Test Plan for the Performance Evaluation of the Severn Trent De Nora BalPure™ BP-2000 Ballast Water Management System

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1. MERC 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 following protocols describe how MERC will evaluate the performance characteristics of the Severn Trent De Nora (STDN) BalPure™ BP-2000 Ballast Water Treatment 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 does 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 Advisory Board and provided to STDN and the MERC 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/University of Maryland Center for Environmental Science.

2. Treatment to be Evaluated

The STDN Ballast Water Treatment System (BWTS) applies established chlorination technology to oxidize and disinfect aquatic invasive species (AIS). The BALPURE® BWTS ia an electrolytic process for the on-site generation of dilute hypochlorite on demand without storage during the ballasting operation and the neutralization of residual oxidants during the de-ballasting operation. Sodium hypochlorite is generated by means of electrolysis of seawater. In this process, seawater flows through the electrolytic cells in a ratio of 1 part for every 100 parts of ballast water. The system can operate effectively in brackish water to 15 PSU. Because the amount of seawater required to effectively treat ballast water is small even fresh water ballast can be treated by BALPURE® BWT with supplemental salt or stored seawater in designated ballast tanks such as aft peak tanks. The current passing through the seawater causes the salt (NaCl) and water (H_2O) to form sodium hypochlorite (NaOCl) and hydrogen (H_2) as a secondary by-product. Hypochlorite solution and Hydrogen are produced and separated immediately upon exit from the electrolytic cells. The weak hypochlorite solution (1 g/L) is injected back into the ballast stream. Hydrogen that is separated from the hypochlorite is immediately diluted to less than 1% hydrogen by forced air blowers and discharged to a safe location. The hypochlorite is generated automatically on demand and is matched to the ballast flow rate and the oxidant demand of the ballast water. Initial oxidant concentration is dosed at 1.5 times the TOC concentration which remains effective for several days. To minimize potential regrowth of (micro) organisms a background level of chlorine is maintained (1 mg/L) until discharge. This will affect toxicity and by-product concentrations at compulsory sampling at day 1 and day 3 of the tests. To reduce potential toxic effects of the chlorine at discharge sodium sulfite or bisulfite is added prior to discharge. Sulfite when reacted forms sulfate which is present in seawater at concentrations 4,000 times stronger than the treated ballast water.

For this land based test, the water is filtered through a 40 micron BallastSafe™ BSFc Automatic Electric Filter, Model BSFc-H-1.6 prior to treatment. The sintered stainless steel screen technology enables it to remove zooplankton. BallastSafe's filter features continuous cleaning of large volumes of dirt during ballasting without interruption, and a reversible screw system for smooth, reliable and rapid cleaning of the entire screen surface.

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 is to conduct independent, scientifically-sound, rigorous, and quality assured evaluations of ballast water treatment systems. Therefore, MERC relies on challenging ambient conditions found in the Chesapeake Bay, and does 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 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 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).

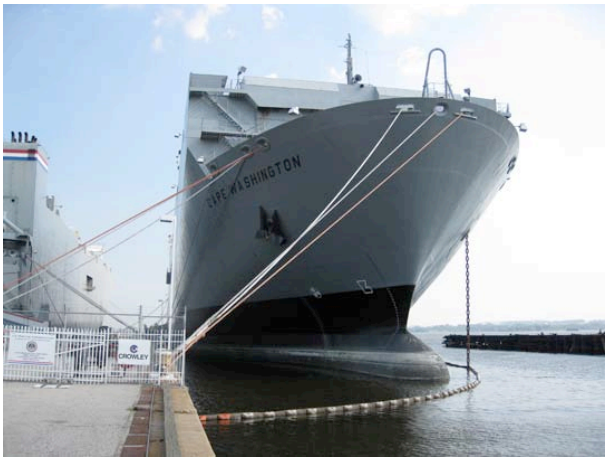
Parameter	Proposed ETV/USCG†	Recommended IMO G8‡	Historic Ranges* Port of Baltimore
Temperature (^{o}C)	10 - 35	–	4 - 28
Salinity (psu)	0 - 31	Two salinities, >10 psu difference	5 - 15
Total Suspended Solids (mg/l)	> 15	> 50	1 - 60
Particulate Organic Carbon (mg/l)	> 1	> 5	0.5 - 6.0
Dissolved Organic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

† 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.

For this specific evaluation of the BalPure treatment system, Severn Trent De Nora has made a special request to MERC to augment intake water to more consistently approach the initial challenge water conditions described in the G8 guidelines during the test trials. While MERC does 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 to increase TSS, POC and DOC levels. 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 BalPure 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 BalPure 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 17.

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 BalPure 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 BalPure 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 18.

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 BalPure 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 19.

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*.

4. Test Trials

MERC will conduct a maximum of six test trials (12 total) of the BalPure 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 BalPure 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 8), 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 BalPure system may result in the termination of

testing prior to the maximum of six test trials depending on the nature of the failures. MERC Senior Management will make a final decision on early termination of the tests, in consultation with STDN 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.

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) to sample each time point and treatment type (Table 2, page 19). 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:

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 19). 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 a combination of CMFDA (5-chloromethylfluorescein diacetate) and FDA (fluorescein diacetate) as a selective live/viable indicator. Samples stained with CMFDA+FDA, 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 and FDA 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.

Quantifying Viable Indicator Pathogens:

A 1 l sample of water for each mesocosm/tub (a 5 l integrated sample) 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 19). 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 BalPure System Discharge Toxicity

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 BalPure 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 STDN.

Summaries of the proposed test methods are given in Tables 4 through 8 (page 20). 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.

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

- Test Plan for STDN BalPure finalized and Evaluation Agreements signed by August 14, 2009
- BalPure ballast water treatment system delivered to *Cape Washington* for testing by MERC Sept. 8
- BalPure system installed and operating on the *Cape Washington* by Sept. 14, 2009.
- Two BalPure calibration run completed by September 21, 2009
- MERC evaluation of the BalPure systems initiated by September 24, 2009
- MERC will complete sample analysis and compile data from the evolution by November 16, 2009
- Draft report on the performance of the BalPure system for review by the Advisory Board/Committee and STDN 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.

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.

9. Quality Assurance/Quality Control

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.

10. Roles and Responsibilities

The evaluation is coordinated and supervised by the MERC Principal Investigator, and Program Coordinator. MERC 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 of any problems encountered. Manufacturer representatives shall train MERC 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 Program Coordinator. In addition to aiding the development of these protocols, the MERC Advisory Board 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 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.
- 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.

Manufacturers shall:

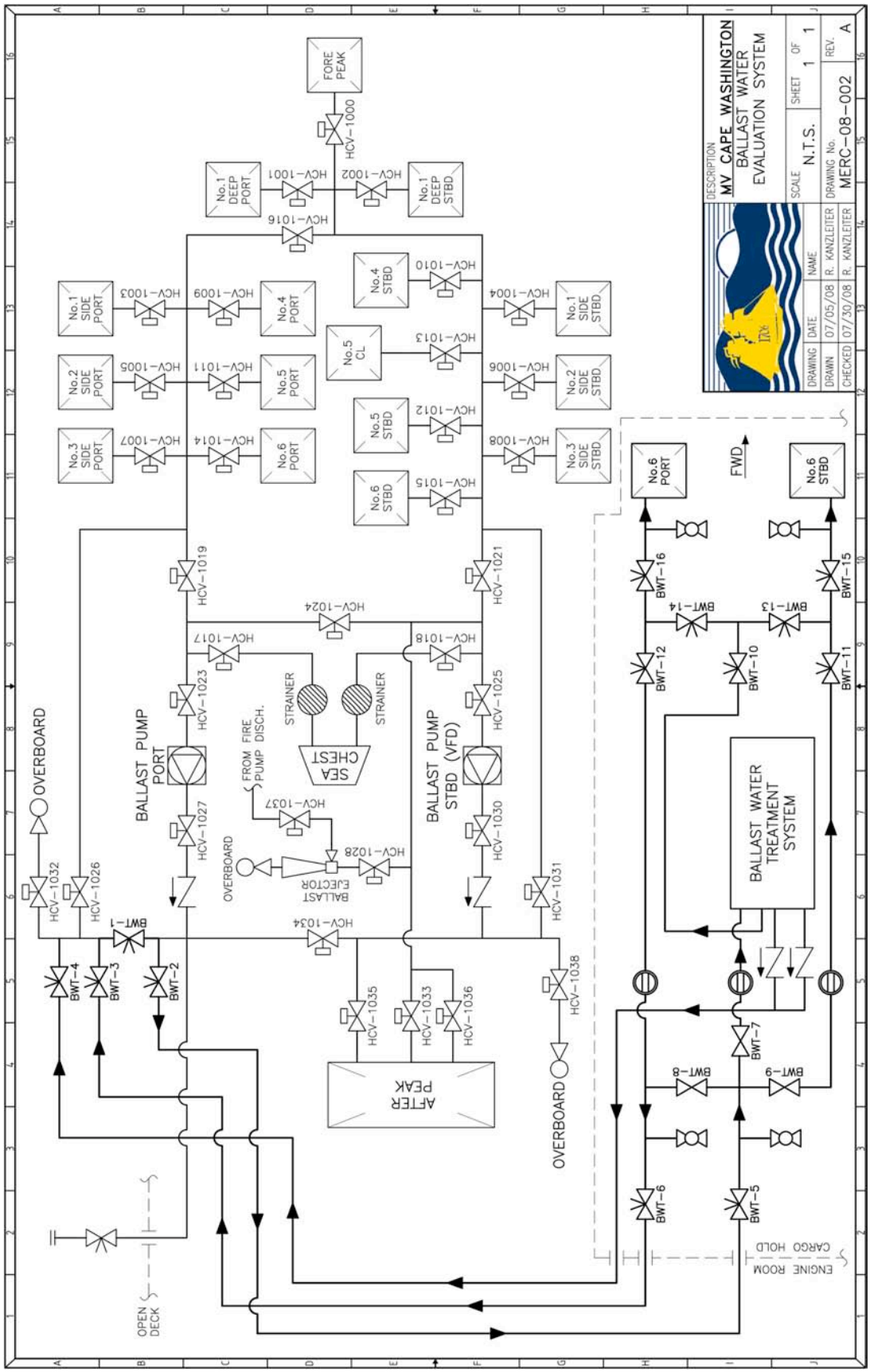
- Review the draft test/QA plan and provide comments and recommendations.
- Work with MERC to commit to a specific schedule for testing.
- Provide an operational treatment systems for the agreed upon test site.
- Oversee and cover the costs of treatment system installation on the MERC *Cape Washington* test platform.
- Aid in calibration and operation of treatment system for testing. Primary operating responsibility.
- Review and comment on draft Performance Report.

Advisory Board/Committee* shall:

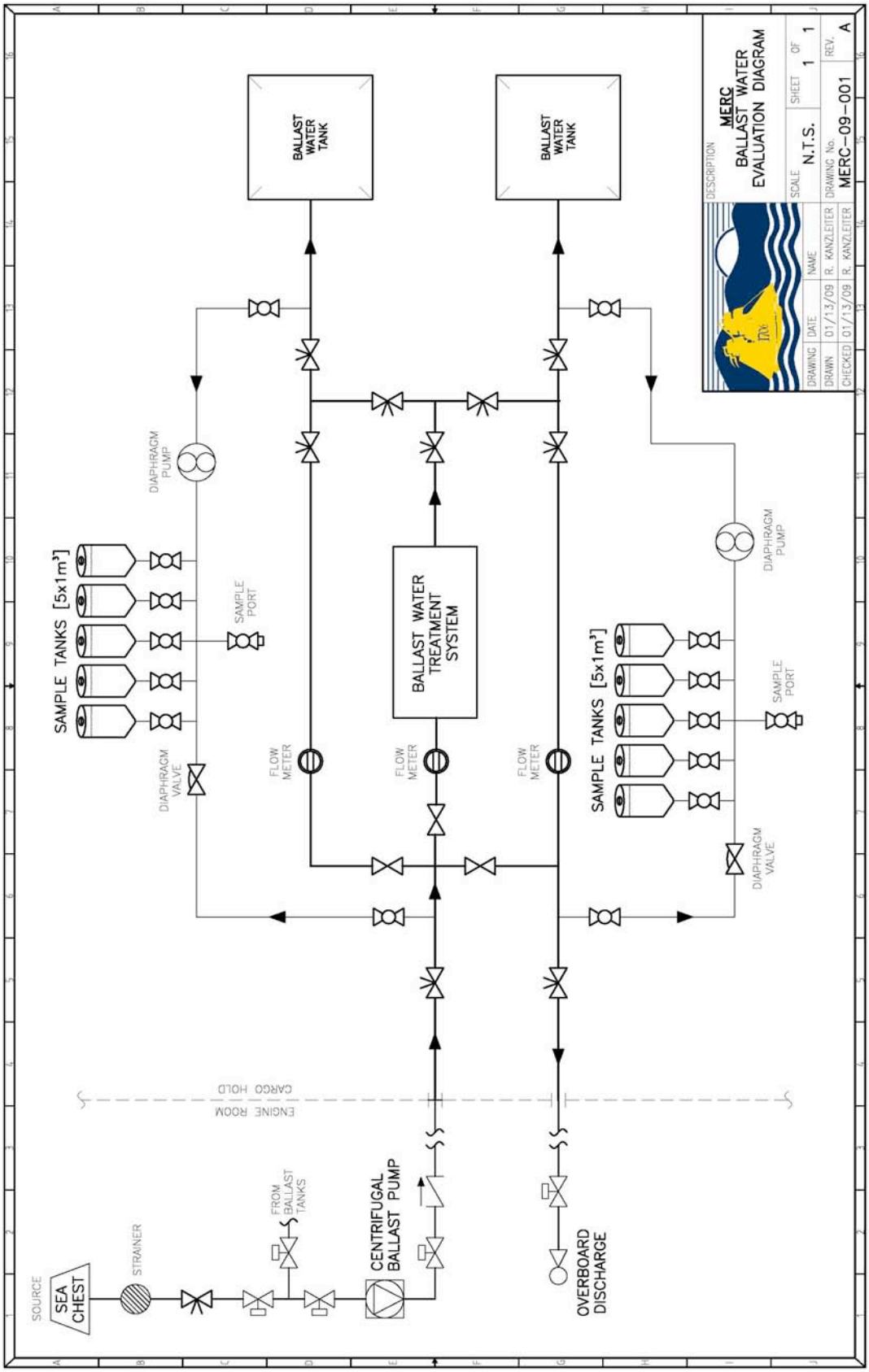
- Assist in developing the Test Protocols/QA Plan.
- Approve the final Test Protocols/QA Plan.
- Provide specific advice during testing.
- Review and comment upon draft Performance Report.

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

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



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



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

MERC 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
Total Suspended Solids (TSS)	Initial filling	Quantify challenge water	500 ml
Particulate Organic Material (POC)	Initial filling	Quantify challenge water	500 ml
Dissolved Organic Material (DOC)	Initial filling	Quantify challenge water	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
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
Bacteria cfu / ml	a. Initial filling, b. After treatment, c. Control and treatment after 5 days	Quantify microbial communities	5 l

14. 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%