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A Study of Ecotoxicity Test for Byproducts of Ozone in the Ballast Water Treatment System with Ozonation

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Abstract : *Ecological toxicity testing of the whole-effluent from the ozone ballast water treatment system was conducted as specified in the quality assurance project plans (QAPP). The growth inhibition test with microalgae, acute aquatic toxicity test with the Rotifer reproduction, toxicity test (or population growth) with the Rotifer,survival and growth toxicity test with larval fish and sediment toxicity test with amphipod were carried out to evaluate ecological toxicity on the movile test barge.*

Key words : *ozone, ballast water treatment, toxicity test, TRO, DBP*

1. Introduction

This study is being sought in accordance with the IMO Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substances (G9), as adopted by Resolution MEPC.169(57) of the IMO Marine Environment Protection Committee (MEPC) in April 2008.

The aims of this study are to address by providing data in relation to the Ozone ballast water treatment system on: TRO and disinfection by-products (DBPs) in the treated ballast water, and ecological toxicity of the treated ballast water at time zero, after two days and after five days following treatment.

2. Materials and methods

2.1 Mobile test barge

The test runs were carried out on the mobile test barge, which is fitted with an Ozone BWTS(Ballast Water Treatment System) and two simulated ballast tanks of 312.5 m^3 volume each, supplied with inlet and outlet arrangements and equipment for proper cleaning. For each test run, one tank was filled with untreated seawater as a control (C), and the other was filled with seawater treated by the Ozone BWTS, as the treatment (T). The two tanks were covered to mimic real ballast tank conditions (e.g. preventing light introduction and limiting air exchange), and the internal surfaces had protective coatings as widely applied for ships' ballast tanks.

The test runs were performed at two different sites with more than 10 ppt difference in salinity, according to the IMO G-8 guidelines. Test runs 1 and 2 for seawater were performed at the Busan Port, and Test runs 3 and 4 for brackish water were performed at the Nakdong River. An overview of the testing scheme is presented in Table 1.

During each test run samples of ballast water were taken from both the control and treatment tanks immediately following treatment, after two days storage and after five days storage. At each sampling event Ozone and TRO (Total Residual Oxidant) levels were measured immediately on site, and the samples were then sent to the DAU laboratory for TRO analysis and WET testing, as well as to the laboratory for TRO and DBPs analysis.

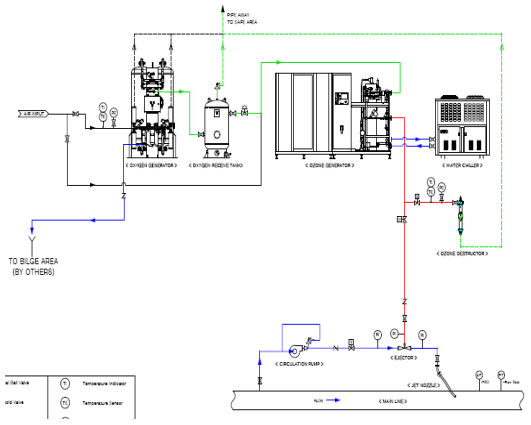

Fig. 1 Schematic of overall Layout of the Ozone BWTS

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Table 1 Overview of the supplementary TRO/DBPs analysis and WET tests carried out Sept-Nov 08.

Test run	1	2	3	4
Site	Busan Port	Busan Port	Nakdong River	Nakdong River
Samples/ Analysis	Ozone TRO DBPs	Ozone TRO DBPs WET- Microalgae WET-acute Rotifer WET-chronic Rotifer WET-acute fish WET-chronic fish WET-sediment	Ozone TRO DBPs	Ozone TRO DBPs WET-Microalgae WET-acute Rotifer WET-chronic Rotifer WET-acute fish WET-chronic fish WET-sediment

2.2 Sampling procedures

Collection of field samples from the test barge was undertaken, using standard water sample collection methods and in accordance with the G8 Guidelines. Standard operating procedures (SOPs) were employed to provide consistency and reproducibility of the sampling methods used by field personnel, as outlined in the Quality Assurance Project Plans (QAPP).

Water samples were collected at three time intervals following treatment - immediately after treatment, two days (48 hours) after treatment, and five days after treatment, and identified as T0 T2 and T5 for the treated ballast water and C0, C2 and C5 for the control (untreated) ballast water.

Water samples were taken directly into a clean bucket after washing with sample water, pre-filtered with 45 μm net to remove large particles, and aliquoted into sterile plastic bags to prevent possible contamination. The analytical laboratory provided pre-labeled sample containers which included the required preservative for each parameter.

2.3 Sample handling

The collected samples were cooled immediately in an ice-box to (<4oC) and those required for WET testing were transported to the DAU test facility by car within six hours of being collected. The samples for DBP analysis were also packed and stored in cooler and transported to the test facility.

The test facilities prepared written standard operating procedures (SOPs) for sample custody, and an example of a sample chain-of-custody form is contained in the Quality Assurance Project Plan (QAPP) for this testing.

When the samples arrived at DAU (Data Acquisition Units) and laboratory, the sample custodian received the samples, signed the form, opened the sample coolers, carefully checked the contents for evidence of leakage and to verify that samples were kept on ice, and then verified that all information on the sample container label was correct and consistent with the information on the chain-of-custody form.

2.4 Analytical methods

Analytical methods for measuring TRO in-situ (on the test barge) were as follows:

· Automatic sampling using installed TRO concentration analyzer (CL17 Chlorine Analyzer, HACH), installed separately in ballast pipe and in ballast tank.
· Manual testing of samples using Standard Method 4500-Cl G - DPD Colorimetric Method

For samples sent to DAU for WET testing, the analytical methods were as described below.

Immediately after verification of field water samples, TRO levels were measured in the first place. For the preparation of test substances, the water samples were passed through the filtering system (pore size 0.45 μm) by using peristaltic pump. After measurements of pH, temperature, salinity, and dissolved oxygen (DO), test substances were diluted with natural seawater, if necessary.

For WET testing, the test substances prepared from the treated ballast water samples (T) were subjected to the concentration-response tests, which consisted of control dilution water (0%) and a minimum of three concentrations commonly selected to approximate a geometric series, i.e. 100%, 50% and 25%.

With respect to the formulation of the dosing preparation, commercial natural sea water was used as a dilutent. For some tests, additional dosing preparations such as 75% solutions were applied. As the test substances prepared from the untreated ballast water samples (C) were likely to be nontoxic, the single concentration(undiluted, 100% solution) was subjected to the limit test.

All WET tests were initiated simultaneously within 24 hours of sample receipt. For some tests which are necessary for the renewal of test substances, samples were kept in secure storage (locked refrigerators) where they were maintained at 4 degrees Celcius.

2.4.1 Determination of TRO

TRO is the sum of ozone plus all halogen species in the

+1 oxidation state, consisting mostly of HOBr/OBr- Determination of TRO was conducted according to the DPD (N,N-diethyl-p-phenylenediamine) colorimetric method based on USEPA 330.5 (Hach method 8167). The level of TRC was measured as the equivalent of mg/L as Cl2, and the level of TRO was calculated as mg/L as Br2 (1 mol Cl2 = 0.44 mol Br2).

2.4.2 Determination of DBPs

Determination of DBPs was carried out at the labs of SGS, as specified in the Study Planand/or according to the procedures presented in the QAPP, as contained in Appendix 1. The following DBPs were tested for:

Bromate

Trihalomethanes (THMs); Trichloromethane, Dichlorobromomethane, Dibromochloromethane, Tribromomethane

Haloacetic acids (HAAs); Monochloroacetic acid (MCAA), Dichloroacetic acid (DCAA), Trichloroacetic acid (TCAA), Dibromoacetic acid (DBAA), Bromochloroacetic acid (BCAA).

2.4.3 Whole Effluent Toxicity tests

Ecotoxicity testing of the whole-effluent from the Ozone ballast water treatment system was conducted as specified in the Study Plans and QAPP, as contained in Appendix 1 of Annex 3 The following toxicity tests were carried out:

Growth Inhibition Test with Microalgae

Acute aquatic Toxicity Test with the Rotifer

Reproduction Toxicity Test (or population growth) with the Rotifer

Survival Toxicity Test with Larval Fish

Growth Toxicity Test with Larval Fish

Sediment Toxicity Test with Amphipod

3. Results and Discussion

3.1 Fulfillment of the water quality criteria

All relevant environmental water parameters are summarized in Table 2.

Test run 2 and 4 fulfilled the G8 requirements for the water quality required for land-based testing, and thus have been taken into further considerations for WET test. However, test run 1 and 3 were evaluated for the measurement of TRO and DPBs, considering only by way of suggestion.

Table 2 Chemical water quality criteria of the inlet water for the test runs 1-4

	Test run 1	Test run 2	Test run 3	Test run 4
Salinity, PSU	32.5	33.3	21.0	21.0
TSS, mg/L	2.6	4.2	25.6	51.0
DOC, mg/L	1.75	1.32	1.91	8.25
POC, mg/L	0.85	1.56	0.45	5.26

3.2 In-situ TRO results

The in-situ TRO results for treated (T0, T2, T5) ballast water samples from test runs 1 to 4 are summarized in Table 3. Results of both the manual and automatic TRO sampling are presented side-by-side, along with Ozone dosage rate, for visual comparison.

Table 3 Concentration of TRO in water samples of test run 1 to 4

Test Cycle	TRO	Unit	MDL	C0	T0	C2	T2	C5	T5
3-1	Manual (Automatic)	mg/L	0.045 (0.08)	ND	4.19 (4.23)	ND	0.18 (0.20)	ND	ND (ND)
1-7	Manual (Automatic)	mg/L	0.045 (0.08)	ND	4.01 (4.01)	ND	ND (ND)	ND	ND (ND)
3-6	Manual (Automatic)	mg/L	0.045 (0.08)	ND	3.04 (2.98)	ND	ND (ND)	ND	ND (ND)
2-7	Manual (Automatic)	mg/L	0.045 (0.08)	ND	2.50 (2.54)	ND	NS (ND)	ND	ND (ND)

ND : Not detected.

The DBP results for untreated (C0, C2, C5) and treated (T0, T2, T5) ballast water samples from test runs 1 to 4 are summarized in Table 3

In seawater where there is a significant concentration of bromide ion, ozone is catalytically destroyed with a half-life of five seconds. As found in the earlier testing conducted to support the original application or Final Approval, there was no ozone observed in any of the ballast water samples that were analyzed. Therefore, ozone per se can be considered a good oxidant for the disinfection of marine ballast water because it is not chemically persistent.

The oxidation of the bromide ion by chlorine (hypochlorous acid/hypochlorite ion, HOCl/OCl-) results in the formation of oxidized bromide ion (bromine). For ozonated seawater, bromine is the residual oxidant most likely to exist for any extended period of time, in concentrations potentially harmful to marine organisms. Bromine rapidly forms hypobromous acid (HOBr), which is in equilibrium with hypobromite ion (OBr-) with a pK of 8.8. The ozonation of hypobromite ion results in bromate ion, and the reaction of this oxidized form of bromine with

naturally occurring organic matter results in the formation of HAAs and THMs including bromoform. No oxidized forms of chlorine are possible under ozonation treatment conditions.

The results from the testing as presented above indicate that bromate ion was always below the method detection limit in all samples, suggesting that the lower pH of the coastal water favored the formation of HOBr that does not react with ozone to form bromate ion. From the tested compounds of HAAs and THMs, only slightly increased amounts of Monochloroacetic acid, Dichloroacetic acid, Trichloroacetic acid, Dibromoacetic acid, and Tribromomethane could be detected in the ballast water treated with the Ozone BWTS. However, the limited toxicity data available from the literature suggests that these compounds are not acutely toxic with LC50 values 1 -2 orders of magnitude higher than oxidized bromide ion.

Even in the case of bromoform, which was the chemical detected at the highest level, the most sensitive species is the sheepshead minnow with 96-hours LC50 values ranging from 7.1 - 18 mg/L. Therefore, even if some compounds were produced as by-products of seawater ozonation, these values are potencies below recent limit values in drinking water and did not approach that, which would result in any toxicity to the receiving waters.

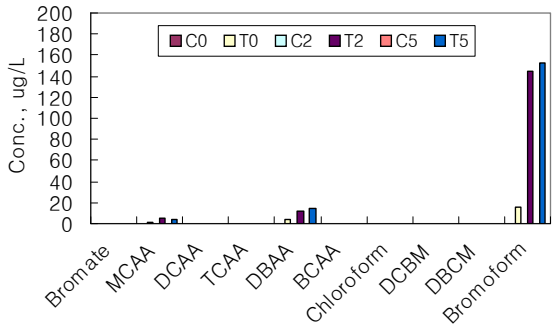

Fig. 2 Concentration of DBPs in water samples of test run 1(high salinity)

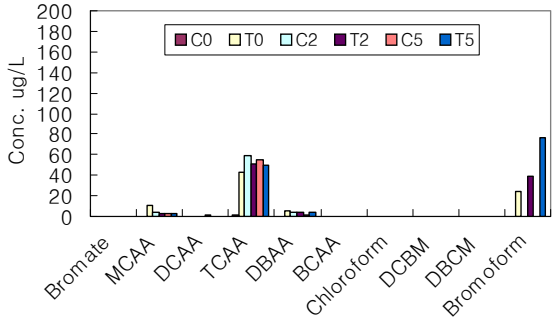

Fig. 3 Concentration of DBPs in water samples of test run 2 (high salinity)

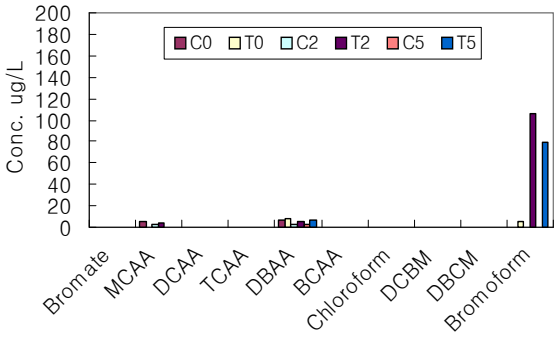

Fig. 4 Concentration of DBPs in water samples of test run 3 (low salinity)

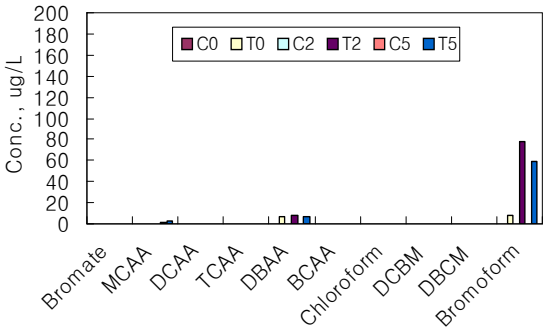

Fig. 5 Concentration of DBPs in water samples of test run 4 (low salinity)

3.3 TRO in WET test substances

The first measurement of TRO levels was performed on the test barge, immediately following sampling, and results are presented in Table 4 above. TRO levels in the WET samples were again measured immediately after verification of field water samples which were delivered to the DAU laboratory. The elapsed time from the completion of ozonation was about 5 hours. As soon as the test substances for WET tests were prepared and diluted, the second measurement of TRO level was conducted shortly prior to the beginning of the WET test. Results are summarized in Table 4.

As shown in test result, the TRO levels measured at field showed an initial increase and subsequent decrease in concentration presumably due to back reactions as the reaction time increased. These results suggest that one of the toxicologically important ozonation by-products is likely to be bromine, and bromine residual would likely be maintain or degrade slowly in ozonated ballast waters for a limited time periodand would continue to have a biocidal effect if ozonated waters remain in dark ballast tanks. However, it should be noted that the HOBr/OBr- are extremely labilein sunlight and would not persist long in

the environment, thus oxidant concentrations diminish quickly in ozonated seawater exposed to the atmosphere.

Table 4 Level of TROs in samples as tested in situ (on barge) and in laboratory

		Test run 1		Test run 2		Test run 3		Test run 4	
		lab	In situ **	lab	In situ **	lab	In situ **	lab	In situ **
T0 (%)	100	(1.521)* 0.568	2.43	(0.976)* 0.295	1.67	(0.704)* 0.227	1.17	(0.545)* 0.159	0.79
	75			0.204				0.068	
	50			0.091				< 0.045	
	25			< 0.045				< 0.045	
C0 (%)	100	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND
T2 (%)	100	(0.091)* 0.068	0.11	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND
	75			< 0.045				< 0.045	
	50			< 0.045				< 0.045	
	25			< 0.045				< 0.045	
C2 (%)	100	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND
T5 (%)	100	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND
	75			< 0.045				< 0.045	
	50			< 0.045				< 0.045	
	25			< 0.045				< 0.045	
C5 (%)	100	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND	(< 0.045)* < 0.045	ND
Dilution Water		(< 0.045)* < 0.045	-	(< 0.045)* < 0.045	-	(< 0.045)* < 0.045	-	(< 0.045)* < 0.045	-

* These values of TROs were measured from the water samples, which were delivered at laboratory 4 hours after sampling (5 hours after completion of ozonation), in advance of the filtering (pore size 0.45 μm) preparation of test substances.

** These values of TROs were measured from the in-situ water samples at 5 hours after completion of ozonation.

The test results indicate that the laboratory TRO level immediately on delivery was lower than the corresponding field level at 5 hours after completion of ozonation. Furthermore, the laboratory TRO level after filtration was dramatically reduced. These results suggest that ozone-produced oxidants may dissipate very quickly from samples that are exposed to sunlight and atmosphere and bubbled with ozone-free ambient air. Therefore, investigators should consider the implications of the loss of oxidants during sampling process and test substance preparation for WET tests in estimating overall or residual toxicity of TRO in ozonated seawater.

To address any possibility of residual TRO and DBP levels of concern in the treated effluent, it will be opted to include a TRO neutralizer as standard in the Ozone BWTS design.

3.4 WET tests

As would be expected, the untreated samples (C0, C2, and C5) exhibited no to minimal toxicity (i.e. less than 10% mortality) in any of the tests. The median lethal concentrations (LC50) were greater than 100% solution, meaning that the untreated seawater showed no toxicity. For the treated samples (T0, T2 and T5), In summary, only the test substances prepared from water samples immediately after treatment of the Ozone ballast water treatment system (T0) showed residual toxicity, of which the data are summarized as shown in Table 5 and 6. No toxicity and no detected TRO were observed in the treated water samples stored for 2 days (T2) and 5 days (T5).

Table 5 Summary of aquatic toxicity data of T0 water samples from Test Run 2 (high salinity)

	Species	NOEC	LOEC	EC50	Notes
Micro algae	*Phaeodactylum tricornutum*	50%	75%	83%	Growth rate, 96 h
	Dunaliella tertiolecta	50%	75%	81%	Growth rate, 96 h
Rotifer	*Brachionus plicatilis*	> 100%	> 100%	> 100%	Survival, 24 h
	Brachionus plicatilis	25%	50%	> 100%	Growth rate, 96 h
Fish	*Cyprinodon variegatus*	> 100%	> 100%	> 100%	Survival, 96 h
	Cyprinodon variegatus	> 100%	> 100%	> 100%	Growth weight, 7 d
Amphipod	*Monocorophium acherusicum*	> 100%	> 100%	> 100%	Survival, 10 d

Table 6 Summary of aquatic toxicity data of T0 water samples from Test Run 4 (medium salinity)

	Species	NOEC	LOEC	EC50	Notes
Micro algae	*Phaeodactylum tricornutum*	50%	75%	> 100%	Growth rate, 96 h
	Dunaliella tertiolecta	50%	75%	> 100%	Growth rate, 96 h
Rotifer	*Brachionus plicatilis*	> 100%	> 100%	> 100%	Survival, 24 h
	Brachionus plicatilis	> 100%	> 100%	> 100%	Growth rate, 96 h
Fish	*Cyprinodon variegatus*	> 100%	> 100%	> 100%	Survival, 96 h
	Cyprinodon variegatus	> 100%	> 100%	> 100%	Growth weight, 7 d
Amphipod	*Monocorophium acherusicum*	> 100%	> 100%	> 100%	Survival, 10 d

The results of the WET tests using test substances of T0 from test run 2 (high salinity seawater) with microalgae indicated that ozonation byproducts caused toxicity against *Phaeodactylum tricornutum* at dilutions of 75% (ED50:

83%). All organisms died when exposed to 100% (non-diluted) test solution. In treatments where 100% mortality occurred, the TRO was 0.295 mg/L. Partial growth inhibition (45%) was seen in 100% test solution of T0 from test run 4 (medium salinity brackish water) at concentration 0.159mg/L of TRO. Microalgae, *Dunaliella tertiolecta*, showed similar growth inhibition to high salinity seawater, but were less sensitive (23% growth inhibition) to medium salinity brackish water.

The study sought to evaluate the effects of exposing Rotifer, Brachionus plicatilis to ozone and/or residual oxidants associated with the use of ozone in seawater indicated thatsurvival of the rotifers was affected significantly only when TRO values were higher than 0.22 mg/L, which would be considered the no observable effect concentration (NOEC). In our all acute toxicity tests with Rotifer, Brachionus plicatilis, no mortality was observed when TRO measurements were less than 0.295 mg/L. However, the growth rate of rotifers resulted from reproduction toxicity test was slightly reduced by exposing them to 100% test solution of T0 (20% for high salinity seawater and 12% for medium salinity brackish water).

Various reports confirm that seawater ozonation can induce rapid mortality to marine organisms, but comparisons of specific effect concentrations were complicated due to the variety of exposure times and oxidant measurement methods used in each study. A recent study was reported to determine the toxicity of ozone in artificial seawater for five species of marine organisms in short-term batch exposures. Larval topsmelt (*Atherinops affinis*) and juvenile sheepshead minnows (*Cyprinodon variegatus)* were the most sensitive to oxidant exposure, and the mysid shrimp (*Americamysis bahia*) was the most sensitive invertebrate. Conversely, benthic amphipods (*Leptocheirus plumulosus* and *Rhepoxinius abronius*) were the least sensitive of all species tested. LC50 values of the most sensitive organism tested, juvenile topsmelt was 0.38 and 0.31 mg/L TRO after only 1 and 2 h of ozone exposure, respectively. Juvenile sheepshead minnows were nearly as sensitive (0.35 mg/L TRO). In our WET tests, however, no mortality of Cyprinodon variegates and Monocorophium acherusicum occurredin any test substance of ozone exposed water samples where maximumTRO concentration was 0.295 mg/L. The lower sensitivity of sheepshead minnows to ozone-produced oxidant might be due to the difference of experimental conditions between the laboratory batch ozonation test with artificial seawater and larger-scale land-based testwith natural sea- or brackish water.

4. Conclusion

This testing indicates that the maximum TRO levels in effluent from the ozone ballast water treatment system(the Ozone BWTS), under the conditions tested, will be 4.23 mg/L as Br2 at T0, 0.18 mg/L at T2 and 0.03 mg/L at T5. Similarly, this supplementary testing indicates that the maximum bromoform levels in effluent from the Ozone BWTS will be 0.024 mg/L at T0, 0.145 mg/L at T2 and 0.152 mg/L at T5

Although the results of WET tests indicate that the latent toxicity is not expected in the discharged ballast water of the Ozone ballast water treatment system after 2 days storage, field TRO data from the test barge and from the literature suggest that some ozone-produced oxidants responsible for marine organism mortality may persist at toxic concentrations in ballast waters 1 - 2 days following ozonation depending on storage conditions and exposure to sunlight.

Risk-based decisions need to be made to set maximum acceptable discharge concentrations and conditions (potentially taking into account discharge rates and dilution or degradation following discharge into receiving waters). Chemical treatments, such as sodium thiosulfate, may provide a fast and effective means of reducing TRO concentrations without the risk of endangering organisms in the vicinity of the vessel undergoing ozonation.

Based on this supplementary testing of TRO, DBP and whole effluent toxicity at T0, T2 and T5, we conclude that the Ozone ballast water treatment system should be fitted with a TRO neutralizer that uses sodium thiosulfate to remove and neutralize any residual TRO prior to discharge of the treated ballast water.

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