Ecotoxicological effects of ballast water effluent teated by an electrolytic method on marine environment

Tae Won Kim[1] · Keun-Yong Kim[2] · Myung-Baek Shon[3] · Young-Soo Kim[4] · Ji Hyun Lee[5] · Chang Ho Moon[†] · Min Ho Son[6]

Abstract: Ballast water effluent treated by an electrolytic method contains reactive chlorine species and disinfection by-products (DBPs). In this study, we conducted whole effluent toxicity (WET) testing and ecological risk assessment (ERA) to investigate its ecotoxicological effects on marine environment. WET testing was carried out for three marine pelagic organisms, i.e., diatom Skeletonema costatum, rotifer Brachionus plicatilis and fish Paralichthys olivaceus. The biological toxicity test revealed that S. costatum was the only organism that showed apparent toxicity to the effluent; it showed no observed effect concentration (NOEC), lowest observable effect concentration (LOEC) and effect concentration of 50% (EC50) values of 12.5%, 25.0% and 83.3%, respectively, at brackish water condition. In contrast, it showed insignificant toxicity at seawater condition. B. plicatilis and P. olivaceus also showed no toxicities to the effluent at the both salinity conditions. Meanwhile, chemical analysis revealed that the ballast water effluent contained total residual oxidants (TROs) below 0.03 µg/L and a total of 20 DBPs including bromate, volatile halogenated organic compounds (VOCs), halogenated acetonitriles (HANs), halogenated acetic acids (HAAs) and chloropicrin. Based on ERA, the 20 DBPs were not considered to have persistency, bioaccumulation and toxicity (PBT) properties. Except monobromoacetic acid, the ratio of predicted environmental concentration (PEC) to predicted no effect concentration (PNEC) of the other 19 DBPs did not exceed 1. Thus, our results of WET testing and ERA indicated that the ballast water effluent treated by electrolysis and subsequently neutralization was considered to have no adverse impacts on marine environment.

Keywords: Ballast water effluent, Ecological risk assessment, Electrolytic method, Disinfection by-products, Reactive chlorine species, Whole effluent toxicity testing

1. Introduction

During last decades, transportation by transoceanic ships has given rise to many ecological, economic and even human health issues at global scale, because their ballast water helps long distance dispersals of invasive species that might exert potentially harmful effects in new foreign environments [1]-[5]. Diplomatic Conference at International Maritime Organization (IMO) in London adopted the International Convention for Control and Management of Ship's Ballast Water and Sediments in 2004 in order to regulate and ultimately eradicate transportations of non-indigenous species via ship's ballast water among countries. The convention obligates installation and operation of a ballast water management system (BWMS). Many BWMSs have thereafter been developed worldwide to disinfect non-indigenous organisms during ballasting and deballasting processes. IMO requires vendors to conduct biological toxicity test as well as efficacy test of BWMSs before applying for basic and final approvals [4][6][7].

Until now ballast water technologies such as physical (e.g., filtration, cavitation, etc.), mechanical (e.g., heating, UV radiation, etc.) or chemical (e.g., biocides, electrolysis, ozone, etc.) methods were certified by IMO and adopted for BWMSs [3][4]. In comparison to other methods, an electrolytic method has superior advantages in terms of on-site generation, cheap running cost, easy up-scaling, no potent toxicity as well as high removal efficacy [8][9]. Among fifty BWMSs that make use of active substances and received approvals from IMO, twenty four adopted

† Corresponding author (ORCID: http://orcid.org/0000-0001-7060-7260): Department of Oceanography, Pukyoung National University, Busan 608-737, Korea, E-mail: chmoon@pknu.ac.kr, Tel: +82-51-629-6573

1 Marine Eco-Technology Institute, E-mail: twkim@marine-eco.co.kr, Tel: +82-70-8630-7117
2 Marine Eco-Technology Institute, E-mail: kykim@marine-eco.co.kr, Tel: +82-70-8630-7116
3 Korean Resister, E-mail: mbshon@krs.co.kr, Tel: +82-70-8799-8269
4 Korea Marine Equipment Research Institute, E-mail: catenatum@komeri.re.kr, Tel: +82-51-400-5143
5 Korea Testing & Research Institute, E-mail: ljh@ktr.or.kr, Tel: +82-52-220-3127
6 Marine Eco-Technology Institute, E-mail: mhson@marine-eco.co.kr, Tel: +82-70-8630-7104
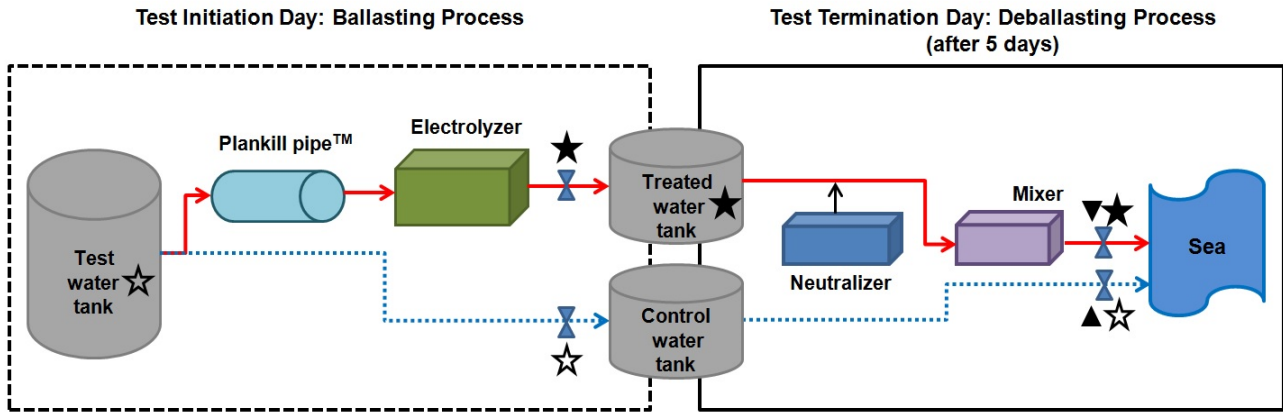
Test Initiation Day: Ballasting Process

Test Termination Day: Deballasting Process (after 5 days)

Figure 1: A layout diagram showing the ballasting and deballasting processes of a ballast water management system (BWMS) used in this study. Unbroken red and dotted blue arrow lines indicate directions of treated and control water flows, respectively. Sampling points for whole effluent toxicity testing and chemical analysis are indicated by arrowheads and stars, respectively. The three sampling points for total residual oxidant measurements are indicated by solid stars.

the electrolytic method as a core disinfection principle [10][11].

Chlorine (Cl_2) is a strong disinfection agent with high oxidizing potential because of their strong electron affinity and electronegativity. Electrolytic treatment of brine water generates chorine that forms reactive chlorine species such as sodium hypochlorite ($NaOCl$), hypochlorous acid ($HOCl$), hypochlorite ion (OCl^-), hypobromous acid ($HOBr$), hypobromite ion (OBr^-), etc. in solution [4][12]−[15]. They are extremely dangerous and poisonous for all living organisms at high enough concentrations and thus exert great efficacy in inactivating aquatic microbes [15]−[18]. Moreover, they are persistent within a ballast water tank while continuously exerting disinfecting activity during shipping time. However, the reactive chlorine species consecutively react with many aquatic inorganic and organic compounds and unavoidably form diverse disinfection by-products (DBPs). The DBPs have apparent toxic effects on aquatic organisms in the marine ecosystem as well as on human being [13][14][19][20].

In this study, we conducted biological toxicity test and chemical analysis of effluent (discharged water) from a BWMS that adopted an electrolytic method as a core disinfection principle to verify its potential ecotoxicological impacts of the ballast water effluent on marine environment. Three test organisms belonging to different taxa, i.e., Skeletonema costatum (Bacillariophyta), Brachionus plicatilis (Rotifera) and Paralichthys olivaceus (Teleostei) were used for the biological toxicity test.

2. Materials and Method

2.1 A BWMS Used and Its Operations

A BWMS, MARIOMATE™ of KT Co., Ltd in Korea used in this study adopted an electrolytic method to generate active substances to disinfect aquatic organisms. The system comprises a filtration unit (Plankill pipe™), a chemical treatment unit (Electrolyzer) and a neutralizing unit (Neutralizer) that are seriately connected (**Figure 1**).

The BWMS was operated at two conditions, i.e., the seawater condition at salinity of 34 and the brackish water condition at salinity of 20 at 24 June 2011 and 7 July 2011, respectively. During the ballasting process seawater was intaken from a fish farm nearby an intensive fish farming area in Busan, Korea. The seawater was diluted with tap water and adjusted to salinities of 34 and 20 to be used for seawater and brackish water conditions, respectively. Thirty tons of test water stored in the test water tank was equally dispensed into two separate tanks; half of the test water was sent to the control water tank without any treatment, while the other half of test water was sent to the treated water tank after being subsequently passed through Plankkill Pipe™ and Electrolyzer for physical and chemical treatments, respectively. The Electrolyzer was controlled to generate total residual oxidants (TRO) at 10 mg/L as Cl_2 equivalent. Thereafter, both control and treated water was incubated for five days, with taking shipping time into account. During the deballasting process, the treated water was subsequently passed through Neutralizer and Mixer for neutralization.

2.2 Sampling of ballast water effluent

For whole effluent toxicity (WET) testing, 800 and 400

L of ballast water effluent discharged from the control and treated water tanks, respectively, was sampled during the deballasting process (**Figure 1**; arrowheads). The effluent was carefully poured into lightproof 200 L-PE bottles and held the head space above the remaining sample to minimum. The bottles were tightly sealed and transferred to the testing laboratory by a refrigerator car within 3 h after.

For chemical analysis, each 1 L of test, control and treated water was collected at the test initiation day during the ballasting process, each 1 L of control and treated water at the test termination day during the de-ballasting process (**Figure 1**; stars).

2.3 WET Testing

2.3.1 Preparation of test solutions

Test solutions for WET testing were prepared by diluting the ballast water effluent with filtered natural seawater through a 0.2 μm pore diameter membrane filter (Whatman™, GE Healthcare Life Sciences, UK) for diatom Skeletonema costatum, a 0.45 μm pore diameter membrane filter (ADVANTEC®, Toyo Roshi Kaisha Ltd, Japan) for rotifer Brachionus plicatilis and a 1 μm pore diameter channel passing (CP) filter (Chisso Filter Co., Ltd, Japan) for fish Paralichthys olivaceus. Each toxicity test consisted of a control and five effluent concentrations (i.e., 0, 6.25, 12.5, 25.0, 50.0 and 100%) that were serially diluted with control water of each operation condition that was prepared by filtration through a 1 μm pore diameter CP filter.

2.3.2 Reference toxicant test

Reference toxicant tests before WET testing were conducted for S. costatum and B. plicatilis using a standard reference toxicant, potassium dichromate ($K_2Cr_2O_7$) (Sigma-Aldrich, USA) according to ISO 10253 [21], ASTM E 1440-91 [22] and Janssen et al. [23]. The sensitivity of those test organisms were verified with reference to ECOTOX Database of US EPA (http://cfpub.epa.gov/ecotox/), and accuracy and validity of the test methods were qualified with reference to the above guidelines.

Water temperature and salinity were measured by using WQC-22A Multiparameter Water Quality Meter (DKK-TOA Co., Japan) and light intensity by using MIC 98201 (MIC Meter Industrial Co., Taiwan).

2.3.3 WET testing of S. costatum

S. costatum was used for a growth inhibition test for 72 h inferred from specific growth rate as endpoint according to [21]. Stock culture of S. costatum has been maintained in Marine Eco-Technology Institute Co., Ltd (Busan, Korea) and subcultured at weekly interval. The growth inhibition test was conducted in triplicate in a 15 mL-test tube containing f/2 medium (Sigma-Aldrich, USA) under culture conditions of 20±1°C, 40.5 μmol photons/m^2/s and 24-h light/0-h dark photoperiod using an incubator (JSCC-150, JSR Co., Ltd, Korea). S. costatum cells were inoculated at an initial cell density of 3,000 cells/mL and counted every 24 h using a Sedgewick-Rafter chamber under a microscope (CKX 31, Olympus, Japan). The test tube was manually agitated every morning.

2.3.4 WET testing of B. plicatilis

B. plicatilis was used for acute (24 h) and chronic (96 h) toxicity tests inferred from survival rate and population growth as endpoints according to [22] and [23], respectively. B. plicatilis cysts were purchased from MicroBioTests Inc. (Gent, Belgium) and hatched according to manufacturer's instruction. B. plicatilis neonates within 2 h just after hatching were used for toxicity tests. Each five neonates were picked up with a micropipette and transferred into each well of a 48-well plate (SPL Life Science Co., Ltd, Korea) containing 1 mL of test solution. The acute and chronic tests were conducted in 12 replicates under culture conditions of 25±1°C and 0-h light/24-h dark photoperiod using an incubator (JSCC-150, JSR Co., Ltd, Korea). For chronic test, Nannochloropsis sp. was fed at cell density of $1×10^6$ cells/mL one time. The number of B. plicatilis individuals alive were counted under a stereomicroscope (SZ, Olympus, Japan) at the end of the test.

2.3.5 WET testing of P. olivaceus

P. olivaceus was used for acute (96 h) and chronic (7 days) toxicity tests inferred from survival rates according to OECD 203 [24] and OECD 212 [25], respectively. Fertilized eggs and larvae of P. olivaceus were purchased from a fish farm, Gyeonggyangsusan at Yeosu in Korea. Fish larvae of 30-day-old after hatching and composed of similar body sizes were used for acute toxicity test after acclimating them more than two weeks to laboratory

Table 1: Validation criteria of each whole effluent toxicity (WET) testing

Test organism	Test type	Validation criteria	Reference
Skeletonema costatum	Growth inhibition (72 h)	- Specific growth ratel: ≥ 0.9/day - Coefficient variation of average growth rate in replicate: $\leq 7\%$ - pH change: < 1.0	ISO, 2006
Brachionus plicatilis	Survival rate (24 h)	- Survival rate: $\geq 90\%$	ASTM, 2004
	Population growth (96 h)	- Population growth rate: > 0.55 - Growth inhibition in the lowest toxicant concentration: $< 50\%$	Janssen et al., 1994
Paralichthys olivaceus	Survival rate (96 h)	- Survival rate: $\geq 90\%$	OECD, 1992
	Survival rate (7 days)	- Survival rate: $\geq 70\%$	OECD, 1998

conditions. Fertilized eggs within 8 h just after hatching were used for chronic toxicity test. Each ten larvae or fertilized eggs were picked up with a dropping pipette and transferred into each beaker containing 800 mL of test solution. The tests were conducted in four replicates with a static, non-renewal type for acute test and a static renewal type for chronic test. Both tests were carried out under growth conditions of 20±1°C, 27.0 µmol photons/m^2/s and 16-h light/8-h dark photoperiod of cool-white fluorescent lights using a custom-made temperature-constant circulating water tank. The number of P. olivaceus larvae alive were counted with naked eyes at the end of both tests.

2.3.6 Statistical analysis

After conducting analysis of variance (ANOVA) tests, the lowest concentration that showed a significant statistical difference between control water and test solutions was used as lowest observed effect concentration (LOEC), and the maximum concentration that showed no statistical difference was used as no observed effect concentration (NOEC), based on specific growth rate of S. costatum, survival and population growth rates of B. plicatilis, and survival rates of P. olivaceus. Effect concentration of 50% (EC50) of S. costatum, and lethal concentration of 50% (LC50) of B. plicatilis and P. olivaceus were calculated using a linear interporation method. All statistical analyses were carried out using ToxCalc 5.0 (Tidepool Scientific Software, McKinleyville, CA, USA).

2.3.7 Validity of toxicity tests

All of the tests conducted in this study met validation criteria for each toxicity test in **Table 1**.

2.4 Chemical Analysis

Reactive chlorine species generated during the electrolytic treatment of test water were expressed as total residual oxidants (TROs) and measured at three sampling points (**Figure 1;** solid stars) using CLX OnLine Residual Chlorine Monitor (HF scientific, Inc., Fort Myers, FL, USA) as the equivalent of Cl_2 mg/L. Meanwhile, DBPs were measured at six sampling points (**Figure 1;** stars).

2.5. ERA

We assessed persistency, bioaccumulation and toxicity (PBT) property targeting 20 DBPs that were produced first time or showed higher concentrations in the effluent than the background concentrations in test water to evaluate ecological risks, when those DPBs were released into sea. They were considered to have PBT properties in terms of persistency in seawater (> 60 days half-life), bioaccumulation (> 3000 L/kg wet weight Log K_{ow}), bioconcentration [> 2000 bioconcentration factor (BCF)] and biological toxicity (< 0.01 mg/L NOEC from chronic test) [6]. EPI Suite^{TM} version 4.10 were used for calculating half-life, Log K_{ow} and BCF values, and ECOTOX Database for NOEC value.

Marine Antifaulant Model for PEC-Ballast Water (MAMPEC-BW) version 3.0 [Institute for Environmental Studies (IVM), Amsterdam, Netherlands] with environmental settings of GESAMP-BWWG Model Harbour was used to calculate PEC values to estimate emissions of chemical substances in the ballast water effluent. Information on each chemical substance was referred to OECD Screening Information Data Set (SIDS) (http://www.chem.unep.ch/irptc/sids/OECD-SIDS/sidspub.html), Joint Research Centre (JRC) of European Commission (http://ec.europa.eu/dgs/ jrc/) and manufacturer's material safety data sheet (MSDS). PNEC values were calculated by utilizing biological toxicity data

collected during PBT prosperity assessment and by adopting assessment factor corresponding to the lowest chronic NOEC value [6]. Finally, the ratio of PEC to PNEC (PEC/PNEC ratio) over 1 was considered to have negative impacts on marine environment.

3. Results

3.1 WET Testing

WET testing of the ballast water effluent treated by an electrolytic method was conducted to calculate NOEC, LOEC and EC50/LC50 values through growth inhibition rate tests of S. costatum cells, survival and population growth rate tests of B. plicatilis neonates, and survival rate tests of P. olivaceus larvae. Those toxicity tests revealed that S. costatum was the only test organisms that was sensitive to the ballast water effluent. Its toxicity was higher at brackish water condition by showing NOEC, LOEC and EC50 values of 12.5%, 25.0% and 83.3%, respectively, than at seawater condition by showing 25.0%, 50.0% and 94.0%, respectively. In contrast, both B. plicatilis and P. olivaceus showed NOEC values of 100%, and LOEC and LC50 values over 100% (**Table 2**) at both operation conditions.

3.2 Chemical Analysis

TRO measurement in the ballast water effluent treated by an electrolytic method was carried out at three sampling points in **Figure 1**. TRO concentrations in treated water just after the electrolytic treatment were 10.70 and 10.40 mg/L at seawater and brackish water conditions at the test initiation day, respectively, and 5.47 and 1.47 mg/L at the test termination day before neutralization, respectively. In contrast, TRO concentrations

in the effluent after neutralization were below the detection limit (< 0.03 mg/L) at both salinity conditions (**Table 3**).

The profile and amounts of DBPs of two points were only presented in this study for brevity, though we analyzed those at six sampling points (**Figure 1**). At the seawater condition, a total of 20 DBPs composed of one bromate, nine volatile halogenated organic compounds (VOCs), two halogenated acetonitriles (HANs), seven halogenated acetic acids (HAAs) and one chloropicrin were detected in the test and treated water at the initiation day and in the effluent at the termination day (**Table 4**). All of them were produced first time in the effluent at the termination day or showed higher concentrations in the effluent than those in the test and treated water at the initiation day. In the effluent, VOCs were detected at relatively wide concentrations from 5.16 µg/L (trichloromethane) to 236.00 µg/L (1,2-dichloroethane), HANs were detected at concentrations in the range of 0.19−5.25 µg/L, HAAs were detected at the widest concentrations from 0.98 µg/L (bromochloroacetic acid) to 381.00 µg/L (monobromoacetic acid), and bromate and chloropiclin were detected at concentrations of 32.30 µg/L and 0.03 µg/L, respectively.

At the brackish water condition, a total of 11 DBPs composed of one bromate, three VOCs, one HANs and six HAAs were detected in the test and treated water at the initiation day and in the effluent at the termination day (**Table 4**). All of them were produced first time in the effluent at the termination day or showed higher concentrations in the effluent than those in test and treated water at the initiation day. In the effluent, VOCs were detected in relatively wide concentrations from 2.50

Table 2: Results of whole effluent toxicity (WET) testing of three test organisms against ballast water effluent treated by an electrolytic method at two operation conditions. LOEC: lowest observed effect concentration, NOEC: no observed effect concentration, EC50: effect concentration of 50% and LC50: lethal concentration of 50%.

Test organism	Operation condition	Test type	NOEC (%)	LOEC (%)	EC50 pr LC50 (%)
Skeletonema costatum	Seawater	Growth inhibition (72 h)	25.0	50.0	94.0
	Brackish water	Growth inhibition (72 h)	12.5	25.0	83.3
Brachionus plicatilis	Seawater	Survival rate (24 h)	100	> 100	> 100
		Population growth (96 h)	100	> 100	> 100
	Brackish water	Survival rate (24 h)	100	> 100	> 100
		Population growth (96 h)	100	> 100	> 100
Paralichthys olivaceus	Seawater	Survival rate (96 h)	100	> 100	> 100
		Survival rate (7 days)	100	> 100	> 100
	Brackish water	Survival rate (96 h)	100	> 100	> 100
		Survival rate (7 days)	100	> 100	> 100

Table 3: Total residual oxidant (TRO) concentrations in ballast water effluent treated by an electrolytic method at two operation conditions (unit: μg/L)

Operation condition	Initiation day	Termination day	
	After electrolysis	Before neutralization	After neutralization
Seawater	10.70	5.47	< 0.03
Brackish water	10.40	1.47	< 0.03

Table 4: Compositions of disinfection by-products (DBPs) generated in the test water at the test initiation day and in the ballast water effluent at the test termination day during operation of a ballast water management system (unit: μg/L). "–" symbol menas below the detection limit.

Disinfection by-product	Seawater		Brackish water	
	Initiation day	Termination day	Initiation day	Termination day
	Test water	Effluent	Test water	Effluent
Bromate	–	32.30	–	20.70
Volatile halogenated organic compounds (VOCs)				
Dichloromethane	2.13	2.18	–	–
Bromochloromethane	5.29	13.20	–	–
Trichloromethane	3.60	5.16	–	–
1,2-dichloroethane	1.52	236.00	–	–
1,2-dichloropropane	18.90	29.80	1.31	2.50
Dichlorobromomethane	–	9.49	–	–
Dibromochloromethane	–	57.50	14.50	25.00
Tribromomethane	5.62	217.00	–	204.00
Bromobenzene	1.11	89.60	–	–
Halogenated acetonitriles (HANs)				
Bromochloroacetonitrile	–	0.19	–	0.03
Dibromoacetonitrile	–	5.25	–	–
Halogenated acetic acids (HAAs)				
Monobromoacetic acid	47.20	381.00	8.45	34.00
Dichloroacetic acid	4.46	4.65	1.06	2.90
Dalapon	4.32	9.49	–	–
Trichloroacetic acid	70.00	139.00	0.83	44.00
Dibromoacetic acid	0.83	30.40	2.61	79.00
Bromodichloroacetic acid	0.91	0.98	0.89	6.85
Tribromoacetic acid	–	13.60	–	61.50
Chloropicrin	–	0.03	–	–

μg/L (dichloropropane) to 204.00 μg/L (tribromomethane), bromochloro- acetonitrile of HANs was detected at concentration of 0.03 μg/L, HAAs were detected at the widest concentrations from 2.90 μg/L (dichloroactic acid) to 79.00 μg/L (dibromoacetic acid), and bromate was detected at concentration of 20.70 μg/L.

3.3 Ecological Risk Assessment

3.3.1 PBT property

Based on EPI Suite^{TM}, 20 DPBs detected in the ballast water effluent was calculated to have half-times of 8.7−37.5 days, Log K_{ow} of 0.41−2.99 L/kg wet weight and BCF of 3.16−43.6 L/kg wet weight (**Table 5**). Their lowest chronic NOEC values were deduced to have in the range of 1,730−433,000 μg/L and 1,600−100,000 μg/L for VOCs and HAAs, respectively, but those values for bromate, HANs and chloropicrin were unable to deduce because of lack of data in ECOTOX Database. The toxicity property of those DPBs could not be calculated because of high uncertainty due to lack of information on chronic NOEC values.

3.3.2 PEC/PNEC ratio

Based on MAMPEC-BW, 20 DPBs detected in the ballast water effluent was calculated to have PEC values in the range of 7.02×10^{-4}−5.39 μg/L and PNEC values in the range of 2.00×10^{-2}−4.24×10^{3} μg/L when estimation factor of 1−1000 was applied to the lowest chronic NOEC values (**Table 6**). The PEC/PNEC ratio of monobromo- acetic acid was 2.70×10^{0}, whereas those of the other 19 DBPs did not exceed 1.

Tae Won Kim · Keun-Yong Kim · Myung-Baek Shon · Young-Soo Kim · Ji Hyun Lee · Min Ho Son · Chang Ho Moon

Table 5: Persistency, bioaccumulation and toxicity (PBT) property of 20 disinfection by-products (DBPs) generated during operation of a ballast water management system (unit: μg/L)

Disinfection by-product	Half-life (day)	Log K_{ow}	BCF (L/kg wet weight)
Bromate	15.0	0.63	3.16
Volatile halogenated organic compounds (VOCs)			
Dichloromethane	37.5	1.34	3.10
Bromochloromethane	15.0	1.43	3.96
Trichloromethane	37.5	1.52	9.26
1,2-dichloroethane	37.5	1.48	4.40
1,2-dichloropropane	37.5	1.98	9.41
Dichlorobromomethane	37.5	2.00	9.70
Dibromochloromethane	37.5	2.16	12.4
Tribromomethane	37.5	2.40	17.8
Bromobenzene	37.5	2.99	43.6
Halogenated acetonitriles (HANs)			
Bromochloroacetonitrile	37.5	0.38	3.16
Dibromoacetonitrile	37.5	0.47	4.16
Halogenated acetic acids (HAAs)			
Monobromoacetic acid	8.7	0.41	3.16
Dichloroacetic acid	15.0	0.92	3.16
Dalapon	37.5	0.78	3.16
Trichloroacetic acid	37.5	1.33	3.16
Bromochloroacetic acid	15.0	0.61	3.16
Dibromoacetic acid	15.0	0.70	3.16
Bromodichloroacetic acid	37.5	1.53	3.16
Tribromoacetic acid	15.0	1.71	3.16
Chloropicrin	60.0	2.09	11.10

4. Discussion

Diverse BWMSs have been developed worldwide in order to regulate international transportation of invasive species by ship's ballast water. They adopted physical, mechanical or chemical method such as filtration, cabitation, UV radiation, electrolysis, ozonation, etc. as a core principle in order to disinfect the non-indigenous species [9]. Among them, an electrolytic method generates highly toxic chlorine by passing brine ballast water through an electrical current and is now widely used for eliminating inorganic and organic micropollutants or disinfecting viral and bacterial contaminants for drinking water supply, sanitary, aquaculture, food industry and so forth [13][14][16][19][26]−[29] as well as for disinfecting aquatic organisms in ballast water [4][8][9][15]. The chlorine generated by electrolytic treatment forms reactive chlorine species such as NaOCl, HOCl, OCl⁻, HOBr, OBr⁻, etc. that subsequently react with inorganic and

organic compounds in water solution and unavoidably form diverse DBPs during storage in a ballast water tank. The DBPs exert apparent toxic effects on marine organisms and have adverse impacts on marine environment [15][20]. Thus, we carried out WET testing and ERA of the ballast water effluent by using the BWMS used in this study that adopted an electrolytic method as a core disinfection principle to investigate its marine ecotoxicological effects.

WET testing revealed that diatom S. costatum was sensitive with the ballast water effluent by showing apparent toxicity of higher NOEC, LOEC and EC50 values at the brackish water condition than those at the seawater condition. Moreover, rotifer B. plicatilis and fish P. olivaceus showed no toxicity with NOEC values of 100%, and LOEC and LC50 values over 100%. This result is congruent with previous MEPC reports [30]−[34] and Shon et al. [35] that showed that S. costatum was the most sensitive test organism to the ballast water effluent treated by an electrolytic method. The reason for such toxicity to S. costatum appears to result from residual minute amounts of chlorine species and DBPs that might have no effects on the other test organisms. Their residual traces might exert apparent toxicity to phytoplankton by causing serious damage to its photosynthetic apparatus [36]−[40], though their toxic effects were assessed to be negligible in our TROs measurement and ERA (see below).

Though the concentrations of TROs were measured below the detection limit in the ballast water effluent at both salinity conditions, the total numbers of DBPs in the effluent that were produced first time or showed higher concentrations than the background concentrations in test water were much more at the seawater condition than the brackish water condition. Moreover, though ERA showed no PBT properties for those 20 DBPs at both operation conditions monobromoacetic acid showed the PEC/PNEC ratio over 1 at the seawater condition, which means to have ecotoxicological effects on marine environment to a certain degree. Thus, the seawater condition could be considered to have higher toxic effects than the brackish water condition in terms of the number and intensity of DBPs. However, this result was incongruent with WET testing; S. costatum showed higher toxicity at the brackish water condition than the seawater condition. Mixture of more than two chlorine species or DBPs could exhibit additive effects on aquatic organisms. Thus, the result of ERA conducted with a single DBP detected in the

Table 6: Predicted environmental concentration (PEC) and predicted no effect concentration (PNEC) values, and PEC/PNEC ratio of 20 disinfection by-products (DBPs) in the ballast water effluent at two operation conditions

Substance	PEC (µg/L)	Toxicity (µg/L)	Assesment factor	PNEC (µg/L)	PEC/PNEC ratio	Test condition
Bromate	1.63×10^{-1}	30,000	1,000	3.00×10^{1}	5.43×10^{-3}	Seawater
Volatile halogenated organic compounds (VOCs)						
Dichloromethane	1.25×10^{-2}	42,400	10	4.24×10^{3}	2.95×10^{-6}	Seawater
Bromochloromethane	8.18×10^{-2}	67,000	1,000	6.70×10^{1}	1.22×10^{-3}	Seawater
Trichloromethane	3.31×10^{-2}	185	1,000	1.85×10^{-1}	1.79×10^{-1}	Seawater
1,2-dichloroethane	1.55×10^{0}	1,820	10	1.82×10^{2}	8.52×10^{-3}	Seawater
1,2-dichloropropane	1.90×10^{-2}	4,090	10	4.09×10^{2}	4.65×10^{-4}	Seawater
Dichlorobromomethane	7.00×10^{-2}	240,000	1,000	2.40×10^{2}	2.92×10^{-4}	Seawater
Dibromochloromethane	4.93×10^{-1}	34,000	1,000	3.40×10^{1}	1.45×10^{-2}	Seawater
Tribromomethane	2.16×10^{0}	1,730	10	1.73×10^{2}	1.25×10^{-2}	Seawater
Bromobenzene	6.45×10^{-1}	1,600	1,000	1.60×10^{0}	4.03×10^{-1}	Seawater
Halogenated acetonitriles (HANs)						
Bromochloroacetonitrile	4.18×10^{-3}	20	1,000	2.00×10^{-2}	2.09×10^{-1}	Seawater
Dibromoacetonitrile	1.17×10^{-1}	550	1,000	5.50×10^{-1}	2.13×10^{-1}	Seawater
Halogenated acetic acids (HAAs)						
Monobromoacetic acid	5.39×10^{0}	200	100	2.00×10^{0}	2.70×10^{0}	Seawater
Dichloroacetic acid	8.22×10^{-2}	23,000	1,000	2.30×10^{1}	3.57×10^{-3}	Seawater
Dalapon	2.11×10^{-1}	750	10	7.50×10^{1}	2.81×10^{-3}	Seawater
Trichloroacetic acid	3.10×10^{0}	1,200	10	1.20×10^{2}	2.58×10^{-2}	Seawater
Bromochloroacetic acid	1.21×10^{-1}	69,000	1,000	6.90×10^{1}	1.75×10^{-3}	Brackish water
Dibromoacetic acid	1.40×10^{0}	69,000	1,000	6.90×10^{1}	2.03×10^{-2}	Brackish water
Bromodichloroacetic acid	2.18×10^{-2}	52,800	1,000	5.28×10^{1}	4.13×10^{-4}	Seawater
Tribromoacetic acid	1.09×10^{0}	101,000	1,000	1.01×10^{2}	1.08×10^{-2}	Brackish water
Chloropicrin	7.02×10^{-4}	17	1,000	1.70×10^{-2}	4.13×10^{-2}	Seawater

effluent might be different from that of the WET testing with the effluent composed of a mixture of 20 DBPs. Moreover, biological toxicity of the effluent to the marine environment could be changed depending on various physicochemical variables such as hardness, pH, temperature, dissolved oxygen (DO), etc. of marine outfall.

By the way, monobromoacetic acid was detected at the highest concentration in the ballast water effluent at seawater condition (381.00 µg/L) and it was the only one DBP that has PEC/PNEC ratio over 1. Its high concentration in the effluent and PEC/PNEC ratio was considered to result from test water; its concentration in test water used for the BWMS operation in this study was 47.20 µg/L and indeed much higher than previous studies that reported to vary in the range of 0−1.67 µg/L [30]−[35]. Monobromoacetic acid is used for diverse synthetic raw materials and one of DBPs generated during chlorine disinfection process of water treatment [41][42]. The test water used in this study was not originated from coastal water, but collected from an intake pipeline of a fish farm nearby an intensive fish farming area. Thus, it could be speculated that residual disinfecting agents used

for cleaning fish tanks or intake pipelines, or high concentration of residual DBPs released from other fish farms might result in the existence of monobromoacetic acid. In another chemical analysis from an independent BWMS operation using test water collected from coastal water monobromoacetic acid was not detected in the test water and was detected at a concentration of 1.78 µg/L in the effluent with the PEC/PNEC ratio below 1 at seawater condition (data not shown). Therefore, the effluent of the BWMS used in this study that adopted an electrolytic method for ballast water treatment was considered to have no adverse effects on marine environment, when results of WET testing, PEC property and ERA were collectively taken into account.

Disinfecting performance of BWMSs that adopt an electrolytic process as a core principle is affected by many factors such as process method (flow-through electrolytic treatment method vs. electrolyzed water injection), raw materials and array method of electrodes, TROs concentration, exposure time, size of organisms, etc. [15][17][18]. Such factors might have great influences on toxicity of the ballast water effluent due to unexpected residual active substances (e.g., TROs and FROs) and

diverse by-products (e.g., DBPs), and subsequently exhibit adverse effects on marine environment. Thus, WET testing and ERA should be carried out upon the ballast water effluent generated by an electrolytic method. When toxic effects were once detected through such assessments additional tests such as toxicity reduction evaluation (TRE) and toxicity identification evaluation (TIE) should be carried out to identify the causative substance(s) and draw up appropriate measures to sequestrate them [43]. In addition, since the ballast water effluent might contain different compositions and amounts of active substances and by-products depending on test water quality assurance (QA) and quality control (QC) of test water must be conducted prior to an efficacy test of a BWMS that prepares to apply for IMO's basic and final approvals.

Acknowledgments

References

[1] G. M. Hallegraeff, "Transport of toxic dino-flagellates via ships' ballast water: bioeco-nomic risk assessment and efficacy of possible ballast water management strategies," Marine Ecology Progress Series, vol. 168, pp. 297‑309, 1998.

[2] A. Ricciardi and H. J. MacIsaac, "Recent mass invasion of the North American Great Lakes by Ponto-Caspian species," Trends in Ecology & Evolution, vol. 15, no. 2, pp. 62‑65, 2000.

[3] E. Tsolaki and E. Diamadopoulos, "Technologies for ballast water treatment: a review," Journal of Chemical Technology and Biotechnology, vol. 85, no. 1, pp. 19‑32, 2010.

[4] A. A. Goncalves and G. A. Gagnon, "Recent technologies for ballast water treatment," Ozone: Science & Engineering, vol. 34, no. 2, pp. 174‑195, 2012.

[5] Ø. Endresen, H. L. Behrens, S. Brynestad, A. B. Andersen, and R. Skjong, "Challenges in global ballast water management," Marine Pollution Bulletin, vol. 48, no. 7, pp. 615‑623, 2004.

[6] IMO, International Convention for the Control and Management of Ship's Ballast Water and Sediments 2004, Methodology for Information Gathering and Conduct of Work of the GESAMP-BWWG.BWM.2/Circ.13/ Rev.1, 2012.

[7] S. Gollasch, M. David, M. Voigt, E. Dragsund, C. Hewitt, and Y. Fukuyo, "Critical review of the IMO international convention on the management of ships' ballast water and sediments," Harmful Algae, vol. 6, no. 4, pp. 585‑600, 2007.

[8] R. C. Matousek, D. W. Hill, R. P. Herwig, J. R. Cordell, B. C. Nielsen, N. C. Ferm, D. J. Lawrence, and J. C. Perrins, "Electrolytic sodium hypochlorite system for treatment of ballast water," Journal of Ship Production, vol. 22, no. 3, pp. 160‑171, 2006.

[9] E. Tsolaki, P. Pitta, and E. Diamadopoulos, "Electrochemical disinfection of simulated ballast water using Artemia salina as indicator," Journal of the Chemical Engineering, vol. 156, no. 2, pp. 305‑312, 2010.

[10] IMO, http://www.imo.org/OurWork/Environment/ BallastWaterManagement/Documents/Table%20of%20B A%20FA%20TA%20udat-ed%20in%20May%202014.pdf, Accessed June 11, 2014.

[11] Class NK, https://www.classnk.or.jp/hp/pdf/activ-ities/statutory/ballastwater/approval_ballast_e.pdf, Accessed June 11, 2014.

[12] G. Patermarakis and E. Fountoukidis, "Disinfection of water by electrochemical treatment," Water Research, vol. 24, no. 12, pp. 1491‑1496, 1990.

[13] M. Deborde and U. von Gunten, "Reactions of chlorine with inorganic and organic compounds during water treatment-kinetics and mechanisms: a critical review," Water Research, vol. 42, no.1, pp. 13‑51, 2008.

[14] C. A. Martínez-Huitle and E. Brillas "Electrochemical alternatives for drinking water disinfection," Angewandte Chemie International Edition, vol. 47, no. 11, pp. 1998‑2005, 2008.

[15] B. Werschkun, Y. Sommer and S. Banerji, "Disinfection by-products in ballast water treatment: An evaluation of regulatory data," Water Research, vol. 46, no. 16, pp. 4884‑4901, 2012.

[16] Y. R. Huang, Y. C. Hung, S. Y. Hsu, Y. W. Huang, and D. F. Hwang, "Application of electrolyzed water in the food industry," Food Control, vol. 19, no. 4, pp. 329‑345, 2008.

[17] R. J. Pascho, M. L. Landolt, and J. E. Ongerth, "Inactivation of renibacterium salmoninarum by free chlorine," Aquaculture, vol. 131, no. 3, pp. 165‑175,

1995.

[18] H. Kiura, K. Sano, S. Morimatsu, T. Nakano, C. Morita, M. Yamaguchi, T. Maeda, and Y. Katsuoka, "Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration," Journal of Microbiological Methods, vol. 49, no. 3, pp. 285‑293, 2002.

[19] Y. T. Woo, D. Lai, J. L. McLain, M. K. Manibusan, and V. Dellarco, "Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products," Environmental Health Perspective (Supplement 1), vol. 110, no. 1, pp. 75‑87, 2002.

[20] N. Zhang, B. Ma, J. Li, and Z. Zhang, "Factors affecting formation of chemical by-products during ballast water treatment based on an advanced oxidation process," Journal of Chemical Engineering, vol. 231, pp. 427‑433, 2013.

[21] "Water quality-marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum," Geneva, Switzerland, ISO 10253, 2006.

[22] "Standard guide for acute toxicity test with the rotifer Brachionus," West Conshohocken, United States, ASTM E1440-91, 2006.

[23] C. R. Janssen, G. Persoone, and T. W. Snell. "Cyst-based toxicity tests. VIII. Short-chronic toxicity tests with the freshwater rotifer Brachionus calyciflorus," Aquatic Toxicology, vol. 28, no. 3, pp. 243‑258, 1994.

[24] "OECD guideline for testing of chemicals, section 2 (fish, acute toxicity test)," OECD 203, 1992.

[25] "OECD guidelines for the testing of chemicals section 2 (fish, short-term toxicity test on embryo and sac-fry stages)," OECD 212, 1998.

[26] G. Patermarakis and E. Fountoukidis, "Disinfection of water by electrochemical treatment," Water Research, vol. 24, no. 12, pp. 1491‑1496, 1990.

[27] N. Tanaka, T. Fujisawa, T. Daimon, K. Fujiwara, M. Yamamoto, and T. Abe, "The cleaning and disinfecting of hemodialysis equipment using electrolyzed strong acid aqueous solution," Artificial Organs, vol. 23, no. 4, pp. 303‑309, 1999.

[28] K. S. Venkitanarayanan, G. O. Ezeike, Y. C. Hung, and M. P. Doyle, "Efficacy of electrolyzed oxidizing water for inactivating Escherichia coli O157:H7, Salmonella enteriditis, and Listeria monocytogenes," Applied Environmental Microbiology, vol. 65, no. 9,

pp. 4276‑4279, 1999.

[29] M. A. Jorquera, G. Valencia, M. Eguchi, M. Katayose, and C. Riquelme, "Disinfection of seawater for hatchery aquaculture systems using electrolytic water treatment," Aquaculture, vol. 207, no. 3, pp. 213‑224, 2002.

[30] MEPC, "Harmful aquatic organisms in ballast water, application for basic approval of AquaStarTM ballast water treatment system submitted by Republic of Korea," Marine Environment Protection Committee, 61/2/1, 2010.

[31] MEPC, "Harmful aquatic organisms in ballast water, application for basic approval of STX Metal Co., Ltd ballast water treatment system submitted by the Republic of Korea," Marine Environment Protection Committee, 62/2/8, 2010.

[32] MEPC, "Harmful aquatic organisms in ballast water, application for final approval of the AquaStarTM ballast water treatment system submitted by the Republic of Korea," Marine Environment Protection Committee, 63/2/3, 2011.

[33] MEPC, "Harmful aquatic organisms in ballast water, application for basic approval of the HS-BALLAST ballast water treatment system submitted by the Republic of Korea," Marine Environment Protection Committee, 64/2/3, 2012.

[34] MEPC, "Harmful aquatic organisms in ballast water, application for final approval of the ballast water treatment system (Smart Ballast BWMS) submitted by the Republic of Korea," Marine Environment Protection Committee, 64/2/2, 2012.

[35] M. B. Shon, M. H. Son, J. H. Lee, Y. J. Son, G. H. Lee, C. H. Moon, and Y. S. Kim, "The study on the marine eco-toxicity and ecological risk of treated discharge water from ballast water management system using electrolysis," Journal of the Korean Society for Marine Environmental and Energy, vol. 16, pp. 88‑101, 2013 (in Korean).

[36] A. J. Brook and A. L. Baker, "Chlorination at power plants: impact on phytoplankton productivity," Science, vol. 176, no. 4042, pp. 1414‑ 1415, 1972.

[37] E. J. Carpenter, B. B. Peck, and S. J. Anderson, "Cooling water chlorination and productivity of entrained phytoplankton," Marine Biology, vol. 16, no. 1, pp. 37‑40, 1972.

[38] R. W. Eppley, E. H. Renger, and P. M. Williams, "Chlorine reactions with seawater constituents and the inhibition of photo- synthesis of natural marine phy-

Tae Won Kim · Keun-Yong Kim · Myung-Baek Shon · Young-Soo Kim · Ji Hyun Lee · Min Ho Son · Chang Ho Moon

toplankton," Estuarine and Coastal Marine Science, vol. 4, no. 2, pp. 147‑161, 1976.

[39] A. S. Brooks and N. E. Liptak, "The effect of inter-mittent chlorination on freshwater phytoplankton," Water Research, vol. 13, no. 1, pp. 49‑52, 1979.

[40] E. H. Poornima, M. Rajadurai, T. S. Rao, B. Anupkumar, R. Rajamohan, S. V. Narasimhan, V. N. R. Rao, and V. P. Venugopalanb, "Impact of thermal discharge from a tropical coastal power plant on phytoplankton," Journal of Thermal Biology, vol. 30, no. 4, pp. 307‑316, 2005.

[41] E. Malliarou, C. Collins, N. Graham, and M. J. Nieuwenhuijsen, "Haloacetic acids in drinking water in the United Kingdom," Water Research, vol. 39, no. 12, pp. 2722‑2730, 2005.

[42] S. E. Kim, Formation and Control of Chlorinated Disinfection By-products in Water Supply System, Ph.D, Thesis, University of Seoul, Seoul, Korea, 2005 (in Korean).

[43] J. A. Fava, D. Lindsay, W. H. Clement, R. Clark, G. M. DeGraeve, J. D. Cooney, S. Hansen, W. Rue, S. Moore, and P. Lankford, "Generalized method-ology for conducting industrial toxicity reduction evaluations (TREs)," EPA/600/2-88/070, The Chemical and Chemical Product Branch Risk Reduction Engineering Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH, USA, 1989.