_4 (COOK) (COOH) – primary standard for organic carbon.
7.4 Sodium Hydrogen Carbonate ($NaHCO_3$) and Sodium Carbonate (Na_2CO_3) – primary standard for inorganic carbon.
7.5 Hydrochloric Acid, 2 N –
Hydrochloric acid (HCl), concentrated, 172 ml
Deionized water, q.s. 1000 ml

In a 1000 ml volumetric flask, add 172 ml of concentrated hydrochloric acid to ~600 ml of deionized water. Dilute to 1000 ml with deionized water.

7.6 Organic Carbon Stock Standard: Potassium Hydrogen Phthalate (KHP) Standard, 1000 mg/l

Potassium hydrogen phthalate ($HOCOC_6H_4COOK$), Dried at 45 C	2.125 g
Deionized water	1000 ml

In a 1000 ml volumetric flask, dissolve 2.125 g of potassium hydrogen phthalate in ~800 ml of deionized water. Dilute to 1000 ml with deionized water. Make fresh every 4 - 6 months. Store at 4 C.

7.7 Inorganic Carbon Stock Standard: Sodium Hydrogen Carbonate/ Sodium Carbonate ($NaHCO_3/Na_2CO_3$) Standard, 1000 mg/l

Sodium Hydrogen Carbonate ($NaHCO_3$)	1.75 g
Sodium Carbonate, Anhydrous (Na_2CO_3)	2.205 g
Deionized H_2O	500 ml

In a 500 ml volumetric flask, dissolve 1.75 g NaHCO3 and 2.205 g Na2CO3 in ~300 ml deionized H2O. Dilute to 500 ml with deionized H2O. Make fresh every 4 months. Store at 4° C.

7.8 Blanks – Two blanks are used in calculations for this analysis.

- 7.8.1 ASTM D1193, Type I water is used for the Laboratory Reagent Blank. The LRB is comprised of the instrument blank plus the organic content found within the TYPE I water. The area of the LRB is subtracted from the area of the standards.
- 7.8.2 For ambient water samples, the LRB cannot be used, because the samples have no added Type I water. Because the instrument has an internal blank that is not constant, the instrument blank is determined to be the **absolute value** of the y intercept. This value is subtracted from the area of the samples.
- 7.8.3 If the ambient water sample is diluted with TYPE I water, then the LRB must be used in the calculation.
- 7.8.4 This does not apply to TIC samples. The instrument blank for inorganic carbon is insignificant, so no blank is subtracted from the samples.

7.9 Quality Control Sample (QCS) – For this procedure, the QCS can be any certified dissolved sample which is obtained from an external source. If a certified sample is not available, then use the standard material (KHP).

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for DOC and/or DIC should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.

8.2 Water collected for DOC should be frozen at -20° C, or acidified with 2N HCl to a pH of ≤2. Water collected for DIC should not be acidified. The sample container should be either borosilicate glass or Teflon. Plastic containers may be used if well cleaned and aged. Freshwater samples should be frozen in Teflon or plastic to prevent breakage.

8.3 Frozen DOC and/or DIC samples may be stored longer than 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits.

8.4 DOC samples acidified with 2N HCL should be frozen, as above, or refrigerated at 4° C for no longer than 28 days.

8.5 DIC samples stored at 4° C should be analyzed within 28 days.

9 QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

9.2.1 The initial demonstration of capability (DOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with analyses.

9.2.3 Method Detection Limits (MDLs) – MDLs should be established for DOC and DIC using a low level ambient water sample. To determine the MDL values, analyzed seven replicate aliquots of water. Perform all calculations defined in the procedure (Section 10) and report the

concentration values in the appropriate units. Calculate the MDL as follows:

MDL = S x 3

Where, S = Standard Deviation of the replicate analyses.

9.2.4 MDLs should be determined yearly.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of Nanopure water treated the same as the samples. LRB data are used to assess contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – when using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3σ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these samples shall be used to determine batch acceptance.

9.3.3 The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4 Control Charts – The SRM data is graphed, and the slope, y-intercept, and r squared data are compiled and tracked.

9.3.5 Continuing Calibration Verification (CCV) – Following every 12-15 samples, one or two CCVs are analyzed to assess instrument performance. The CCVs are made from the same material as calibration standards (KHP), and are to be within TV ± 3σ. Failure to meet the criteria constitutes correcting the problem and reanalyzing the samples. If not enough sample exists, the data must be qualified if reported.

9.4 Assessing Analyte Recovery

9.4.1 Matrix spikes are performed on a 20% QA/QC basis.

9.4.2 0.5 ml of the highest KHP standard in the curve is added to 5.0 ml of sample for a total volume of 5.5 ml.

9.4.3 0.5 ml standard 0.5/5.5 = 0.09

9.4.4 0.09 X STD conc.
9.4.5 5.0 ml sample 5.0/5.5 = 0.91
9.4.6 (original sample conc. X 0.91) + (0.09 x std conc.) = (expected conc.) mg/L

9.5 Data Assessment and Acceptance Criteria for Quality Control Measures
9.5.1 The Acceptance Criteria for DOC is 0.9990. If the r^2 is less than acceptable, all blanks and standards analyzed during the run may be averaged into the curve.

9.6 Corrective Actions for Out of Control Data
9.6.1 If the acceptance criteria are still not met, the samples are to be rerun.

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Daily calibration must be performed before sample analysis may begin. Four point calibration is used with the Shimadzu TOC 5000/5000A.

10.1.1 Type I water is used as the "zero point" in the calibration. Because even Type I water contains some organic carbon, the area count response is subtracted from the standards as the water blank, forcing the curve through zero. The standards are calculated by the following equation:

$$\text{mg TOC/L} = (A_{STD} - A_{H2OBLK})/ m$$

Where: A_{STD} = Area of the standard
A_{H2OBLK} = Area of water blank
m = slope of the regression line

TOC sample concentration is calculated using the following equation:

$$\text{mg TOC/L} = (A_S - | b |)/ m$$

Where: A_S = area of the sample,
b = y-intercept, and
m = slope of the regression line

11 PROCEDURE

11.1 How to run the Shimadzu 5000

11.1.1 Turn on the gas.

11.1.2 Turn on instrument. The power switch is located on the right side of the instrument.

11.1.3 Instrument checks when turning on: Check level of liquid in the humidifier, located in the lower right corner inside. The level should be between the two lines. If low—add DHOH. Unscrew cap on the side and squirt in up to upper line.

11.1.4 Turn on ASI. Press F5 to initialize the ASI. Make sure the turntable is in place to avoid error messages. If the ASI is turned on after opening the MAIN MENU, the instrument will automatically initialize the ASI. Samples may be loaded onto the carousel while the TOC-5000 is warming up. Just make sure the turntable is in place before turning on the ASI.

11.1.5 Use the F keys to navigate. Go to NEXT (F1) which opens the MAIN MENU. Press 3 and Enter for General Conditions.

11.1.6 Using arrow keys, scroll down to TOC furnace. Press 1 and Enter to turn on furnace. Return to MAIN MENU (F2). Make sure liquid in the TIC chamber —plastic reservoir inside in upper center— is bubbling. If not, there is a leak.

11.1.7 Press 6 and Enter to get to MONITOR SCREEN. This screen allows you to monitor instrument conditions. It takes 20-30 minutes to come to temperature.

11.1.8 Loading Samples: Decide on standard curve.

11.1.8.1 NPOC conditioning curve: Place a std vial of DHOH is position S1. (This vial may also be used as the zero std of the calibration curve.) Load at least 3 sample vials of DHOH in positions 1-3. 100 µl injection, range 1, 9 max 10 injections, 3 minute sparge.

11.1.8.2 NPOC: I use 2 curves: Low — 0-10 ppm ---- DHOH, 2.0, 5.0, 10.0 as calibration standards, and adding 0.5, 1.0, and 7.5 as well for stds as samples. 60 µL injection, range 1, 6 min sparge.
High----- 0-20 ppm ----- DHOH, 5.0, 10.0, 20.0 as calibrating stds and adding 1.0, 2.0, & 15.0 as stds as samples. 30 µL injection, range 1, 6

min sparge. The instrument allows up to a 4 point curve.

11.1.8.3 TIC: This analysis is not performed on a regular basis; therefore the samples are analyzed using a curve of 0-30 ppm Na_2CO_3/$NaHCO_3$ and an injection volume of 25 µl in range 1. If the samples fall within the low end of the curve, the curve range and injection volume are adjusted accordingly. If the samples fall off scale, they are diluted.

11.1.9 Use the large sample vials for the curve stds. Examples of sample protocol can be found in the data sheet notebook.

11.1.10 I always load several DHOH samples (at least 3) as conditioning samples.

11.1.11 Fill std vials ~ 1/3 to 1/2 full.

11.1.12 Fill sample vials to within 3/4 - 1" of the top. Acidify the NPOC samples by adding 100 µl of 2N HCl. The instrument can be set up to automatically add acid to the standard cups. Make sure a standard cup filled with 2N HCl is in position S8. DO NOT acidify TIC samples.

11.1.13 Data sheets are found in the LOTUS TOC5000 folder, labeled TOC datasheet, high, low std. Print as many as needed. (Low std set to print 5; high std prints 2, and plain datasheet prints 1.) Examples are found before section 12.

11.1.14 When the turntable is loaded, place in the ASI. Line up pins to align properly. Put top on lining up pins carefully. Sample needles will Z if the top is not properly aligned.

11.1.15 Go to the MAIN MENU — Press 9 and Enter for the AUTOSAMPLER.

11.1.16 Determine analysis type: Press 1 for TC, 2 for IC, 3 for TOC, 4 for NPOC on each to be used, one line per curve. The instrument can store up to 18 curves.

11.1.17 Create 2 std curves for NPOC. Using the number keys, toggle the sample type to NPOC. Enter sample positions under IS and FS, and curve # under C1. Curve #1 is used as the conditioning curve. At least vials 1 -3 of DHOH and the std DHOH (S1). (9 max 10 injections, 100 µl, range = 1, 3 minute sparge, and curve not through zero.) Curves 2-18 are used as sample curves. See 11.1.19.

11.1.18 If analyzing TIC, create 1 std curve for TIC. Using the number keys, toggle the sample type to TIC.

11.1.19 Enter curve # under C1 and ENTER. This opens the screen to input curve data. Use arrow keys to navigate. Enter std conc. & position (S1, S2, etc.)

11.1.19.1 NPOC: Range = 1, inj vol 60 µL for low curve and 30 µL for high curve. 3 max 5 injections, 200 SD, 2.0% CV, 6 minute sparge, acid addition on, and curve forced through zero.

11.1.19.2 TIC: Range = 1, inj. vol 25 µl for 0-30 ppm curve. 3 max 5 injections, 200 SD, 2.0% CV.

11.1.20 Return to SAMPLE CONDITIONS. Make sure sample conditions match curve conditions. Ex: # of inj, sparge time, etc.

11.1.21 When all is set, press F1 (NEXT)

11.1.22 Decide whether to leave instrument in 1 (Finish), 2 (Running), or 3 (No Change)

11.1.23 Press F1 (NEXT)

11.1.24 Press Start

11.1.25 Check paper supply

11.1.26 Make sure the Rinse Reservoir is full.

11.1.27 Make sure the waste bottle has room.

11.2 Shutdown procedure for the TOC5000.

11.2.1 Make sure the Autosampler needles are in the home position.

11.2.2 Open MAIN MENU (F2).

11.2.3 Enter 7 for STANBY OPTIONS

11.2.4 Press STANBY (F1) to shutdown. This turns the furnace off and closes the main pressure valve.

11.2.5 Wait 30 minutes before turning off power.

11.3 How to run the Shimadzu 5000A

11.3.1 Turn on gas.

11.3.2 Turn on instrument.

11.3.3 Turn on laptop and open TOCControl. There is no password, so just hit ENTER. Turn on TOC5000A. The power switch is located on the left hand side behind the Autosampler.

11.3.4 Open communication to instrument by clicking on the connect button on top button bar. (Looks like two plugs connecting). The computer will go through a series of checks before connecting.

11.3.5 Instrument checks when turning on: Check level of liquid in the humidifier, located in the lower right corner inside. The level should be between the two lines. If low—add DHOH. Unscrew cap on the side and squirt in up to upper line. Make sure liquid in the TIC chamber —plastic reservoir in upper center— is bubbling. If not, there is a leak.

11.3.6 The furnace automatically turns on when connected. It takes 20-30 minutes to come to temperature.

11.3.7 To view instrument conditions, click on View /Background Monitor. When all conditions are OK, the system is ready to run.

11.3.8 Loading Samples: Decide on standard curve. I use 2 curves: Low — 0-10 ppm ---- DHOH, 2.0, 5.0, 10.0 as calibration standards, and adding 0.5, 1.0, and 7.5 as well for stds as samples. 60 µL injection. High----- 0-20 ppm ----- DHOH, 5.0, 10.0, 20.0 as calibrating STDs and adding 1.0, 2.0, & 15.0 as STDs as samples. 30 µL injection. The instrument allows up to a 4 point curve.

11.3.9 Use the large sample vials for the curve stds. Examples of sample protocol can be found in the data sheet notebook.

11.3.10 I always load several DHOH samples (at least 3) as conditioning samples

11.3.11 Fill std vials ~ 1/3 to 1/2 full.

11.3.12 Fill sample vials to within 3/4 - 1" of the top.

11.3.13 Data sheets are found in the LOTUS TOC5000 folder on the desktop, labeled TOC datasheet, high, low std. Print as many as needed. (Low STD set to print 5; high STD prints 2, and plain datasheet prints 1.)

11.3.14 Acidify the samples by adding 100 µl of 2N HCl. The standard curve files are set up to automatically add acid to the standard cups. Make sure a standard cup filled with 2 N HCl is in position S8.

11.3.15 When the turntable is loaded, place in the ASI. Line up pins to align properly. Put top on lining up arrows carefully. Sample needles will Z if the top is not properly aligned.

11.3.16 Close the Background Monitor and a blank sample table will be opened.

11.3.17 Enter sample table by clicking on Edit/Insert Std. Click on CONDBLK.CAL. Then click on Edit/Autogenerate. In the method name slot, type CONDBLK.MET. Using the TAB, toggle through to fill in the blanks. This is an NPOC file. I usually put conditioning DHOH vials in positions 1-3. The standard curve is next. Several curves are already stored. Click on Edit/ insert STD again to choose the proper curve. LOSTD10 is a 0-10 ppm curve with DHOH, 2.0, 5.0, & 10 in positions S1, 2, 3, 4. HISTD20 is a 0-20 ppm curve with DHOH, 5.0, 10.0, 20.0 in positions S1, 2, 3, 4.

11.3.18 Click on Edit/ Insert Sample next. Most likely this is a new file, so click on NEW. You will then be prompted enter the conditions of the samples to be run. Enter a file name (up to 8 characters), and TAB to the next slot. Enter the sample information (ex. DNR Potomac 3/30/08). TAB again to enter

sample name (ex. Potomac), TAB to next and hit backspace to clear. This leaves the SAMPLE ID column blank so ID's can be entered when the table is completed. TAB again, and using the down arrow, choose the NPOC option. Click on NPOC tab at top to enter standard information. Click on Browse to choose standard curve. (LOSTD10 or HISTD20). Injection volumes and other stored info will be displayed. Click OK on each of the screens that pop up. You will be prompted to save the method file. (Up to 8 characters. Ex: POT0330). At this point, one line is added to the sample table. The sample vial position is blank. Type in the vial position (usually 4) from the bench sheet and hit enter. Move the cursor to the next line down, first column and click. To enter the rest of the sample table, click on Edit/ Autogenerate. Enter the file name.met and TAB to enter vial positions (starting with 5.) If you have more than one group of samples, you may use autogenerate several times to enter each group. EX: Potomac uses vial positions 5-46, Chincoteague 47-60, and MDE Loch Raven 61-78. Each time you enter autogenerate, be sure to change the name of the group. The method file remains the same.

11.3.19 When the sample table is entered, go back and enter the sample ID's. Save the table using the same name as the method file.

11.3.20 Click Start.

11.3.21 Choose whether to keep instrument on at end of run or to finish. Click OK. Click OK again to acknowledge that the acid container is in position S8.

11.3.22 Do not run instrument with computer hooked up to the network. It increases the chances of the system locking up.

11.3.23 Make sure the Rinse Reservoir is full.

11.3.24 Make sure the waste bottle has room.

11.3.25 When the run is finished, reconnect to the network and save the file to P:drive/Kaumeyer, so it can be accessed by the desktop.

11.4 Shutdown Procedure for the TOC5000A

11.4.1 Make sure the Autosampler needles are in the home position.

11.4.2 Make sure the software is in Standby. (Should say Standby in lower left corner of screen.)

11.4.3 Click on connection button (next to ? button, 2nd from right)

11.4.4 Click OK to disconnect instrument.

11.4.5 Close program - shutdown computer.

11.4.6 Turn off instrument (on left behind ASI).

11.4.7 If instrument is not in standby:
11.4.8 Click on Halt to stop program
11.4.9 .The ASI needles should rise and go to home position. (They don't always)
11.4.10 Click on Measure, then Standby.
11.4.11 Click on OK in screen to shutdown. Wait at least 30 minutes for the furnace to cool.
11.4.12 Click Close to remove that screen.
11.4.13 After 30 minutes, follow shutdown procedure above.

SHIMADZU DATA SHEET			CRUISE :				
TODAY'S DATE:							
INSTRUMENT USED:							
MANUAL/PC CONTROL			ANALYST: NLK		OTHER:		
			INJECTION		FILE		
SPIKE CONC.:			VOLUME:		NAME:		
VIAL/STD	AREA		VIAL/STD	AREA		VIAL/STD	AREA
cond std	S1		S4			S7	
	S2		S5			S8	ACID
	S3		S6				
VIAL/ID	AREA	VIAL	ID	AREA	VIAL	ID	AREA
1 COND		27			53		
2 COND		28			54		
3 COND		29			55		
4 DHOH		30			56		
5 DHOH		31			57		
6		32			58		
7		33			59		
8		34			60		
9		35			61		
10		36			62		
11		37			63		
12		38			64		
13		39			65		
14		40			66		
15		41			67		
16		42			68		
17		43	DHOH		69		
18		44			70		
19		45			71		
20		46			72		
21		47			73		
22		48			74		
23		49			75		
24		50			76		
25		51			77		
26		52			78		

SHIMADZU DATA SHEET CRUISE :
TODAY'S DATE:
INSTRUMENT USED:

ANALYST: NLK
MANUAL/PC CONTROL **OTHER:**
INJECTION VOLUME: 60 FILE
SPIKE CONC.: 10 mg/L KHP uL NAME:

VIAL/STD	AREA	VIAL/STD	AREA		VIAL/STD	AREA
cond std	**S1 DHOH**	**S4 10.0 KHP**			**S7**	
	S2 2.0 KHP	**S5**			**S8**	**ACID**
	S3 5.0 KHP	**S6**				

VIAL/ID	AREA	VIAL	ID	AREA	VIAL	ID	AREA
1 COND		27			53		
2 COND		28			54		
3 COND		29			55		
4 DHOH		30			56		
5 DHOH		31			57		
6 0.5 KHP		32			58		
7 1.0 KHP		33			59		
8 2.0 KHP		34			60		
9 5.0 KHP		35			61		
10 7.5 KHP		36			62		
11 10.0 KHP		37			63		
12 DHOH		38			64		
13 DHOH		39			65		
14		40			66		
15		41			67		
16		42			68		
17		43	DHOH		69		
18		44			70		
19		45			71		
20		46			72		
21		47			73		
22		48			74		
23		49			75		
24		50			76		
25		51			77		
26		52			78		

SHIMADZU DATA
SHEET CRUISE :
TODAY'S DATE:
INSTRUMENT USED:
MANUAL/PC
CONTROL ANALYST: NLK OTHER:
INJECTION VOLUME: 30 uL **FILE NAME:**
SPIKE CONC.: 20 mg/L KHP

VIAL/STD	AREA	VIAL/STD	AREA		VIAL/STD	AREA
cond std	**S1 DHOH**	**S4 20.0 KHP**			**S7**	
	S2 5.0 KHP	**S5**			**S8**	**ACID**
	S3 10.0 KHP	**S6**				

VIAL/ID	AREA	VIAL	ID	AREA	VIAL	ID	AREA
1 COND		27			53		
2 COND		28			54		
3 COND		29			55		
4 DHOH		30			56		
5 DHOH		31			57		
6 1.0 KHP		32			58		
7 2.0 KHP		33			59		
8 5.0 KHP		34			60		
9 10.0 KHP		35			61		
10 15.0 KHP		36			62		
11 20.0 KHP		37			63		
12 DHOH		38			64		
13 DHOH		39			65		
14		40			66		
15		41			67		
16		42			68		
17		43	DHOH		69		
18		44			70		
19		45			71		
20		46			72		
21		47			73		
22		48			74		
23		49			75		
24		50			76		
25		51			77		
26		52			78		

12. Maintenance Schedule for the TOC5000/5000A

12.1 **Daily**:

12.1.1 Check liquid level in humidifier. Add DHOH to top line if level is too low. Keep level between top and bottom scored lines on vessel.

12.1.2 Check paper supply if using either instrument in Stand Alone Mode.

12.1.3 Make sure that the liquid in the IC pot is bubbling once the furnace is on. Lack of bubbling means a leak is present.

12.1.4 Main gas pressure setting @ 4.5 kg/cm^2.

12.1.5 Carrier gas setting @ 150 cc.

12.1.6 Sparge gas setting @ ~30-60 cc when in use.

12.1.7 Check the level of the rinse container.

12.1.8 Carrier gas pressure. Use Ultra Zero grade Air from Airgas or comparable grade. UZ Air is a synthetic blend containing 20-22% oxygen, < 1 ppm CO + CO_2 combined, < 2% H_2O, < 0.1% THC.

12.2 **Monthly**, approximately or after 15-18 analytical batches:

12.2.1 Consumables parts list:

12.2.1.1 PN 017-42801-01 TC catalyst, regular sensitivity

12.2.1.2 PN 036-11209-84 Black o-rings, injection port; 5/pk

12.2.1.3 PN 036-11408-84 Teflon o-rings (white), 5/pk

12.2.1.4 PN 630-01565-00 injection port needle

12.2.1.5 PN 638-41323-00 TC combustion tube

12.2.1.6 PN 220-91101-00 syringe plunger w/tip

12.2.1.7 PN 630-00105-01 platinum screens, 2/pk

12.2.1.8 PN 630-02674-01 mist trap filter ball

12.2.1.9 PN 035-62994-03 Teflon ferrules, TOC5000 only

12.2.1.10 PN 630-00707-00 cooling coil, TOC5000 only

12.2.1.11 PN 200-91532-02 printer paper

12.2.1.12 PN 638-41314-00 cooling coil, TOC5000A only

12.2.1.13 PN 638-41284-00 ASI sampling needles

12.2.1.14 PN 630-01566-00 Teflon coated o-ring

12.2.1.15 PN 630-00635-01 KHP, primary std

12.2.1.16 PN 630-00962-01 Na_2CO_3, primary std

12.2.1.17 PN 630-00963-01 NaHCO3, primary std

12.2.1.18 JT Baker PN 1820-01 Cupric Oxide Wire, Baker Analyzed, A.C.S. Reagent grade or equivalent.

12.2.2 Make sure oven is off and cooled to room temperature.

12.2.3 Remove the old column by unscrewing the two side screws on the mounting plate.

12.2.4 Remove the injection port slide, and the injection block.

12.2.5 Release the TC gas line from the side of the block.

12.2.6 Remove the syringe from under the 4 port valve.

12.2.7 Rinse the syringe and replace the old plunger and tip with a new plunger and tip.

12.2.8 Remove the old mist trap filter ball and replace with a new filter, taking care not to touch with bare fingers.

12.2.9 Remove the ultra pure water trap, rinse well, and return. There is no need to fill with water.

12.2.10 The catalyst and the cupric oxide may be used straight from the bottle, but it is recommended that each be pre-combusted between 680° and 850° C for 90 minutes. This reduces the time needed to condition the column. Left over pre-combusted catalyst and cupric oxide may be stored in a desiccator.

12.2.11 Place 2 platinum screens in the bottom of the column. Cover with a very thin layer of quartz wool. Note: pressure problems may arise if the quartz wool is too thick.

12.2.12 Pour 120 mm of catalyst into the tube. Top with 2-4 mm of cupric oxide. Note: The CuO is used to prevent back splashing of the vaporized sample, which causes doublet peaks.

12.2.13 Smear a thin layer of high vacuum silicone grease 1-2 mm below the top of the column. Set aside.

12.2.14 Remove the old orings from the top of the injection block. Rinse the block. Put a thin layer of silicone grease on the new black oring and put into place. Lay a new (white) Teflon oring on top. Do not grease. Note: the Teflon coated oring on the underside of the injection block needs to be replaced twice yearly.

12.2.15 Remove the injection needle from the injection slide. Rinse the slide, Replace with a new needle (remove the wire from inside the new needle). Adjust the needle so that only a millimeter or so is showing through the slide, and then tighten the knurled nut. The tip of the needle should not be visible when holding the slide on a horizontal plane. It will score the Teflon oring if it is out too far.

12.2.16 Slide the air tubing back onto the needle.

12.2.17 Insert the column into the bottom of the injection block. Place into the furnace opening, making sure that the drain

tube is properly aligned. Insert into the cooling coil and hand tighten. Note: the TOC5000 requires new Teflon ferrules at this point.

12.2.18 Adjust the column height with mounting plate screws. On the 5000, allow enough space to slide a folded piece of paper underneath the coil. On the 5000A, adjust the height by the bottom platform.

12.2.19 Return the TC gas line to its proper position.

12.2.20 Secure the injection port slide.

12.2.21 Return the syringe to its proper position.

12.2.22 Turn gas and instrument on. Liquid in the IC block should bubble. If not, check and tighten everything that was loosened.

12.2.23 Turn the furnace on. Refer to Section 11.1 for TOC5000, using Curve #1, and Section 11.2 for TOC5000A instrument instructions, using the file COLCOND.CAL and COLCOND.MET.

12.2.24 Fill auto-sampler tubes 1-78 with ASTM Type I water, and acidify with 100 µl 2N HCl. Acidify the water in position S1 standard cup with 300 µl 2N HCl.

12.2.25 Pull up the maintenance screen and do a Zero Point Detection.

12.3 Semi-annual maintenance:

12.3.1 Replace the Teflon coated oring.

12.3.2 Replace the NaOH solution in the humidifier with a 0.3N NaOH solution: 1.2 g NaOH/100 ml H_2O.

12.4 Annual maintenance:

12.4.1 Replace the halogen scrubber and acrodisc filter.

12.4.2 Replace the soda lime scrubber.

12.4.3 Replace the 4 port valve.

12.4.4 If not used frequently, replace the IC port orings and needle. If used regularly, follow the monthly schedule.

12.4.5 Replace the cooling coil.

12.4.6 Replace the ASI needles.

12.5 Pollution Prevention and Waste Management:

12.5.1 Liquids generated by this method are safe to put down the sink.

12.5.2 Spent catalyst may be disposed of in the trash.

12.5.3 Spent CO_2 absorber (Soda Lime) must be disposed in a proper manner. It should be taken to the Storage Facility on campus to be dealt with as hazardous waste.

13.0 References:

13.1 EPA Method 415.1. Determination of Total Organic Carbon in Water using Combustion or Oxidation.

13.2 Sugimura, Y. and Y. Suzuki. 1988. A high temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar. Chem. 24:105-131.

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Chesapeake Biological Laboratory
University of Maryland Center for Environmental Science
Nutrient Analytical Services Laboratory

Fluorometric Determination of Chlorophyll α in waters and sediments of Fresh/Estuarine/Coastal Areas.

1. SCOPE and APPLICATION

1.1 This is an acetone extraction method to determine chlorophyll α in water and sediments.
1.2 A Method Detection Limit (MDL) of 0.90 µg/L total chlα, 1.01 µg/L phaeophytin, 0.82 µg/L active chlα, and 1.17 µg/L non-acid chlα was determined using 3X the standard deviation of 7 replicates.
1.3 The quantitation limit for chlα is dependent upon sample volume.
1.4 This procedure should be used by analysts experienced in the theory and application of chlorophyll analysis. Three months experience with an experienced analyst, certified in the analysis using the fluorometer, is required.
1.5 This method can be used for all programs that require fluorometric analysis of chlorophyll α.
1.6 This procedure conforms to EPA Method 445.0

2. SUMMARY

2.1 Chlorophyll α is extracted from the cells using a 90% solution of acetone. The samples are refrigerated in the dark from 2 to 24 hours (over night is preferable). After the appropriate time, the samples are allowed to come to room temperature, and then centrifuged to separate the sample material from the extract. The extract is analyzed on a fluorometer. To determine phaeophytin and active chlα, the extract is then acidified using 5% HCl, and reread. The concentrations are then calculated.

3. DEFINITIONS

3.1 Acceptance Criteria – Specified limits placed on characteristics of an item, process, or service defined in a requirement document. (ASQC)
3.2 Accuracy – The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components which are due to sampling and analytical operations; a data quality indicator. (QAMS)
3.3 Aliquot – A discrete, measured, representative portion of a sample taken for analysis. (EPA QAD Glossary)
3.4 Analytical Range – The analytical range is dependent on the volume of water filtered and the volume of acetone used in the extraction.

3.5 Batch – Environmental samples, which are prepared and /or analyzed together with the same process and personnel, using the same lot(s) of reagents. A **preparation batch** is composed of one to 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An **analytical batch** is composed of prepared environmental samples (extracts, digestates, or concentrates) and/or those samples not requiring preparation, which are analyzed together as a group using the same calibration curve or factor. An analytical batch can include samples originating from various environmental matrices and can exceed 20 samples. (NELAC/EPA)

3.6 Blank- A sample that has not been exposed to the analyzed sample stream in order to monitor contamination during sampling, transport, storage or analysis. The blank is subjected to the usual analytical and measurement process to establish a zero baseline or background value and is sometimes used to adjust or correct routine analytical results. (ASQC)

3.7 Calibrate- To determine, by measurement or comparison with a standard, the correct value of each scale reading on a meter or other device, or the correct value for each setting of a control knob. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.8 Calibration – The set if operations which establish, under specified conditions, the relationship between values indicated by a measuring device. The levels of the applied calibration standard should bracket the range of planned or expected sample measurements. (NELAC)

3.9 Calibration Curve – The graphical relationship between known values, such as concentrations, or a series of calibration standards and their analytical response. (NELAC)

3.10 Calibration Method – A defined technical procedure for performing a calibration. (NELAC)

3.11 Calibration Standard – A substance or reference material used to calibrate an instrument. (QAMS)

- 3.11.1 Initial Calibration Standard (STD) – A series of standard solutions used to initially establish instrument calibration responses and develop calibration curves for individual target analytes.
- 3.11.2 Initial Calibration Verification (ICV) – An individual standard, analyzed initially, prior to any sample analysis, which verifies acceptability of the calibration curve or previously established calibration curve.
- 3.11.3 Continuing Calibration Verification (CCV) – An individual standard which is analyzed after every 10-15 field sample analysis.

3.12 Certified Reference Material – A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body. (ISO 17025

3.13 Corrective Action – Action taken to eliminate the causes of an existing nonconformity, defect or other undesirable situation in order to prevent recurrence. (ISO 8402)

3.14 Deficiency – An unauthorized deviation from acceptable procedures or practices. (ASQC)

3.15 Demonstration of Capability – A procedure to establish the ability of the analyst to generate acceptable accuracy. (NELAC)

3.16 Detection Limit – The lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated degree of confidence.

3.17 Duplicate Analysis – The analyses of measurements of the variable of interest performed identically on two sub samples (aliquots) of the same sample. The results from duplicate analyses are used to evaluate analytical or measurement precision but not the precision of sampling, preservation or storage internal to the laboratory. (EPA-QAD)

3.18 Epiphytic growth – Fouling organisms which adhere to solid surfaces in natural waters.

3.19 External Standard (ES) – A pure analyte (anacystis nidulans algae, or equivalent) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard is used to calibrate the instrument response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the unknown sample.

3.20 Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout filed and laboratory procedures. Analyses of FD1 and FD2 provide a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.21 Field Reagent Blank (FRB) – An aliquot of reagent water or other blank matrix that is places in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.22 Fluorescence – Fluorescence is a physical property of certain atoms and molecules. A fluorescent molecule has the ability to absorb light at one wavelength and almost instantly emit light at a new and usually longer wavelength. (Turner Designs TD700 manual)

3.23 Holding time – The maximum time that samples may be held prior to analysis and still be considered valid. (40 CFR Part 136) The time elapsed from the time of sampling to the time of extraction or analysis, as appropriate.

3.24 Instrument Detection Limit (IDL) – The minimum quantity of analyte of the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time absorbance line, etc.

3.25 Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.26 Laboratory Reagent Blank (LRB) – A matrix blank (i.e., 90% acetone) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the instrument.

3.27 Laboratory Control Sample (LCS) – A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes from a source independent of the calibration standard or a material containing known and verified amounts of analytes. The LCS is generally used to establish intra-laboratory or analyst-specific precision and bias or to assess the performance of all or a portion of the measurement system. (NELAC)

3.28 Limit of Detection (LOD) – The lowest concentration level that can be determined by a single analysis and with a defined level of confidence to be statistically different from a blank. (ACS)

3.29 Limit of Quantitation (LOQ) – The minimum levels, concentrations, or quantities of a target variable (target analyte) that can be reported with a specified degree of confidence. The LOQ is set at 3 to 10 times the LOD, depending on the degree of confidence desired.

3.30 Linear Dynamic Range (LDR) – The absolute quantity over which the instrument response to an analyte is linear. This specification is also referred to as the Linear Calibration Range (LCR).

3.31 Material Safety Data Sheets (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.32 May – Denotes permitted action, but not required action. (NELAC)

3.33 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 98% confidence that the analyte concentration is greater than zero.

3.34 Must – Denotes a requirement that must be met. (Random House College Dictionary)

3.35 Precision – The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves; a data quality indicator. Precision is usually expressed as standard deviation, variance or range, in either absolute or relative terms. (NELAC)

3.36 Preservation – Refrigeration, freezing, and/or reagents added at the time of sample collection (or later) to maintain the chemical and or biological integrity of the sample.

3.37 Quality Control Sample (QCS) – A sample of analytes of known and certified concentrations. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.38 Quenching – A phenomenon which occurs when the fluorescence measurements decrease even though the analyte concentration is increasing. (Turner Designs TD700 manual)

3.39 Raw fluorescence – Refers to the "relative" fluorescence of a substance being read, rather than the actual concentration. (Turner Designs TD700 manual)

3.40 Run – One sample analysis from start to finish, including printout.

3.41 Run Cycle – Typically a day of operation – the entire analytical sequence of runs from the first run to the last run and including the transfer of run cycle data to the disc.

3.42 Sample Volume – Volume of water filtered.

3.43 Sensitivity – The capability of a test method or instrument to discriminate between measurement responses representing different levels (concentrations) of a variable of interest.

3.44 Shall – Denotes a requirement that is mandatory whenever the criterion for conformance with the specification requires that there be no deviation. (ANSI)

3.45 Should – Denotes a guideline or recommendation whenever noncompliance with the specification is permissible. (ANSI)

3.46 Standard Reference Material (SRM) – Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers, or compositional standards. The materials are used as an indication of the accuracy of a specific analytical technique.

4. INTERFERENCES

4.1 Light and heat cause the chlorophyll molecule to break down. Therefore, the samples should be kept cold in the dark and care should be taken when grinding the samples so as not to overheat the sample. Chlorophyll *b* also fluoresces within an overlapping range of wavelengths, possibly leading to an overestimation of chlorophyll α.

5. SAFETY

5.1 Safety precautions must be taken when handling reagents, samples and equipment in the laboratory. Protective clothing including lab coats, safety glasses and enclosed shoes should be worn. In certain situations, it will be necessary to also use gloves and/or a face shield. If solutions come in contact with eyes, flush with water continuously for 15 minutes. If solutions come in contact with skin, wash thoroughly with soap and water. Contact Solomons Rescue Squad (911) if emergency treatment is needed and also inform the CBL Business Manager of the incident. Contact the CBL Business Manager if additional treatment is required.

5.2 The toxicity or carcinogenicity of each reagent used in this procedure may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known hazardous materials and procedures.

5.3 Do not wear jewelry when troubleshooting electrical components. Even low voltage points are dangerous and can injure if allowed to short circuit.

5.4 The following hazard classifications are listed for the chemicals used in this procedure. Detailed information is provided on Material Safety Data Sheets (MSDS).

Chemical	Health	Flammability	Reactivity	Contact	Storage
Hydrochloric Acid	3	0	2	4	White
Acetone	2	3	0	3	Red

On a scale of 0 to 4 the substance is rated on four hazard categories: health, flammability, reactivity, and contact. (0 is non-hazardous and 4 is extremely hazardous)
STORAGE
Red – Flammability Hazard: Store in a flammable liquid storage area.
Blue – Health Hazard: Store in a secure poison area.
Yellow – Reactivity Hazard: Keep separate from flammable and combustible materials.
White – Contact Hazard: Store in a corrosion-proof area.
Green – Use general chemical storage (On older labels, this category was orange).
Striped – Incompatible materials of the same color class have striped labels. These products should not be stored adjacent to substances with the same color label. Proper storage must be individually determined.

6. EQUIPMENT AND SUPPLIES

6.1 A fluorometer equipped with the proper light source and filters for chlorophyll analysis. This laboratory uses a Turner Designs TD700 fluorometer equipped with a daylight white lamp, 340-500 nm excitation filter and >665 nm emission filter, and a Turner Designs Trilogy fluorometer equipped with either the non-acid or the acid optical module.
6.2 Freezer, capable of maintaining -20° ± 5° C.
6.3 Lab ware – All reusable lab ware (glass, Teflon, plastic, etc) should be sufficiently clean for the task objectives.
6.4 A centrifuge.
6.5 A Teflon pestle for grinding, either by hand or power, and/or a sonicator.

7. REAGENTS AND STANDARDS

7.1 Purity of Water – Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Specification D 1193, Type I. Freshly prepared water should be used for making the standards intended for calibration. The detection limits of this method will be limited by the purity of the water and reagents used to make the standards.
7.2 Purity of Reagents – Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without compromising the accuracy of the determination.
7.3 Acetone ($H_2C=O=CH_2$), 90% v/v

Acetone, reagent grade	900 ml
De-ionized water	100 ml

Using a graduated cylinder, add 100 ml de-ionized water to 900 ml acetone.
7.4 Hydrochloric Acid, 5 % (v/v) –

Hydrochloric acid (HCl), concentrated,	5.0 ml
De-ionized water, q.s.	100 ml

In a 100 ml volumetric flask, add 5.0 ml of concentrated hydrochloric acid to ~60 ml of de-ionized water. Dilute to 100 ml with de-ionized water.
7.5 Blanks – A reagent blank of 90% acetone is used.
7.6 Standards – Standards used are one of the following:
7.6.1 Turner Designs Fluorometer standards, PN 10-850. These include a set of 2 ampoules, 1 high and 1 low, accompanied by certification from Turner Designs.

7.6.2 Chlorophyll α from Anacystis nidulans algae, PN C6144-1MG, ordered from Sigma. If chlorophyll from algae is not available, chlorophyll α from spinach may be substituted.

7.7 Quality Control Sample (QCS) – For this procedure, the QCS can be any certified sample which is obtained from an external source. If a certified sample is not available, then use the standard material. A solid secondary standard from Turner Designs is used.

8 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Water collected for chlα should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent.

8.2 Sediments collected for chlα should be sampled to a known depth and area, or known wet weight.

8.3 Solid matrices used to determine epiphytic deposition, should not be soluble in acetone and should be of a size to fit within a 50 ml centrifuge tube and fit below the 40 ml mark. Mylar sheets are commonly used.

8.4 Water collected for chlα should be filtered as soon as possible. If immediate filtration is not possible, the water samples should be kept on ice in the dark and filtered within 24 hours.

8.5 The sample is kept frozen at -20° C or lower. Filter pads may be stored in folded aluminum foil pouches. Sediments and solid matrix samples may be stored in 50 ml polypropylene centrifuge tubes. Do NOT use polystyrene tubes.

8.6 Frozen chlα samples should be analyzed within 4 weeks.

9 QUALITY CONTROL

9.1 The laboratory is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory instrument blanks and calibration standard material, analyzed as samples, as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data generated.

9.2 Initial Demonstration of Capability

9.2.1 The initial demonstration of capability (DOC) – is used to characterize instrument performance (MDLs) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this procedure.

9.2.2 Quality Control Sample (QCS/SRM) – When using this procedure, a quality control sample is required to be analyzed at the beginning and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 10% of the

certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with analyses.

9.2.3 Method Detection Limits (MDLs) – MDLs should be established for chlα using a low level ambient water sample. To determine the MDL values, analyze seven replicate aliquots of water. Perform all calculations defined in the procedure (Section 11) and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = S x 3

Where, S = Standard Deviation of the replicate analyses.

9.2.4 MDLs should be determined yearly.

9.3 Assessing Laboratory Performance

9.3.1 Laboratory Reagent Blank (LRB) – The laboratory must analyze at least one LRB with each batch of samples. The LRB consists of 90% acetone treated the same as the samples. LRB data are used to assess contamination from the laboratory environment.

9.3.2 Quality Control Sample (QCS)/ Standard Reference Material (SRM) – when using this procedure, a quality control sample is required to be analyzed at the beginning of the run and end of the run, to verify data quality and acceptable instrument performance. If the determined concentrations are not within ± 3σ of the certified values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with the analyses. The results of these samples shall be used to determine batch acceptance.

9.3.3 The QCS will be obtained from a source external to the laboratory and different from the source of calibration standards.

9.3.4 Continuing Calibration Verification (CCV) – The CCV is the solid secondary standard which is also the quality control sample. . If the high std is off by 2.5% or more, reset the calibration to the high solid standard reading acquired at calibration, using the calibration function. Failure to meet the criteria constitutes correcting the

problem and reanalyzing the samples. If not enough sample exists, the data must be qualified if reported.

9.4 Data Assessment and Acceptance Criteria for Quality Control Measures

9.4.1 The Acceptance Criteria for chlα is 0.9990. If the r^2 is less than acceptable, the standards must be made again.

9.5 Corrective Actions for Out of Control Data

9.5.1 If the samples do not fall within the range of the standards, they must be diluted.

10 CALIBRATION AND STANDARDIZATION

10.1 Calibration – Quarterly calibrations with standards of known concentration are performed. Checks with a solid secondary standard are made daily. If the solid standard has drifted more than 2.5 % from the original calibration, the instrument is reset to that setting.

10.2 If using Anacystis nidulans algae, the concentration must be determined by spectrophotometer.

10.3 The fluorometric standards received from Turner Designs are certified for concentration, and are ready for use on the fluorometer.

10.4 To calibrate either the TD700 or the Trilogy, refer to the operating manuals.

11 PROCEDURE

11.1 Sample Preparation – water column

11.1.1 Filter a known volume of water through a Whatman GF/F filter pad (nominal pore size 0.7 μm). Only a faint color is needed on the pad. Do not rinse the pad.

11.1.2 Fold pad in half, sample inside, wrap in aluminum foil, label and freeze for analysis within 4 weeks.

11.1.3 Before analysis, briefly thaw pads, and then place in a 15 ml centrifuge tube. Add 10 ml of 90% acetone.

11.1.4 Write all information in the lab notebook.

11.1.5 Using a Teflon pestle, grind the filter against the side of the tube until the filter is well ground. If hand grinding, 10-15 seconds is all that is necessary. Power grinding requires vigilance, because excess heat will degrade the chlorophyll. Allow the sample to extract for 2 - 24 hours in the dark under refrigeration. Overnight is recommended.

11.1.6 Remove tubes from refrigerator and allow them to warm to room temperature.

11.1.7 Shake tubes, and then centrifuge at ~2300 rpm for 5-10 minutes. If a refrigerated centrifuge is used, a longer spin time may be used, but not necessary. Again, allow the samples to come to room temperature. Pipette liquid into a 13 mm round cuvette for reading on the TD700 fluorometer or into a 2 ml vial for the Trilogy. Avoid pipetting the fines within the sample tube.

11.1.8 If phaeopigments are to be measured, add 2 drops of 5% Hydrochloric Acid and read on the fluorometer again. If using the Welschmeyer (non-acid) technique, **do not** acidify the sample.

11.2 Chlorophyll α in Sediments and Epiphytes

Along with filtered water samples, sediments and epiphyte Mylar strips are also analyzed for chlorophyll α and phaeophytin. The method is modified in the volume of extract used, and the samples are not ground.

11.2.1 Procedure - Sediments:

11.2.1.1 A known volume of sediment, usually the top 1 cm section of a core, or a known wet weight is placed in a polypropylene 50 ml centrifuge tube and frozen. The sample may be kept frozen for up to 4 weeks before analysis.

11.2.1.2 Forty milliliters of 90% acetone are delivered into the centrifuge tube. The tube is shaken and placed in a dark box and put in the refrigerator to extract for 2-24 hours. Overnight is recommended.

11.2.1.3 After extraction, weigh the tubes to balance the centrifuge. Spin for 5-10 minutes @ ~2300 rpm. Note: If using a refrigerated centrifuge, spin time may be longer.

11.2.1.4 Allow the samples to come to room temperature before analyzing.

11.2.1.5 Pipette liquid into a cuvette for analysis on the fluorometer, being careful not to aspirate any particles. Dilute the sample as needed. Take initial reading. Add 2 drops of 5% HCl to the cuvette and read again to determine phaeopigments and corrected chlα.

11.2.2 Epiphyte Mylar strips – Mylar strips are deployed in the water column for a set length of time to allow epiphytic algae to attach and grow.

11.2.2.1 Cut Mylar strips short enough to stay below the 40-ml mark on a plastic 50-ml centrifuge tube. Freeze until ready to analyze, up to 4 weeks.

11.2.2.2 Add 40 ml of 90% acetone, shake well, and place in a dark box in the refrigerator for 2-24 hours. Overnight is recommended.

11.2.2.3 Follow procedure for sediments after extraction. Dilute the sample as needed.

11.3 Pollution Prevention and Waste Management

11.3.1 This method generates hazardous waste.

11.3.2 Acetone waste is stored in 4 liter jugs in the cabinet under the hood and transferred to the hazardous waste area of the Storage Facility on campus.

11.3.3 Do not pour acetone down the sink.

11.3.4 Decant the waste acetone into the waste jugs, and then allow the remaining ground filter pad or sediment to dry in the hood.

11.3.5 The dried waste may then be put in the trash.

11.4 How to run the Turner Designs TD700

11.4.1 The TD700 is set up to utilize the acidification method, with the filters located in B on the filter holder. If the non-acid method is to be used, the filters are located in A on the filter holder, which must then be moved to that orientation and calibrated.

11.4.1 Turn on power and allow fluorometer to warm up for a minimum of 1 hour.

11.4.2 Take samples out of the refrigerator and allow them to come to room temperature.

11.4.3 Centrifuge samples for 5-10 minutes at ~2300 rpm.

11.4.4 Fill the cuvette at least 2/3 full (approx. 5-6 ml) with 90% acetone. Wipe the cuvette with lens paper to clean and insert the cuvette with acetone blank into sample well.

11.4.5 Press the "*" key on the keypad. This is the "discrete averaging" function allowing all samples to be analyzed for the same amount of time. Take the reading when the instrument reports END.

11.4.6 Remove the acetone blank and the cuvette adaptor. Place the solid secondary standard within the sample well. Press "*". Read both the low and the high sides. Compare the readings to the original solid standard reading during calibration. If the high std is off by 2.5% or more, reset the calibration to the high solid standard reading acquired at calibration, using the calibration function.

11.4.7 Samples may now be analyzed.

11.4.8 Optical Kit: extractive acidification method

11.4.8.1 Daylight White Lamp PN 10-045

11.4.8.2 Excitation filter 340-500 nm PN 10-050R

11.4.8.3 Emission filter >665 nm PN 10-051R

11.4.9 Optical Kit: non-acid method

11.4.8.1 Blue mercury Vapor Lamp PN 10-089

11.4.8.2 Excitation filter 436 nm PN 10-113

11.4.8.3 Emission filter 680 nm PN 10-115

11.5 How to operate the Turner Designs Trilogy

11.5.1 Connect the Trilogy to the desktop computer by the serial port cable(s).

11.5.2 Open the SIS for Trilogy software on the desktop. Click START. An EXCEL spreadsheet will open. Minimize the SIS file.

11.5.3 Turn on the instrument. The instrument has a touch screen. The non-acid optical module is in place. Touch the CHL-NA button to confirm this. (There is also available the acidification optical module (CHL-A). Change the modules to analyze samples by the acidification method. The Trilogy must be recalibrated each time an optical module is changed.)

11.5.4 The Trilogy does not need a long warm up period.

11.5.5 Confirm that the instrument and the computer are talking to each other. A line of gibberish and Turner Designs will appear in the spreadsheet.

11.5.6 Press the CALIBRATE button to select a standard curve, new or existing. Up to 5 standards may be used. Once selected, press View Curve, and then select it to record in the spreadsheet.

11.5.7 Pipette the samples into the 2 ml screw-cap vials, and cap. Wipe the vial with lens paper to insure it is dry and has no smudges. Enter the sample name by pressing the sample ID button on the upper left side of the screen.

11.5.8 A solid secondary standard is available as a check on the calibration. Take out the vial adaptor to insert the solid standard.

11.5.9 To read direct concentration, press the MEASURE FLUORESCENCE button. Enter the volume filtered and press OK. (For standards and blanks enter 1.) Enter the extract volume. (For standards and blanks enter 1.) The instrument will then confirm the choices and begin the analysis. This is the direct concentration mode, so only dilutions need to be factored into the extract volume.

11.5.10 When all samples are run, SAVE the file to the desktop in the Trilogy chla folder. Do not close the SIS software until the EXCEL spreadsheet has been saved.

11.5.11 It is now safe to close the SIS software. It will also close the spreadsheet.

11.5.12 Turn off the instrument.

11.6 Calculations for the Turner Designs TD700

11.6.1 Filtered water samples:

11.6.1.1 Total chla:

$(R_B * F_S * 10 * dil.)/vol.\ filtered\ (ml) = conc.\ \mu g/L$

11.6.1.2 Active chla:

$(R_B - R_A) * F_S * (r/(r-1)) * 10 * dil.)/vol.\ filtered\ (ml) = \mu g/L$

11.6.1.3 Phaeophytin:

$((R_A * r) - R_B) * F_S * (r/(r-1)) * 10 * dil.)/vol.\ filtered\ (ml) = \mu g/L$

Where: R_B = Reading before adding acid
R_A = Reading after adding acid
10 = extract volume in ml
r = acid ratio: R_B/R_A of calibrating std
F_S = calibrating std conc. / reading of std

11.6.2 Sediments:

11.6.2.1 Total chlα:

$$\frac{R_B \times F_s \times 40 \times dil.}{area.of.core \times 1000} = mg/m^2$$

11.6.2.2 Active or corrected chlα:

$$\frac{(R_B - R_A) \times F_s \times (\frac{r}{r-1}) \times 40 \times dil.}{area.of.core \times 1000} = mg/m^2$$

11.6.2.3 Phaeopigments:

$$\frac{((R_A \times r) - R_B) \times F_s \times (\frac{r}{r-1}) \times 40 \times dil.}{area.of.core \times 1000} = mg/m^2$$

11.6.2.4 To determine mg/g total sample:

$$\frac{R_B \times F_S \times 40 \times dil. \times \frac{total.wt(g)}{extract.wt(g)}}{\frac{extract.wt(g)}{1000}} = mg / g$$

11.6.3 Epiphyte Mylar strips:

11.6.3.1 Total chlα:

$$\frac{R_B \times F_S \times 40 \times dil.}{1000} = \mu g / strip$$

By dividing by the area of the strip, the units then become μg/m^2.

Where: R_B = reading before adding acid
R_A = reading after adding acid
40 = extract volume in milliliters
r = acid ratio: R_B/R_A
F_S = calibrating std/reading of std

12 References:

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12.3 EPA Method 445.0: In Vitro Determination of Chlorophyll a and Phaeophytin a in Marine and Freshwater Algae by Fluorescence.

12.4 Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnol. Oceanogr., 39: 1985-1992.