제 출 문

한국조선기자재연구원 원장 귀하

본 보고서는 '주식회사 'KT Marine Co., Ltd.' 선박평형수처리장치의 국제해사기구 기본승인 및 최종승인 취득을 위한 수중생물 급성 및 만성독성평가'의 '기본승인 취득'을 위한 시험결과 최종보고서입니다.

2011년 7월

기관 책임자: 손민호

시험 책임자: 김진희 · 박정임 · 손명백

시험원: 김태원 · 조소은

㈜ 해 양 생 태 기 술 연 구 소

4.2.1 Properties and Actions of the Preparation

4.2.1.1 Data on effects on aquatic plant, invertebrate and fish including sensitive and representative organisms

The most sensitive organism for de-ballast water treated by the KTM-BWMS was the algae, *Skeletonema costatum* that gave the LOEC value of 25.00 %, NOEC value of 12.50 % and 72h-EC_{50} value of 83.32 % from aquatic toxicity test result for the 3-32 psu de-ballast water. For >32 psu aquatic toxicity test with algae, LOEC, NOEC and 72h-EC_{50} value were 50.00 %, 25.00 % and 93.97 %, respectively. For the other test organisms, no significant acute and/or chronic toxic effects were observed (see report in Appendix Ⅳ, section 3.1 and 3.2). The results are summarized in Tables 5 ~ 9.

Algae (Growth inhibition test): Study to assess the effects of the seawater treated by the KTM-BWMS on the diatom, *Skeletonema costatum*, for 72 hours, NOEC and LOEC values from Hypothesis Test and EC_{50} value based on the cell densities at the end of the experiment were calculated for > 32 and 3-32 psu de-ballast water from the KTM-BWMS.

Acute aquatic toxicity

Crustacean: Study to assess the effects of the seawater treated by the KTM-BWMS on the Rotifer, *Brachionus plicatilis*, for 24 hours, NOEC, LOEC and LC_{50} values based on survival data at the end of the experiment were calculated for > 32 and 3-32 psu de-ballast water from the KTM-BWMS.

Fish: Study to assess the effects of the seawater treated by the KTM-BWMS on the olive flounder, *Paralichthys olivaceus*, for 96 hours, NOEC, LOEC and LC_{50} values based on survival data at the end of the experiment were calculated for > 32 and 3-32 psu de-ballast water from the KTM-BWMS.

Chronic aquatic toxicity

Crustacean: Study to assess the effects of the seawater treated by the KTM-BWMS on the Rotifer, *Brachionus plicatilis*, for 96 hours, NOEC, LOEC and EC_{50} value based on population growth rate data at the end of the experiment were calculated for > 32 and 3-32 psu de-ballast water from KTM-BWMS.

Fish: Study to assess the effects of the seawater treated by the KTM-BWMS on the olive flounder, *Paralichthys olivaceus*, for 7 days, NOEC, LOEC and LC_{50} value based on survival data at the end of the experiment were calculated for both > 32 and 3-32 psu de-ballast water from the KTM-BWMS.

Table 5: Growth inhibition test results on > 32 and 3-32 psu de-ballast water from KTM-BWMS

	Species	Test water (psu)	NOEC	LOEC	LC_{50} and/or EC_{50}		Reference
			Conc. (%)		End point	Conc. (%)	
Algae	*Skeletonema costatum*	> 32	25.00	50.00	EC_{50} 72 h	93.97	OECD 201
		3-32	12.50	25.00	EC_{50} 72 h	83.32	OECD 201

Table 6: Acute aquatic toxicity of > 32 psu de-ballast water from KTM-BWMS

	Species	NOEC	LOEC	LC_{50} and/or EC_{50}		Reference
		Conc. (%)		End point	Conc. (%)	
Crustacean	*Brachionus plicatilis*	100.00	> 100.00	LC_{50} 24 h	> 100.00	ASTM E1440-91
Fish	*Paralichthys olivaceus*	100.00	> 100.00	LC_{50} 96 h	> 100.00	OECD 203

Table 7: Acute aquatic toxicity of 3-32 psu de-ballast water from KTM-BWMS

	Species	NOEC	LOEC	LC_{50} and/or EC_{50}		Reference
		Conc. (%)		End point	Conc. (%)	
Crustacean	*Brachionus plicatilis*	100.00	> 100.00	LC_{50} 24 h	> 100.00	ASTM E1440-91
Fish	*Paralichthys olivaceus*	100.00	> 100.00	LC_{50} 96 h	> 100.00	OECD 203

Table 8: Chronic aquatic toxicity of > 32 psu de-ballast water from KTM-BWMS

	Species	NOEC	LOEC	LC_{50} and/or EC_{50}		Reference
		Conc. (%)		End point	Conc. (%)	
Crustacean	*Brachionus plicatilis*	100.00	> 100.00	EC_{50} 96 h	> 100.00	- ASTM E1440-91 - Janssen *et al.*,1994
Fish	*Paralichthys olivaceus*	100.00	> 100.00	LC_{50} 7 D	> 100.00	OECD 212

Table 9: Chronic aquatic toxicity of 3-32 psu de-ballast water from KTM-BWMS

	Species	NOEC	LOEC	LC_{50} and/or EC_{50}		Reference
		Conc. (%)		End point	Conc. (%)	
Crustacean	*Brachionus plicatilis*	100.00	> 100.00	EC_{50} 96 h	> 100.00	- ASTM E1440-91 - Janssen *et al.*,1994
Fish	*Paralichthys olivaceus*	100.00	> 100.00	LC_{50} 7 D	> 100.00	OECD 212

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(Aquatic Toxicity Test)

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1. REQUEST

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

REQUEST

1. Client information (Record the information of client/monitor for test plan and final report)

Organization	KT Marine Co., Ltd.	**Name**	Og Yeol Bag
Department	Business Management	**Position**	CEO
Address	Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.		
Tel	+82-51-441-0692~3	**Fax**	+82-51-441-0695
C.P	+82-10-9343-1135	**E-mail**	ktm@ktmarine.co.kr

2. Monitor information (Real monitoring charge or contact charge)

Organization	KT Marine Co., Ltd.	**Name**	Seong Ho Park
Department	New Project Team	**Position**	General Manager
Address	Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.		
Tel	+82-51-441-0692~3	**Fax**	+82-51-441-0695
C.P	+82-10-3049-0722	**E-mail**	ktm@ktmarine.co.kr

3. Component and information of test (Mark with ■ sign on the components which you want)

Subject	□ Permitting for use (permit organization) □ Research and Development □ Scientific paper ■ etc.(IMO basic and final approval with ballast-water management system)
Division	□ Chemical substance ( ) □ Food □ Agricultural chemicals □ An addition (food/prey) ■ etc. (Sea water)
Object	Acute and chronic effects of the de-ballasting water for aquatic organisms

4. Test component (Mark with ■ sign on the components which you want)

Toxicity test	□ Acute toxicity test □ Chronic toxicity test ■ All of above test
	Test organism
Acute toxicity test	■ Algae (Phytoplankton: Diatom etc.) ■ Invertebrate (Zooplankton: Rotifer) ■ Fish (Olive flounder) □ Echinoderm (Sea urchin) □ Mollusk (Mussel, Oyster, etc) □ All of above division
Chronic toxicity test	■ Algae (Phytoplankton: Diatom etc.) ■ Invertebrate (Zooplankton: Rotifer) ■ Fish (Olive flounder) □ Echinoderm (Sea urchin) □ Mollusk (Mussel, Oyster, etc) □ All of above division

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan 608-830, Republic of Korea
Tel +82-51-611-6200
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Final Report	■ Final report (3 copies of bookbinding and 1 CD recording) □ etc. ( )
Appling guideline (Test method)	■ OECD □ ISO □ EPA ■ etc.(ASTM)

5. Information on the test substance (Record apparent information for Study Plan and Report)
*** Have to record "none", if it dose not have the any information.**

① Division	□ Chemical compound (□Organic □Inorganic) □ Subject matter into biological □ Chemical substance (□Existing substance □New substance) □ Natural substance ■ etc. (Seawater treated by **AquaStar™ BWMS**)				
② Transit	□ Mail □ Direct delivery ■etc.(Sampling and transit of the Test facility)				
③ Sending date	From 1st June 2010 to 30th November 2010				
④ Offer quantity	Net weight: (g or L)×(□ea □box(es) □bottle(s)) Gross weight: (g or L)×(□ea □box(es) □bottle(s)) ■ etc.(treated water: 0.4 ton; Control water: 0.6 ton)				
⑤ Condition of transit	■ Cooling □ Freezing(- ℃) □ Room Temp. □ etc.( )				
⑥ Danger	□ Volatility □ Flammable □ Spontaneous combustion □ Oxydant □ Corrosiveness ■ etc. (None)				
⑦ Substance name or code name	Korean: > 32 PSU 처리수, 3-32 PSU 처리수 English: > 32 PSU treated ballast water, 3-32 PSU treated ballast water				**※ It will directly apply test plan and report.**
CAS No. (If you get it)	None	**Chemical name or common name (If you know it)**		seawater	
⑧ Exterior and type	□ A solid (□ Powder □ Crystallization □ etc. ( )) ■ Liquid (□ Transparency □ Suspension □ Mucous □ etc. ( )) Color:				
⑨ Principal ingredients	Seawater				
⑩ Condition of custody	■ Cooling □ Freezing (- ℃) □ Room Temp. □ Cooling & Darkness □ etc. ( )				
⑪ Purity	none	**⑫ Molecular formula and/or molecular weight**	none		
⑬ pH	7~8	**⑭ Structural formula**	none	**⑮ Specific gravity**	none
※ Lot No.	none	※ Making date	20 . .		
※ Available period	until 20 . . ( ) or from making date to ■ week(s) □ month(s) □ year(s)				
※ Stability	-				
※ Compatibility	■ Hydrophilic □ Hydrophobic	□ Lipophilic □ Lipophobic			
※ Grade sheet of test substance	MSDS: □ Y □ N	COA: □ Y □ N □ Later			
※ Handle with care	None				
※ Disposal of rest test substance	□ Return ■ Disposal				

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

6. Information on the preparation test substance and condition of exposure (for reference)

① Type	Liquid	② pH	6 ~ 8
③ Component	Seawater		
④ Solvent &solubility	□ Acetone □ t-butyl alcohol □ dimethyl formamide □ etc. ( )		
⑤ Condition of custody	□ Cooling at 4 °C □ Freezing(- °C) □ Room Temp. ■ Cooling at 4 °C& Darkness □etc.( )		
⑥ Stability	Stable		
⑦ Homogeneity	-		
⑧ Method of preparation of test solutions	Dilution and control water: Non-treated ballast water		
⑨ Boiling point	ca. 100 °C (Maximum Boiling Temp.)		
⑩ Vessels	□ Glass ■ Plastic □ Rubber □ Metal		
⑪ Handle with care(as preparation)	Light, heat, air and water (stable/unstable) □ Shade □ Hermetical seal □ Dehumidification ■ etc. (none)		
⑫ Analysis method	OECD guideline 201, 203, 212. ASTM Standard E1440-91, USEPA or published paper		
⑬ Handle with care (as use)	none		

7. Annex

□ Toxicity test data □ Test report of other organization □ Reference of test substance □ Annex of reference substances □ Reference (magazine, journal or paper, etc.) ■ etc. (None)

8. Standard or positive reference substance

□ existence ■ none	Name	

9. Certificate of Analysis :

Offering Date	2011. 06. 29 ~ end of the experiment (2011.07.18)

10. Other reference

(Description of the detail Condition, other information and other components)

None

I request that MEI (Marine Eco-Technology Institute Co., Ltd.) would conduct the test for above substance.

Date: 2011. 04. 12

Name: Og Yeol Bag (Sign in)

485-1 Yongdang, Namgu, Busan 608-830, Korea, Marine Eco-Technology Institute Co., Ltd.
Tel. +82-51-611-6200, Fax +82-51-611-0588,
Home page: http://marine-eco.co.kr, e-mail : mhson@marine-eco.co.kr

2. STUDY PLAN

2.1 Acute Toxicity

2.1.1 ANNEX 1

***Skeletonema costatum*, Growth Inhibition Test**

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

STUDY PLAN

Acute Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- *Skeletonema costatum*, Growth Inhibition Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

STUDY PLAN PREPARATION AND APPROVAL

Study title: Acute toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward algae growth inhibition

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Test facility Marine Eco-technology Institute Co., Ltd.

Study Director 2011. 04. 13.

Myung-Baek Shon (signature)

Test site manager (Lab. manager) 2011. 04. 13.

Myung-Baek Shon (signature)

Test facility manager 2011. 04. 13.

Min-Ho Son (signature)

Organization KT Marine Co., Ltd.

2011. 04. 13.

Monitor: Seong Ho Park (signature)

Sponsor: Og Yeol Bag (signature)

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1. OUTLINE OF STUDY

1.1 Purpose

The purpose of this study is to assess the acute toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS (or the >32 and 3-32 PSU de-ballast water from KTM-BWMS) on the growth of diatom, *Skeletonema costatum*.

1.2 Principle

The system response is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations of a treated ballast water. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicates, unexposed control cultures. For full expression of the system response to toxic effects, the cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate.

1.3 Good Laboratory Practice

All procedure of this study is complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

1.4 Guideline

This study is conducted on the basis of principle of following standard method and applied for *Skeletonema costatum*:

- "OECD Guideline for Testing of Chemicals, 201, Freshwater alga and Cyanobacteria, Growth Inhibition test"

Organization for Economic Co-operation and Development, (Adopted: Mar. 23, 2006)

1.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

1.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax: + 82-51-611-0588

1.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.8 Study director

Name: Myung-Baek Shon

Department: BAT (Bio-assay Team for aquatic organisms)

1.9 Study timetable

1.9.1 Treated ballast water (> 32PSU) and non-Treated ballast water (> 32PSU)

Study initiation	2011. 04. 13
Batch culture of test organisms	2011. 04. 13
Acclimation	2011. 04. 13 ~ 06. 26
Pre-culture for test	2011. 06. 26
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation of cell density	2011. 06. 30 ~ 07. 03
Evaluation of chlorophyll *a*	2011. 07. 01 ~ 07. 04
Experimental completion	2011. 07. 04
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Batch culture of test organisms	2011. 04. 13
Acclimation	2011. 04. 13 ~ 07. 23
Pre-culture for test	2011. 07. 23
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation of cell density	2011. 07. 13 ~ 07. 16

Evaluation of chlorophyll *a*	2011. 07. 14 ~ 07. 17
Experimental completion	2011. 07. 17
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.10 Responsible personnel

Laboratory manager (Test site manager) Statistical analysis Observation and record	Myung-Baek Shon
Culture of test organisms Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Check the chemical condition	Tae Won Kim

1.11 Retention of records and data

1.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

1.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

2. MATERIALS AND METHODS

2.1 Test substance-1 (Treated ballast water (> 32PSU) -Treated)

2.1.1 Substance name

Treated water

2.1.2 Lot No.

none

2.1.3 Type

liquid

2.1.4 Principal ingredients

Seawater

2.1.5 Temperature

12~26 ˚C

2.1.6 Salinity

> 32 PSU

2.1.7 pH

6~8

2.1.8 Custody condition

Constant room at 4 ˚C for 7 days

2.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.1.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.1.11 Disposal of the resting substances

Scrap after test

2.1.12 Certificate of analysis

Client supplies to test organization

2.2 Test substance-2 (Non-treated ballast water (> 32PSU) -Control)

2.2.1 Substance name

Non-treated water

2.2.2 Lot No.

none

2.2.3 Type

liquid

2.2.4 Principal ingredients

Seawater

2.2.5 Temperature

12~26 ˚C

2.2.6 Salinity

> 32 PSU

2.2.7 pH

6~8

2.2.8 Custody condition

Constant room at 4 ˚C for 7 days

2.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.2.11 Disposal of the resting substances

Scrap after test

2.2.12 Certificate of analysis

Client supplies to test organization

2.3 Test substance-3 (Treated ballast water (3-32 PSU) -Treated)

2.3.1 Substance name

Treated water

2.3.2 Lot No.

none

2.3.3 Type

liquid

2.3.4 Principal ingredients

Seawater

2.3.5 Temperature

12~26 ˚C

2.3.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.3.8 Custody condition

Constant room at 4 ˚C for 7 days

2.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.3.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.3.11 Disposal of the resting substances

Scrap after test

2.3.12 Certificate of analysis

Client supplies to test organization

2.4 Test substance-4 (Non-treated ballast water (3-32 PSU) -Control)

2.4.1 Substance name

Non-treated water

2.4.2 Lot No.

none

2.4.3 Type

liquid

2.4.4 Principal ingredients

Seawater

2.4.5 Temperature

12~26 ˚C

2.4.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.4.8 Custody condition

Constant room at 4 ˚C for 7 days

2.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.4.11 Disposal of the resting substances

Scrap after test

2.4.12 Certificate of analysis

Client supplies to test organization

2.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance is diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid. The six concentrations of test substance including 0.00 % (only non-treated ballast water by the KTM-BWMS) and 100.00 % are arranged for the final definitive experiment.

2.6 Culture condition (SOP#TO-001)

2.6.1 Room

Incubator

2.6.2 Vessel type / volume

Glass flask / 250 mL

2.6.3 Medium volume / vessel volume & medium

100 mL/250 mL, f/2 (see Annex 2)

2.6.4 Inoculum density

5×10^3 cells/mL

2.6.5 Temperature

20 ˚C

2.6.6 Interval of inoculum

Every a week

2.6.7 L:D cycle

Continuous light

2.6.8 Intensity of light

3,000 lux

2.7 Test system

2.7.1 Test organisms

.1 Scientific name	*Skeletonema costatum* (VBA-007)
.2 Producer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

2.7.2 Ground of selected test organisms

Skeletonema costatum is important as primary producer in trophic structure and widely distributed phytoplankton species (Phlylum Bacillariophyta) in estuarine and coastal areas. It is used to evaluate the aquatic toxicity and has a large database.

2.7.3 Isolation of phytoplankton (SOP#TO-001)

Isolation method Dilution techniques

2.7.4 Preparation of pre-culture and inoculation (SOP#TP-008)

A pre-culture is started 2 to 4 days before the beginning of the experiment. In order to maintain the exponential growth phase until the start of the experiment, a pre-culture is made to inoculate 1×10^4 cells/mL from the algal stock culture to the growth medium. The pre-culture is incubated under the same conditions as those in the experiment. And the pre-culture for the experiment with 3-32 PSU treated ballast water is carried out same way after acclimation of test organisms for more than 14 days.

2.7.5 Check the test organisms for the experiment (SOP#TP-001)

The cell density in the pre-culture is immediately measured before use, in order to calculate the required inoculation volume and appearance of cells.

2.7.6 Disposal of the resting test organisms (SOP#TO-006)

The resting test organisms are scrapped after sterilization by the autoclave.

2.8 Study design

2.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

2.8.2 Exposure conditions (SOP#TP-001)

The final cell density (3×10^3 cells/mL) is added from the algal pre-culture to the each concentration in the test vessels. And then the test vessels are incubated for 72 hours at 22 ± 1˚C.

Experiment conditions and criteria are shown Annex 3 and application site is shown Annex 5.

2.8.3 Observation item and measurement components

.1 Biomass (SOP#TP-009, SOP#TP-012)

Measurement of biomass is conducted by measuring chlorophyll *a* extracted with 90% acetone using Tuner Designs AU-10. The measurement of the chlorophyll *a* is immediately done within 2 hours after extraction of it. To obtain the regression equation between chlorophyll *a* and cell density, cell count in the

control is done by manual cell counting used the microscope (CKX 31, Olympus) with counting chamber.

Calculations of the growth and the inhibition growth rate are shown Annex 4.

.2 Other observations (SOP#TP-001)

Microscopic observation is performed to verify normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae at the end of the experiment.

.3 pH (SOP#TE-002)

pH of the solutions at the beginning and at the end of the experiment is measured.

2.9 Statistical procedures (SOP#SP-101, SOP#SP-102)

For satisfactory correlation with biomass, chlorophyll *a* and cell density in the control are analyzed by linear correlation analysis with Excel program and the greatest R^2 value is yielded. Cell density is calculated by equation from the linear correlation analysis.

NOEC, LOEC and EC50 values of the end point are estimated by statistical analysis procedure referred from USEPA (2002). TOXCALC 5.0 program (Tidepool scientific software, USA) is used for statistic analysis.

3. REFERENCES

OECD guidelines for the testing of chemicals-201. 2006. Freshwater alga and Cyanobacteria, growth inhibition test. 25pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

ANNEX 1

Definitions

1. Coefficient of variation is a dimensionless measure of the variability of a parameter, defined as the ratio of the standard deviation to the mean. This can also be expressed as a percent value. Mean coefficient of variation of average specific growth rate in replicate control cultures should be calculated as follows:

1. Calculate % CV of average specific growth rate out of the daily/section by section growth rates for the respective replicate;
2. Calculate the mean value out of all values calculated under point 1 to get the mean coefficient of variation of the daily/section by section specific growth rate in replicate control cultures.

2. EC_x is the concentration of the test substance dissolved in test medium that results in an x % (e.g. 50 %) reduction in growth of the test organisms within a stated exposure period.

3. Growth rate (average specific growth rate) is the logarithmic increase in biomass during the exposure period.

4. Lowest Observed Effect Concentration (LOEC) is the lowest tested concentration at which the substance is observed to have a statistically significant reducing effect on growth (at $p < 0.05$) when compared with the control, within a given exposure time.

5. No Observed Effect Concentration (NOEC) is the test concentration immediately below the LOEC.

6. Specific growth rate is a response variable defined as quotient of the difference of the natural logarithms of a parameter of observation and the respective time period.

7. Pre-culture is intended to give an amount of algae suitable for the inoculation of test cultures. The pre-culture is incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of 2 to 4 days.

8. Biomass is the dry weight of living matter present in a population expressed in terms of a given volume; e.g., mg algae/litre test solution. Usually “biomass” is defined as a mass, but in this study this word is used to refer to mass per volume. Also in this study, surrogates for biomass as cell density (cells/mL) from fluorescence method.

ANNEX 2

Composition of f/2 (Guillard & Ryther 1962, Guillard 1975)

Component	Stock solution	Quantity Used	Concentration in Final Medium (M)
$NaNO_3$	75.0 g/L dH_2O	1.0 mL	8.82×10^{-4}
$NaH_2PO\cdot4H_2O$	5.0 g/L dH_2O	1.0 mL	3.62×10^{-5}
$Na_2SiO_3\cdot9H_2O$	30.0 g/L dH_2O	2.0 mL	1.06×10^{-4}
*f/2 Trace metal solution	(see recipe below)	1.0 mL	-
**f/2 Vitamin solution	(see recipe below)	0.5 mL	-
*f/2 Trace metal solution			
Component	Stock solution	Quantity Used	Concentration in Final Medium (M)
$FeCl_3\cdot6H_2O$	-	3.15 g	1.17×10^{-5}
$Na_2EDTA\cdot2H_2O$	-	4.36 g	1.17×10^{-5}
$CuSO_4\cdot5H_2O$	9.8 g/L dH_2O	1.0 mL	3.93×10^{-8}
$Na_2MoO_4\cdot2H_2O$	6.3 g/L dH_2O	1.0 mL	2.60×10^{-8}
$ZnSO_4\cdot7H_2O$	22.0 g/L dH_2O	1.0 mL	7.65×10^{-8}
$CoCl_2\cdot6H_2O$	10.0 g/L dH_2O	1.0 mL	4.20×10^{-8}
$MnCl_2\cdot4H_2O$	180.0 g/L dH_2O	1.0 mL	9.10×10^{-7}
**f/2 Vitamin solution			
Component	Stock solution	Quantity Used	Concentration in Final Medium (M)
Cyanocobalamin (Vitamin B_{12})	1.0 g/L dH_2O	1.0 mL	3.69×10^{-10}
Biotin (Vitamin H)	0.1 g/L dH_2O	10.0 mL	2.05×10^{-9}
Thiamine HCl (vitamin B_1)	-	200.0 mL	2.96×10^{-7}

ANNEX 3

Experiment conditions and acceptability criteria for diatom, *Skeletonema costatum*, acute growth toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Static
Temperature	22 ± 1 ˚C
Light quality	"Cool white" fluorescent lamp
Light intensity	3,000 lux
Photoperiod	Continuous illumination
Test chamber size	15 mL
Test solution volume	10 mL
Renewal of test solutions	None
Age of test organismss	2~4 days
Initial cell density in test chambers	3,000 cells/mL
No. replicate chambers per concentration	3
Shaking rate	Twice daily by hand
Aeration	None
Dilution water	Filtered seawater (Control seawater) by 0.2μm membrane filter
Test concentrations	Effluents: 5 including receiving water 100.00% and a control
Test dilution factor	Effluents: 0.5
Test duration	72 hours
Endpoint	Growth (Cell counts)
Test acceptability criteria	Specific growth rate: ≥ 0.92 day^{-1} Variation coefficient of the control specific growth rate: ≤ 10% Change of the control pH: ≤ 1.5

ANNEX 4

1. The average specific growth rate for a specific period is calculated from equation [1]:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} (\text{day}^{-1}) \qquad [1]$$

where:

μ_{i-j} is the average specific growth rate from time I to j;

X_i is the biomass at time *i*;

X_j is the biomass at time *j*;

2. The percent inhibition of growth rate for each treatment replicate is calculated from equation [2]:

$$\%I_r = \frac{\mu_C - \mu_T}{\mu_C} \times 100 \qquad [2]$$

where:

$\%I_r$ is percent inhibition in average specific growth rate;

μ_C is mean value for average specific growth rate (μ) in the control group ;

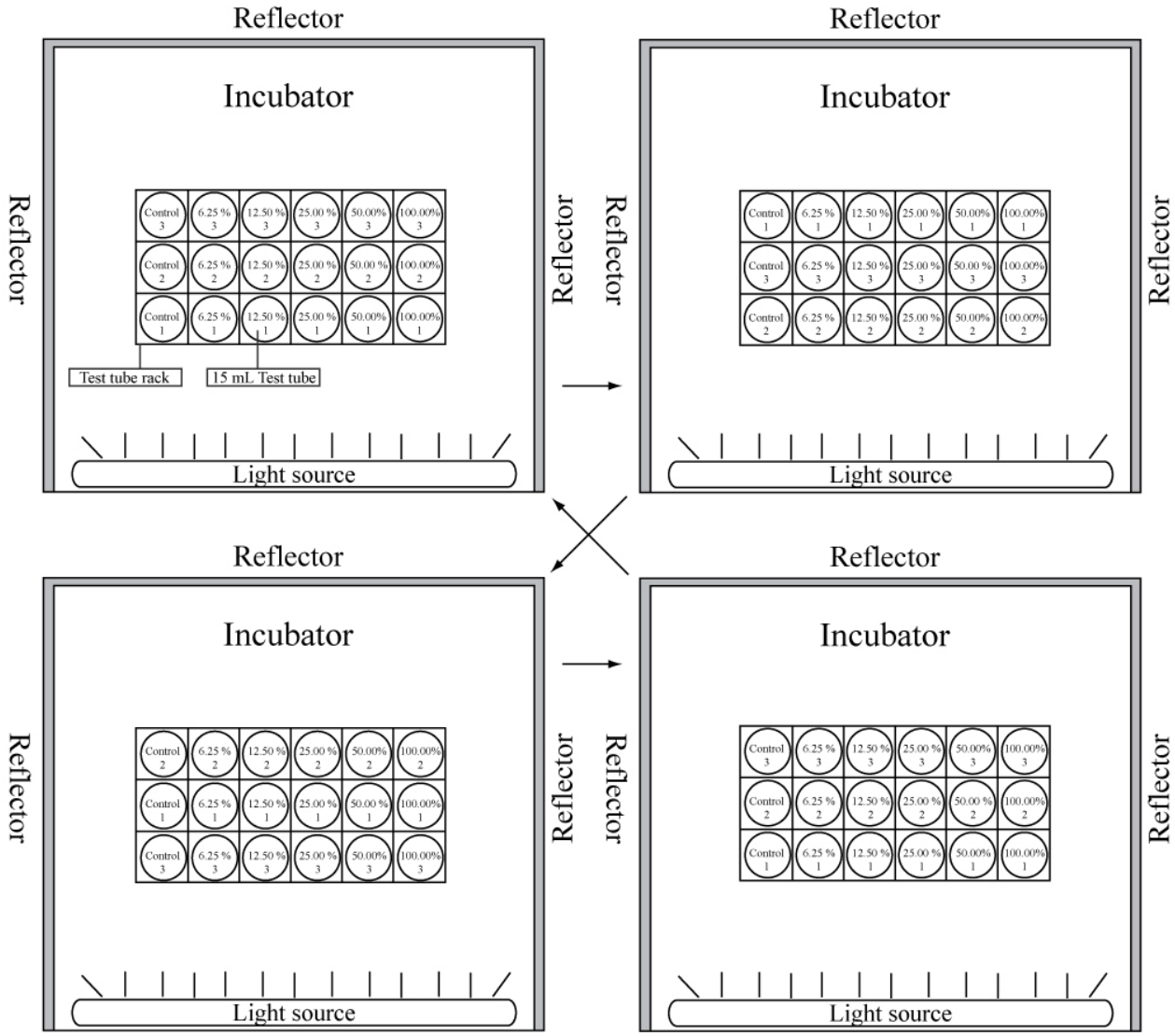
μ_T is average specific growth rate (μ) in treatment replicate

Each treatment group and control group are calculated a mean value for growth rate along with variance estimates.

ANNEX 5

Application Site

Application site for the acute toxicity test with diatom, *Skeletonema costatum* is shown as follow. The arrowheads indicate the systematical rotation of the test vessels.



2.1.2 ANNEX 2

***Brachionus plicatilis*, Acute Toxicity Test**

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

STUDY PLAN

Acute Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- Rotifer, *Brachionus plicatilis* Acute Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

STUDY PLAN PREPARATION AND APPROVAL

Study title: Acute toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward survival of *Brachionus plicatilis*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Test facility Marine Eco-technology Institute Co., Ltd.

Study Director 2011. 04. 13.

Jung-Im Park (signature)

Test site manager (Lab. manager) 2011. 04. 13.

Myung-Baek Shon (signature)

Test facility manager 2011. 04. 13.

Min Ho Son (signature)

Organization KT Marine Co., Ltd.

2011. 04. 13.

Monitor: Seong Ho Park (signature)

Sponsor: Og Yeol Bag (signature)

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1. OUTLINE OF STUDY

1.1 Purpose

The purpose of this study is to assess the acute toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS (or the > 32 and 3-32 PSU de-ballast water from the KTM-BWMS) on the survival of *Brachionus plicatilis*.

1.2 Principle

Neonate of *Brachionus plicatilis* aged less than 2 hours at the start of test is exposed to the treated ballast water by the KTM-BWMS at a range of concentrations including 100.00 % effluent, for a period of 24 hours. Mortality are recorded at 24 hours and compared with control values. The results are analyzed in order to calculate the LC50 at 24 hours.

1.3 Good Laboratory Practice

All procedure of this study is complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

1.4 Guideline

This study using *Brachionus plicatilis* was complied with principle and conducted on the basis of following standard method:

-"Standard Guide for Acute Toxicity Test with the Rotifer *Brachionus*. E 1440-91"

American Society of Testing Materials, (Reapproved 2004)

1.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

1.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax + 82-51-611-0588

1.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.8 Study director

Name: Jung-Im Park

Department: 2nd research team

1.9 Study timetable

1.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 06. 29
Separation of neonates for test	2011. 06. 30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation survival	2011. 07. 01
Experimental completion	2011. 07. 01
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 07. 25
Separation of neonates for test	2011. 07. 26
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation survival	2011. 07. 14
Experimental completion	2011. 07. 14
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.10 Responsible Personnel

Laboratory manager (Test site manager) Statistical analysis	Myung-Baek Shon
Culture of test organisms Observation and record Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Check the chemical condition	Tae Won Kim

1.11 Retention of records and data

1.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

1.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

2. MATERIALS AND METHODS

2.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

2.1.1 Substance name

Treated water

2.1.2 Lot No.

none

2.1.3 Type

liquid

2.1.4 Principal ingredients

Seawater

2.1.5 Temperature

12~26 ˚C

2.1.6 Salinity

> 32 PSU

2.1.7 pH

6~8

2.1.8 Custody condition

Constant room at 4 ˚C for 7 days

2.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.1.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.1.11 Disposal of the resting substances

Scrap after test

2.1.12 Certificate of analysis

Client supplies to test organization

2.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

2.2.1 Substance name

Non-treated water

2.2.2 Lot No.

none

2.2.3 Type

liquid

2.2.4 Principal ingredients

Seawater

2.2.5 Temperature

12~26 ˚C

2.2.6 Salinity

> 32 PSU

2.1.7 pH

6~8

2.2.8 Custody condition

Constant room at 4 ˚C for 7 days

2.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.2.11 Disposal of the resting substances

Scrap after test

2.2.12 Certificate of analysis

Client supplies to test organization

2.3 Test substance-3 (Treated ballast water (3-32 PSU) -Treated)

2.3.1 Substance name

Treated water

2.3.2 Lot No.

none

2.3.3 Type

liquid

2.3.4 Principal ingredients

Seawater

2.3.5 Temperature

12~26 ˚C

2.3.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.3.8 Custody condition

Constant room at 4 ˚C for 7 days

2.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.3.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.3.11 Disposal of the resting substances

Scrap after test

2.3.12 Certificate of analysis

Client supplies to test organization

2.4 Test substance-4 (Non-treated ballast water (3-32 PSU) -Control)

2.4.1 Substance name

Non-treated water

2.4.2 Lot No.

none

2.4.3 Type

liquid

2.4.4 Principal ingredients

Seawater

2.4.5 Temperature

12~26 ˚C

2.4.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.4.8 Custody condition

Constant room at 4 ˚C for 7 days

2.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.4.11 Disposal of the resting substances

Scrap after test

2.4.12 Certificate of analysis

Client supplies to test organization

2.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance is diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid. The six concentrations of test substance including 0.00 % (only non-treated ballast water by the KTM-BWMS) and 100.00 % are arranged for the final definitive experiment.

2.6 Culture condition

2.6.1 Hatching of test organisms (SOP#TO-007)

Test organisms	*Brachionus plicatilis*
Vessel	6 hole plate
Medium	20 PSU filtered seawater (0.45μm membrane filter, Whatman)
Temperature	25 ˚C
Light source	"cool-white" fluorescent lamp
Light cycle	Continuous light
Light cycle	1,000 ~ 3,000 lux

The cysts were obtained from commercial supplier (See '2.7 Test system').

2.6.2 Feeding regime

None

2.7 Test system

2.7.1 Test organisms

.1 Scientific name	*Brachionus plicatilis*
.2 Producer	MicroBioTests Inc., Belgium
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

2.7.2 Ground of selected test organisms

Marine rotifer, *Brachionus plicatilis* have the advantages of cosmopolitan distribution, wide salinity adaptability, importance trophic roles as primary consumer in marine ecosystem, short generation time and easy culture in small spaces. For this reason *Brachionus plicatilis* is commonly used as a toxicity test.

2.7.3 Preparation of the test organisms (SOP#TO-007)

Hatching of rotifer cysts are initiated for approximately 24 h before the start of the test, and have to be carried out in seawater of reduced salinity (20 PSU). They are incubated at 25 ˚C and 1,000 to 3,000 lux continuous illumination. They are checked for the hatching hourly after 24 h incubation in order to collect

the test animals within 2 hours of their hatching. For the > 32 PSU ballast water treated, rotifer neonates are acclimated for approximately 1 hour to adapt to the eventual salinity change.

2.7.4 Check the test organisms for the test (SOP#TO-007)

Health of the test organisms such as the mortality, appearance and abnormal behavior is observed with a microscope (SZ51, Olympus).

2.7.5 Disposal of the resting test organisms (SOP#TO-006)

The resting test organisms are scrapped after sterilization by the autoclave.

2.8 Study design

2.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %:	Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %:	Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %:	Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %:	Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %:	Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %:	Seawater treated by KTM-BWMS	100.00 %

2.8.2 Exposure conditions (SOP#TP-013)

Brachionus plicatilis is placed in test vessels divided into five neonates each replication of concentration including the control. And then the test vessels are placed in incubator for 24 hours at 25 ± 1˚C.

Experiment conditions and criteria are shown Annex 2 and application site is shown Annex 3.

2.8.3 Observation item and measurement components

.1 Mortality (SOP#TP-013)

Each test vessel is checked for mortality of *Brachionus plicatilis* at 24 hours after the beginning of the experiment. Justification of mortality is that animals do not exhibit any internal or external movement in 5 seconds of observation.

.2 Other observations (SOP#TP-013)

In addition to mortality, any abnormal behavior and appearance are recorded.

.3 Temperature (SOP#TE-002)

Temperature of the solutions is measured at the beginning and end of the experiment.

.4 Dissolved oxygen (SOP#TE-002)

Dissolved oxygen (DO) of the solutions is measured at the beginning of the experiment.

(Test vessels contain only 1 mL, it is technically difficult to measure DO at the end of the experiment. It is also known that brachionid rotifers are not sensitive to low oxygen levels.)

.5 pH (SOP#TE-002)

pH of the solutions is measured at the beginning and end of the experiment.

2.9 Statistical procedures (SOP#SP-101)

LC50 values of the end point are estimated by statistical analysis procedure referred from USEPA (2002). TOXCALC 5.0 program (Tidepool scientific software, USA) is used for statistic analysis.

3. REFERENCES

ASTM. 2004. Standard guide for acute toxicity test with the rotifer *Brachionus*. E-1440-91. 8pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

ANNEX 1

Definitions

1. Rotifer cyst is that a rotifer embryo arrested at an early stage in development, enclosed in an envelope and resistant to desiccation and temperature extremes. Rotifer cysts are often incorrectly referred to as resting eggs. Upon hydration, embryonic development resumes until a neonate female emerges from the cyst.

2. Rotifer neonate is a newly hatched, freely swimming rotifer. All neonates hatched from cysts are females.

3. LCx (Lethal concentration) is the concentration estimated to die x percent of the test organisms within a stated exposure period.

ANNEX 2

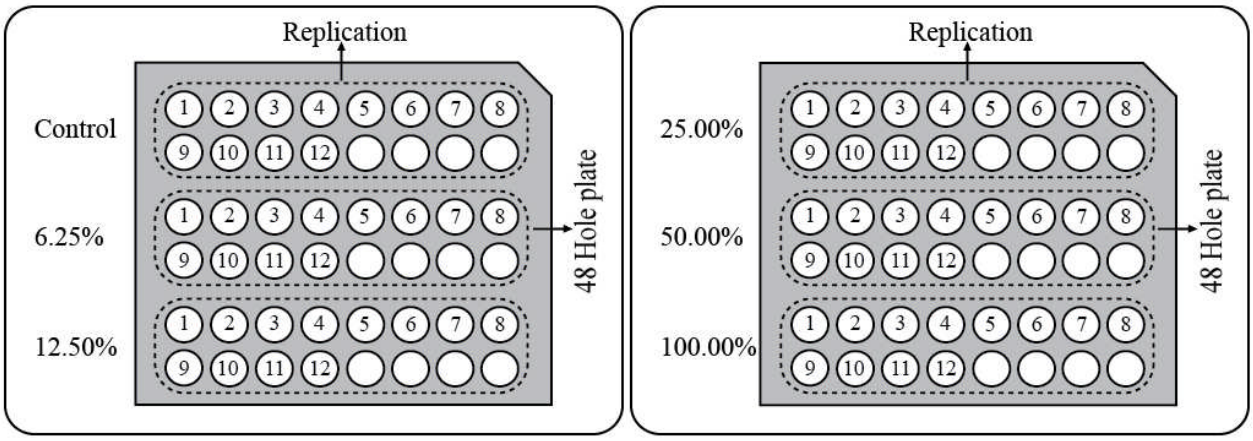
Experiment conditions and acceptability criteria for the rotifer, *Brachionus plicatilis* acute immobilization and survival toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Static (non-renewal)
Temperature	25 ± 1˚C
Light quality	None
Light intensity	Darkness
Photoperiod	0h light: 24 h dark
Test chamber size	1.5 mL (48 hole plate)
Test solution volume	1mL
Renewal of test solutions	None
Age of test organisms	Less than 2 hours
Number of individuals per test chamber	5 inds.
Replicate test chambers per concentration	12
Feeding regime	None
Cleaning	None
Aeration	None
Dilution water	Filtered seawater (Control seawater) by 0.45 µm membrane filter
Test concentration	5 including 100.00% effluents and a control
Dilution factor	Effluents: 0.5
Test duration	24 hours
Endpoints	survival
Test acceptability criteria	90% or greater survival of all control organisms

ANNEX 3

Application Site

Application site for the acute toxicity test with the rotifer, *Brachionus plicatilis* is shown as follow.



2.1.3 ANNEX 3

The olive flounder fish, *Paralichthys olivaceus*, Acute Toxicity Test

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

STUDY PLAN

Acute Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- The olive flounder fish, *Paralichthys olivaceus*, Acute Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

STUDY PLAN PREPARATION AND APPROVAL

Study title: Acute toxicity to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward survival of olive flounder fish, *Paralichthys olivaceus*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Test facility Marine Eco-technology Institute Co., Ltd.

Study Director 2011. 04. 13.

Jin Hee Kim (signature)

Test site manager (Lab. manager) 2011. 04. 13.

Myung-Baek Shon (signature)

Test facility manager 2011. 04. 13.

Min Ho Son (signature)

Organization KT Marine Co., Ltd.

2011. 04. 13.

Monitor: Seong Ho Park (signature)

Sponsor: Og Yeol Bag (signature)

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1. OUTLINE OF STUDY

1.1 Purpose

The purpose of this study is to assess the acute toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS (or the > 32 and 3-32 PSU de-ballast water from the KTM-BWMS) on the survival of juvenile olive flounder, *Paralichthys olivaceus*.

1.2 Principle

The juvenile olive flounder, *Paralichthys olivaceus* is exposed to the treated ballast water of the KTM-BWMS for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentration which kills 50 percent of the fish (LC50) are determined where possible.

1.3 Good Laboratory Practice

All procedure of this study is complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

1.4 Guideline

This study is conducted on the basis of principle of following standard method and applied for *Paralichthys olivaceus*:

- "OECD Guideline for Testing of Chemicals, 203, Fish, Acute Toxicity Test"

Organization for Economic Co-operation and Development, (Adopted: July. 17, 1992)

1.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

1.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611- 6200 Fax: + 82-51-611- 0588

1.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.8 Study Director

Name: Jin Hee Kim

Department: 3rd research team

1.9 Study timetable

1.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Receipt of test organisms	2011. 06. 16
Acclimatization on Lab Condition (> 32 PSU)	2011. 06. 16 ~ 06. 23
Settling in the test vessel	2011. 06. 23
Observation of mortality	2011. 06. 23 ~ 06.30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation of mortality	2011. 06. 30 ~ 07. 04
Experimental completion	2011. 07. 04
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2010. 05. 02
Receipt of test organisms	2011. 06. 16
Acclimatization on Lab Condition (20 PSU)	2011. 06. 16 ~ 07. 05
Settling in the test vessel	2011. 07. 05
Observation of mortality	2011. 07. 05 ~ 07. 12
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13

Evaluation of mortality	2011. 07. 13 ~ 07. 17
Experimental completion	2011. 07. 17
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.10 Responsible Personnel

Laboratory manager (Test site manager) Statistical analysis	Myung-Baek Shon
Culture of test organisms Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Observation and record Check the chemical condition	Tae Won Kim

1.11 Retention of records and data

1.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

1.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

2. MATERIALS AND METHODS

2.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

2.1.1 Substance name

Treated water

2.1.2 Lot No.

none

2.1.3 Type

liquid

2.1.4 Principal ingredients

Seawater

2.1.5 Temperature

12~26 ˚C

2.1.6 Salinity

> 32 PSU

2.1.7 pH

6~8

2.1.8 Custody condition

Constant room at 4 ˚C for 7 days

2.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.1.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.1.11 Disposal of the resting substances

Scrap after test

2.1.12 Certificate of analysis

Client supplies to test organization

2.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

2.2.1 Substance name

Non-treated water

2.2.2 Lot No.

none

2.2.3 Type

liquid

2.2.4 Principal ingredients

Seawater

2.2.5 Temperature

12~26 ˚C

2.2.6 Salinity

> 32 PSU

2.2.7 pH

6~8

2.2.8 Custody condition

Constant room at 4 ˚C for 7 days

2.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.2.11 Disposal of the resting substances

Scrap after test

2.2.12 Certificate of analysis

Client supplies to test organization

2.3 Test substance-3 (Treated ballast water (3-32 PSU) -Treated)

2.3.1 Substance name

Treated water

2.3.2 Lot No.

none

2.3.3 Type

liquid

2.3.4 Principal ingredients

Seawater

2.3.5 Temperature

12~26 ˚C

2.3.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.3.8 Custody condition

Constant room at 4 ˚C for 7 days

2.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.3.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.3.11 Disposal of the resting substances

Scrap after test

2.3.12 Certificate of analysis

Client supplies to test organization

2.4 Test substance-4 (Non-treated ballast water (3-32 PSU) -Control)

2.4.1 Substance name

Non-treated water

2.4.2 Lot No.

none

2.4.3 Type

liquid

2.4.4 Principal ingredients

Seawater

2.4.5 Temperature

12~26 ˚C

2.4.6 Salinity

3-32 PSU

2.4.7 pH

6~8

2.4.8 Custody condition

Constant room at 4 ˚C for 7 days

2.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.4.11 Disposal of the resting substances

Scrap after test

2.4.12 Certificate of analysis

Client supplies to test organization

2.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance is diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid. The six concentrations of test substance including 0.00 % (only non-treated ballast water by the KTM-BWMS) and 100.00 % are arranged for the final definitive experiment.

2.6 Culture condition (SOP#TO-005)

2.6.1 Holding of test organisms

Test organisms	*Paralichthys olivaceus*
Culture type	Semi- recycle filter system
Vessel	1,000 L cubic aquarium
Medium	600 L filtered seawater (1μm CP Filter, Chisso Filter)
Temperature	20 ± 1 ˚C
Light source	“cool-white” fluorescent lamp
Light cycle	L : D = 16 h :8 h
Light intensity	1,000 lux
Dissolved oxygen	6.0~8.0 mg/L

2.6.2 Feeding regime

Type of diet	Su hyup feed for fish 1-1S
Supplier	National Federation of Fisheries Cooperatives
Diet supply period	once a day

2.7 Test system

2.7.1 Test organisms

.1 Scientific name	*Paralichthys olivaceus*
.2 Producer	KyeongYang Fisheries, Yo-Su city, Jeolla province, Republic of Korea
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

2.7.2 Ground of selected test organisms

Pleuronectiformes Paralichthyidae *Paralichthys olivaceus* called olive flounder is widely distributed in coastal water of Korea and is important as economic marine products. *P. olivaceus* plays in marine trophic structure as a top predator.

2.7.3 Preparation of the test organisms (SOP#TP-006)

Paralichthys olivaceus aged 30 ~ 50 days is acclimated under the laboratory and test condition for two or three weeks until 24 hours before the beginning of the experiment. Following a 48 hours settling-in period, mortality is recorded and the following criteria applied:

1. Mortality of greater than 10 percent of population in seven days: rejection of entire batch.
2. Mortality of between 5 and 10 percent of population: acclimatization continued for seven additional 7 days.
3. Mortality of less than 5 percent of population: acceptance of batch.

2.7.4 Check the test organisms for the experiment (SOP#TP-006)

Health of the test organisms such as the mortality, appearance and abnormal behavior is observed.

2.7.5 Disposal of the remaining test organisms (SOP#TO-006)

The resting test organisms are euthanized under tricaine methanesulfonate (MS-222).

2.8 Study design

2.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

2.8.2 Exposure conditions (SOP#TP-006)

20 fish of *Paralichthys olivaceus* on the > 32 and 3-32 PSU treated ballast water is placed in the each replication of test vessel. The test vessels are placed in the constant room for 96 hours at 20 ± 1 ˚C.

Experiment conditions and criteria are shown Annex 2 and application site is shown Annex 3.

2.8.3 Observation item and measurement components

.1 Mortality (SOP#TP-006)

Fish will be considered dead if there is no visible movement and if touching of the caudal peduncle produces no reaction. Mortalities are recorded and then dead animals are removed.

.2 Other observations (SOP#TP-006)

Records are kept of visible abnormalities, such as loss of equilibrium, swimming behavior,

respiratory function and pigmentation.

.3 Temperature (SOP#TE-002)

Temperature of the solutions on each vessel during the experiment period is measured daily.

.4 Dissolved oxygen (SOP#TE-002)

DO of the solutions on each vessel during the experiment period is measured daily.

.5 pH (SOP#TE-002)

pH of the solutions on each vessel during the experiment period is measured daily.

2.9 Statistical procedures (SOP#SP-101)

LC25 and LC50 values of the end point are estimated by statistical analysis procedure referred from USEPA (2002). TOXCALC 5.0 program (Tidepool scientific software, USA) is used for statistic analysis.

3. REFERENCES

OECD guidelines for the testing of chemicals-203. 1992. Fish, Acute toxicity test. 9 pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

ANNEX 1

Definitions

1. Semi-static test is a test without flow of solution, but with occasional batch wise renewal of the test solution after prolonged periods.

2. LC25 and LC50 in this study is the median lethal concentration, i.e. that concentration of the test substance in water which kills 25 percent and 50 percent of a test batch of fish within a particular period of exposure.

ANNEX 2

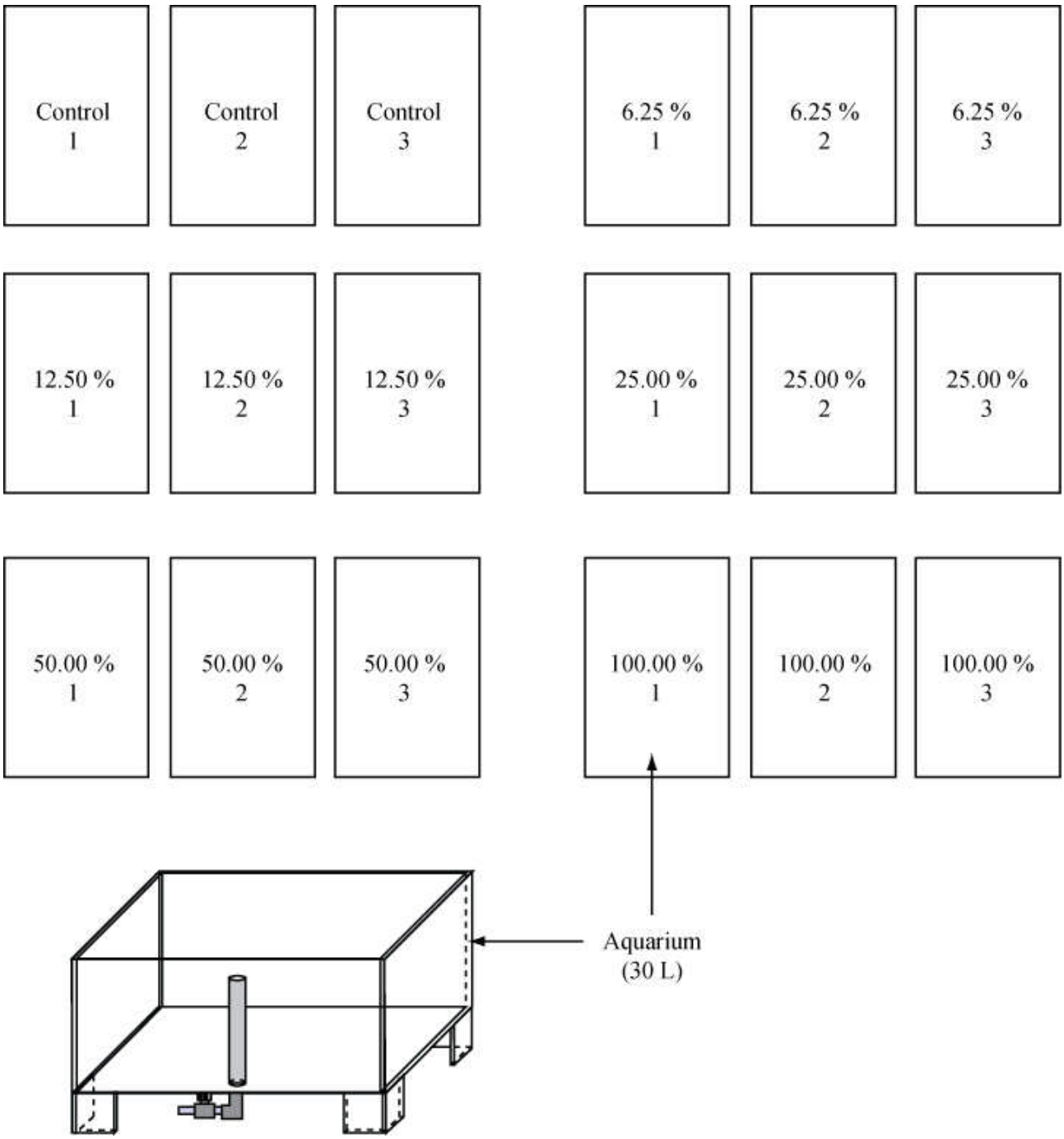
Experiment conditions and acceptability criteria for olive flounder, *Paralichthys olivaceus*, acute mortality toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Semi-static
Temperature	20±1˚C
Light quality	“Cool white” fluorescent lighting
Light intensity	1,000 lux
Photoperiod	16 h light: 8 h dark
Test chamber size	30 L
Test solution volume	20 L
Renewal of test solutions	Every other day
Age of test organisms	Less than 50 days old
Number of individuals per test chamber	20
Replicate test chambers per concentration	3
Feeding regime	None
Cleaning	None
Aeration	To maintain DO (> 60 % of the air saturation value)
Dilution water	Filtered seawater (Control seawater) by 1μm CP Filter, Chisso Filter
Test concentration	5 including 100.00% effluents and a control
Dilution factor	Effluents: 0.5
Test duration	96 hours
Endpoints	Survival
Test acceptability criteria	90% or greater survival of all control organisms

ANNEX 3

Application Site

Application site for the acute toxicity test with olive flounder, *Paralichthys olivaceus* is shown as follow.



2.2 Chronic Toxicity

2.2.1 ANNEX 1

***Brachionus plicatilis*, Chronic Toxicity Test**

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

STUDY PLAN

Chronic Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- Rotifer, *Brachionus plicatilis*, Chronic Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

STUDY PLAN PREPARATION AND APPROVAL

Study title: Chronic toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward population growth rate of *Brachionus plicatilis*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Test facility Marine Eco-technology Institute Co., Ltd.

Study Director 2011. 04. 13.

Jung-Im Park (signature)

Test site manager (Lab. manager) 2011. 04. 13.

Myung-Baek Shon (signature)

Test facility manager 2011. 04. 13.

Min Ho Son (signature)

Organization KT Marine Co., Ltd.

2011. 04. 13.

Monitor: Seong Ho Park (signature)

Sponsor: Og Yeol Bag (signature)

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1. OUTLINE OF STUDY

1.1 Purpose

The purpose of this study is to assess the chronic toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS (or the > 32 and 3-32 PSU de-ballast water from the KTM-BWMS) on the population growth rate of *Brachionus plicatilis*.

1.2 Principle

Neonate of *Brachionus plicatilis* aged less than 2 hours at the start of test is exposed to the treated ballast water of the KTM-BWMS at a range of concentrations including 100.00% effluent, for a period of 96 hours. The rotifer density is counted every 24 hours until 96 hours. At the end of the experiment, the population growth rate (*r*) is calculated that count the number of live rotifers in each test concentration compared with control values. And the percentage inhibition (*I*%) of the rotifer growth for each test concentration is evaluated. The results are analyzed in order to calculate the NOEC, LOEC and EC50 at 96 hours.

1.3 Good Laboratory Practice

All procedure of this study is complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

1.4 Guideline

This study is conducted on the basis of principle of following standard method and applied for *Brachionus plicatilis*:

- "Cyst-based toxicity tests. VIII. Short-chronic toxicity tests with the freshwater rotifer *Brachionus calyciflorus*" (Janssen et al., 1994)

The test preparation in this study is complied on the basis of following standard method:

- "Standard Guide for Acute Toxicity Test with the Rotifer *Brachionus*. E 1440-91"

American Society of Testing Materials, (Reapproved 2004)

1.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

1.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax + 82-51-611-0588

1.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.8 Study director

Name: Jung-Im Park

Department: 2nd research team

1.9 Study timetable

1.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 06. 29
Separation of neonates for test	2011. 06. 30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation population growth rate	2011. 06. 30 ~ 07. 04
Experimental completion	2011. 07. 04
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 07. 12
Separation of neonates for test	2011. 07. 13
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13

Exposure date	2011. 07. 13
Evaluation population growth rate	2011. 07. 13 ~ 07. 17
Experimental completion	2011. 07. 17
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

1.10 Responsible Personnel

Laboratory manager (Test site manager) Statistical analysis	Myung-Baek Shon
Culture of test organisms Observation and record Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Check the chemical condition	Tae Won Kim

1.11 Retention of records and data

1.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

1.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

2. MATERIALS AND METHODS

2.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

2.1.1 Substance name

Treated water

2.1.2 Lot No.

none

2.1.3 Type

liquid

2.1.4 Principal ingredients

Seawater

2.1.5 Temperature

12~26 ˚C

2.1.6 Salinity

> 32 PSU

2.1.7 pH

6~8

2.1.8 Custody condition

Constant room at 4 ˚C for 7 days

2.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.1.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.1.11 Disposal of the resting substances

Scrap after test

2.1.12 Certificate of analysis

Client supplies to test organization

2.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

2.2.1 Substance name

Non-treated water

2.2.2 Lot No.

none

2.2.3 Type

liquid

2.2.4 Principal ingredients

Seawater

2.2.5 Temperature

12~26 ˚C

2.2.6 Salinity

> 32 PSU

2.1.7 pH

6~8

2.2.8 Custody condition

Constant room at 4 ˚C for 7 days

2.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.2.11 Disposal of the resting substances

Scrap after test

2.2.12 Certificate of analysis

Client supplies to test organization

2.3 Test substance-3 (Treated ballast water (3-32 PSU) -Treated)

2.3.1 Substance name

Treated water

2.3.2 Lot No.

none

2.3.3 Type

liquid

2.3.4 Principal ingredients

Seawater

2.3.5 Temperature

12~26 ˚C

2.3.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.3.8 Custody condition

Constant room at 4 ˚C for 7 days

2.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.3.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.3.11 Disposal of the resting substances

Scrap after test

2.3.12 Certificate of analysis

Client supplies to test organization

2.4 Test substance-4 (Non-treated ballast water (3-32 PSU) -Control)

2.4.1 Substance name

Non-treated water

2.4.2 Lot No.

none

2.4.3 Type

liquid

2.4.4 Principal ingredients

Seawater

2.4.5 Temperature

12~26 ˚C

2.4.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.4.8 Custody condition

Constant room at 4 ˚C for 7 days

2.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.4.11 Disposal of the resting substances

Scrap after test

2.4.12 Certificate of analysis

Client supplies to test organization

2.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance is diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid. The six concentrations of test substance including 0.00 % (only non-treated ballast water by the KTM-BWMS) and 100.00 % are arranged for the final definitive experiment.

2.6 Culture condition

2.6.1 Hatching of test organisms (SOP#TO-007)

Test organisms	*Brachionus plicatilis*
Vessel	6 hole plate
Medium	20 PSU filtered seawater (0.45μm membrane filter, Whatman)
Temperature	25 ˚C
Light source	"cool-white" fluorescent lamp
Light cycle	Continuous light
Light cycle	1,000 ~ 3,000 lux

The cysts were obtained from commercial supplier (See '2.7 Test system').

2.6.2 Feeding regime

None

2.7 Test system

2.7.1 Test organisms

.1 Scientific name	*Brachionus plicatilis*
.2 Producer	MicroBioTests Inc., Belgium
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

2.7.2 Ground of selected test organisms

Marine rotifer, *Brachionus plicatilis* have the advantages of cosmopolitan distribution, wide salinity adaptability, importance trophic roles as primary consumer in marine ecosystem, short generation time and easy culture in small spaces. For this reason *Brachionus plicatilis* is commonly used as a toxicity test.

2.7.3 Preparation of the test organisms (SOP#TO-007)

Hatch of rotifer cyst is initiated for approximately 24 h before the start of the test, and have to be carried out in seawater of reduced salinity (20 PSU). They are incubated at 25 ˚C and 1,000 to 3,000 lux continuous illumination. They are checked the hatching hourly after 24 h incubation in order to collect the test animals

within 2 hours of their hatching. For the > 32 PSU ballast water treated, rotifer neonates are acclimated for approximately 1 hour to adapt to the eventual salinity change.

2.7.4 Check the test organisms for the test (SOP#TO-007)

Health of the test organisms such as the mortality, appearance and abnormal behavior is observed with a microscope (SZ51, Olympus).

2.7.5 Disposal of the resting test organisms (SOP#TO-006)

The resting test organisms are scrapped after sterilization by the autoclave.

2.8 Study design

2.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

2.8.2 Exposure conditions (SOP#TP-014)

Brachionus plicatilis is placed in test vessels containing food (*Chlorella vulgaris* , 1×10^6 cells/mL) divided into five neonates each replication of concentration including the control. And then the test vessels are placed in incubator for 96 hours at 25 ± 1˚C.

Experiment conditions and criteria are shown Annex 2 and application site is shown Annex 4.

2.8.3 Observation item and measurement components

.1 Population growth rate (SOP#TP-014)

Each test vessel is checked for density of *Brachionus plicatilis* every 24 hours after the beginning of the experiment. Mortalities are recorded and then dead animals are removed.

Calculations of the population growth rate and the percentage inhibition growth are shown Annex 3.

.2 Other observations (SOP#TP-014)

In addition to immobility and mortality, any abnormal behavior and appearance are recorded.

.3 Temperature (SOP#TE-002)

Temperature of the solutions is measured at the beginning and end of the experiment.

.4 Dissolved oxygen (SOP#TE-002)

Dissolved oxygen (DO) of the solutions is measured at the beginning of the experiment.

(Test vessels contain only 1mL, it is technically difficult to measure DO at the end of the experiment. It is also known that brachionid rotifers are not sensitive to low oxygen levels.)

.5 pH (SOP#TE-002)

pH of the solutions is measured at the beginning and end of the experiment.

2.9 Statistical procedures (SOP#SP-101)

LC50 values of the end point are estimated by statistical analysis procedure referred from USEPA (2002). TOXCALC 5.0 program (Tidepool scientific software, USA) is used for statistic analysis.

3. REFERENCES

ASTM. 2004. Standard guide for acute toxicity test with the rotifer *Brachionus*. E-1440-91. 8pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

Janssen, C.R., G. Persoone and T.W. Snell. 1994. Cyst-based toxicity tests. VIII. Short-chronic toxicity tests with the freshwater rotifer *Brachionus calyciflorus*. Aquatic Toxicology, 28, 243-258.

ANNEX 1

Definitions

1. Rotifer cyst is that a rotifer embryo arrested at an early stage in development, enclosed in an envelope and resistant to desiccation and temperature extremes. Rotifer cysts are often incorrectly referred to as resting eggs. Upon hydration, embryonic development resumes until a neonate female emerges from the cyst.

2. Rotifer neonate is a newly hatched, freely swimming rotifer. All neonates hatched from cysts are females.

3. ECx is the concentration of the test substance dissolved in water that results in a x percent reduction in reproduction of *Brachionus plicatilis* within a stated exposure period.

4. Lowest Observed Effect Concentration (LOEC) is the lowest tested concentration at which the substance is observed to have a statistically significant effect on reproduction (at $p < 0.05$) when compared with the control, within a stated exposure period.

5. No Observed Effect Concentration (NOEC) is the test concentration immediately below the LOEC.

ANNEX 2

Experiment conditions and acceptability criteria for the rotifer, *Brachionus plicatilis* chronic population growth rate toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Static (non-renewal)
Temperature	25 ± 1˚C
Light quality	None
Light intensity	Darkness
Photoperiod	0h light: 24 h dark
Test chamber size	1.5 mL (48 hole plate)
Test solution volume	1 mL
Renewal of test solutions	None
Age of test organisms	Less than 2 hours
Number of individuals per test chamber	5 inds.
Replicate test chambers per concentration	12
Feeding regime	Once at the beginning of the experiment (*Chlorella vulgaris* , 1×10^6 cells/mL)
Cleaning	None
Aeration	None
Dilution water	Filtered seawater (Control seawater) by 0.45 µm membrane filter
Test concentration	5 including 100.00% effluents and a control
Dilution factor	Effluents: 0.5
Test duration	96 hours
Endpoints	population growth rate
Test acceptability criteria	- Population growth rate in the control : ≥ 0.55 - Percentage growth inhibition in the lowest toxicant concentration: < 50%

ANNEX 3

1. The population growth rate for a specific period is calculated from equation [1]:

$$r_m = \frac{\ln N_t - \ln N_0}{t} \qquad [1]$$

where:

r_m is the intrinsic rate of natural increase in the population growth experiment.

N_0 and N_t are represented the population size at t=0 and t=4, respectively.

2. The percentage growth inhibition for each treatment replicate is calculated from equation [2]:

$$I\% = \frac{N_{control} - N_{toxicant}}{N_{control}} \times 100 \qquad [2]$$

where:

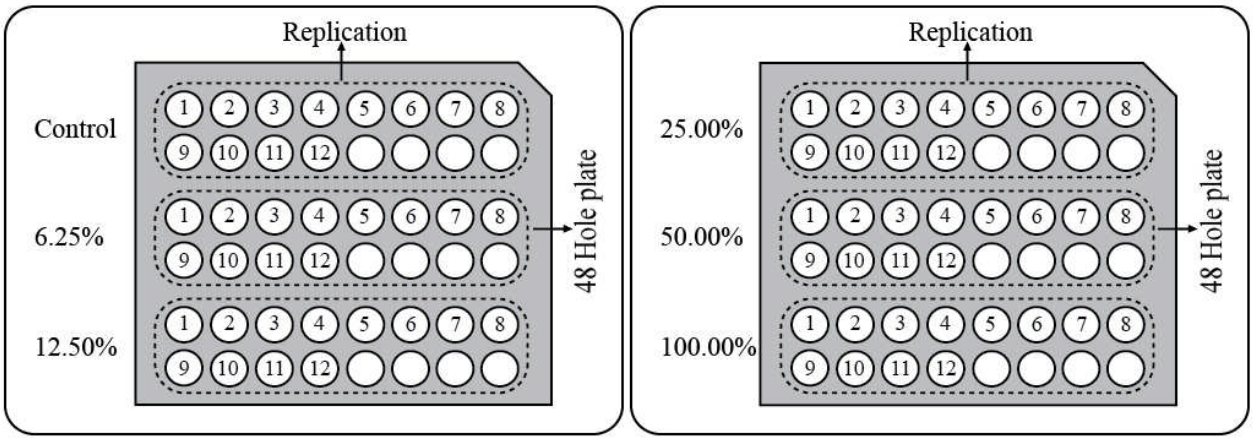
$I\%$ is percent inhibition in average growth rate.

N are represented the population in each test concentrations.

ANNEX 4

Application Site

Application site for the chronic toxicity test with the rotifer, *Brachionus plicatilis* is shown as follow.



2.2.2 ANNEX 2

The olive flounder fish, *Paralichthys olivaceus*, Chronic Toxicity Test

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

STUDY PLAN

Chronic Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- The olive flounder fish, *Paralichthys olivaceus*, Chronic Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

STUDY PLAN PREPARATION AND APPROVAL

Study title: Chronic toxicity to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward survival of olive flounder fish, *Paralichthys olivaceus*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Test facility Marine Eco-technology Institute Co., Ltd.

Study Director 2011. 04. 13.

Jin Hee Kim (signature)

Test site manager (Lab. manager) 2011. 04. 13.

Myung-Baek Shon (signature)

Test facility manager 2011. 04. 13.

Min Ho Son (signature)

Organization KT Marine Co., Ltd.

2011. 04. 13.

Monitor: Seong Ho Park (signature)

Sponsor: Og Yeol Bag (signature)

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1. OUTLINE OF STUDY

1.1 Purpose

The purpose of this study is to assess the chronic toxic effects of the > 32 and 3-32 PSU ballast water treated by KTM-BWMS (or the > 32 and 3-32 PSU de-ballast water from the KTM-BWMS) on the survival concerning early life stage of olive flounder, *Paralichthys olivaceus*.

1.2 Principle

The embryo and sac-fry stages of olive flounder fish, *Paralichthys olivaceus* are exposed to the treated ballast water by the KTM-BWMS for 7 days. The test is begun by placing fertilized eggs in the test chambers and terminated just before complete absorption of the yolk-sac of any larvae or mortalities by starvation start in any of the test chambers in the control. Lethal and sub-lethal effects are assessed and compared with the control.

1.3 Good Laboratory Practice

All procedure of this study is complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

1.4 Guideline

This study is complied with principle and conducted on the basis of following standard method and applied for olive flounder, *Paralichthys olivaceus*:

- "OECD Guideline for Testing of Chemicals, 212, Fish, Short-term Toxicity Test on the Embryo and Sac-fry Stages"

Organization for Economic Co-operation and Development, (Adopted: September. 21, 1998)

1.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

1.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax: + 82-51-611-0588

1.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.8 Study director

Name: Jin Hee Kim

Department: 3rd research team

1.9 Study timetable

1.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Receipt of test organisms	2011. 06. 30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation of mortality	2011. 06. 30 ~ 07. 07
Experimental completion	2011. 07. 07
Draft of final report	2011. 07. 21
Final Report	2011. 07. 22
Study completion	2013. 07. 31

1.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Receipt of test organisms	2011. 07. 13
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation of mortality	2011. 07. 13 ~ 07. 20
Experimental completion	2011. 07. 20
Draft of final report	2011. 07. 21
Final Report	2011. 07. 22
Study completion	2013. 07. 31

1.10 Responsible Personnel

Laboratory manager (Test site manager) Statistical analysis	Myung-Baek Shon
Culture of test organisms Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Observation and record Check the chemical condition	Tae Won Kim

1.11 Retention of records and data

1.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

1.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

1.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

2. MATERIALS AND METHODS

2.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

2.1.1 Substance name

Treated water

2.1.2 Lot No.

none

2.1.3 Type

liquid

2.1.4 Principal ingredients

Seawater

2.1.5 Temperature

12~26 ˚C

2.1.6 Salinity

> 32 PSU

2.1.7 pH

6~8

2.1.8 Custody condition

Constant room at 4 ˚C for 7 days

2.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.1.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.1.11 Disposal of the resting substances

Scrap after test

2.1.12 Certificate of analysis

Client supplies to test organization

2.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

2.2.1 Substance name

Non-treated water

2.2.2 Lot No.

none

2.2.3 Type

liquid

2.2.4 Principal ingredients

Seawater

2.2.5 Temperature

12~26 ˚C

2.2.6 Salinity

> 32 PSU

2.2.7 pH

6~8

2.2.8 Custody condition

Constant room at 4 ˚C for 7 days

2.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.2.11 Disposal of the resting substances

Scrap after test

2.2.12 Certificate of analysis

Client supplies to test organization

2.3 Test substance-3 (Treated ballast water (3-32 PSU) -Treated)

2.3.1 Substance name

Treated water

2.3.2 Lot No.

none

2.3.3 Type

liquid

2.3.4 Principal ingredients

Seawater

2.3.5 Temperature

12~26 ˚C

2.3.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.3.8 Custody condition

Constant room at 4 ˚C for 7 days

2.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.3.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.3.11 Disposal of the resting substances

Scrap after test

2.3.12 Certificate of analysis

Client supplies to test organization

2.4 Test substance-4 (Non-treated ballast water (3-32 PSU) -Control)

2.4.1 Substance name

Non-treated water

2.4.2 Lot No.

none

2.4.3 Type

liquid

2.4.4 Principal ingredients

Seawater

2.4.5 Temperature

12~26 ˚C

2.4.6 Salinity

3-32 PSU

2.3.7 pH

6~8

2.4.8 Custody condition

Constant room at 4 ˚C for 7 days

2.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

2.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.4.11 Disposal of the resting substances

Scrap after test

2.4.12 Certificate of analysis

Client supplies to test organization

2.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance is diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid. The six concentrations of test substance including 0.00 % (only non-treated ballast water by the KTM-BWMS) and 100.00 % are arranged for the final definitive experiment.

2.6 Culture condition

2.6.1 Holding of test organisms

Test organisms	*Paralichthys olivaceus*
Culture type	-
Vessel	-
Medium	-
Temperature	-
Light source	-
Light cycle	-
Light intensity	-
Dissolved oxygen	-

The eggs were obtained from commercial supplier (See '2.7 Test system').

2.6.2 Feeding regime

None

2.7 Test system

2.7.1 Test organisms

.1 Scientific name	*Paralichthys olivaceus*
.2 Producer	KyeongYang Fisheries, Yo-Su city, Jeolla province, Republic of Korea
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

2.7.2 Ground of selected test organisms

Pleuronectiformes Paralichthyidae *Paralichthys olivaceus* called olive flounder is widely distributed in coastal water of Korea and is important as economic marine products. *P. olivaceus* plays in marine trophic structure as a top predator.

2.7.3 Preparation of the test organisms (SOP#TP-007)

The fertilized eggs are obtained from commercial supplier. As the sensitivity of the experiment can be seriously influenced by delaying the start of the test, the experiment is initiated within 8 hours after fertilization.

2.7.4 Check the test organisms for the experiment (SOP#TP-007)

Health of the eggs such as the color and appearance is observed.

2.7.5 Disposal of the remaining test organisms (SOP#TO-006)

The resting test organisms are scrapped after test.

2.8 Study design

2.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %:	Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %:	Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %:	Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %:	Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %:	Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %:	Seawater treated by KTM-BWMS	100.00 %

2.8.2 Exposure conditions (SOP#TP-007)

30 eggs are placed in the each replication of test vessel for the > 32 and 3-32 PSU treated ballast water. And then the test vessels are placed in the constant room for 7 days at 20 ± 1˚C.

Experiment conditions and criteria are shown Annex 2 and application site is shown Annex 4.

2.8.3 Observation item and measurement components

.1 Hatching and survival (SOP#TP-007)

Eggs are considered dead if they are a marked loss of translucency and change in coloration. Embryos are considered dead if they are absence of body movement and/or absence of heart-beat and/or opaque discoloration. Larvae are considered dead if they have immobility and/or absence of respiration movement and/or absence of heart-beat and/or opaque coloration of central nervous system and/or lack of reaction mechanical stimulus. Dead eggs, embryos and larvae are removed after record.

.2 Length and weight (SOP#TP-010)

Total length and dry weights are measured at the end of the experiment.

.3 Stage of embryonic development (SOP#TP-007)

Embryonic development is observed and recorded as remark.

.4 Temperature (SOP#TE-002)

Temperature of the solutions on each vessel during the experiment period is measured daily.

.5 Dissolved oxygen (SOP#TE-002)

DO of the solutions on each vessel during the experiment period is measured daily.

.6 pH (SOP#TE-002)

pH of the solutions on each vessel during the experiment period is measured daily.

.7 Salinity (SOP#TE-002)

Salinity of the solutions during the experiment period is measured daily.

2.9 Statistical procedures (SOP#SP-101)

NOEC, LOEC, LC25 and LC50 values of the end point are estimated by statistical analysis procedure referred from USEPA (2002). TOXCALC 5.0 program (Tidepool scientific software, USA) is used for statistic analysis.

3. REFERENCES

OECD guidelines for the testing of chemicals-212. Fish, Short-term toxicity test on the embryo and sac-fry stages. 20pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

ANNEX 1

Definitions

1. Semi-static test is a test without flow of solution, but with occasional batch wise renewal of the test solution after prolonged periods.

2. LC25 and LC50 in this study is the median lethal concentration, i.e. that concentration of the test substance in water which kills 25 percent and 50 percent of a test batch of fish within a particular period of exposure.

3. Lowest Observed Effect Concentration (LOEC) is the lowest tested concentration at which the substance is observed to have a statistically significant reducing effect on survival (at $p < 0.05$) when compared with the control, within a given exposure time. However, all test concentrations above the LOEC must have a harmful effect equal to or greater than those observed at the LOEC

4. No Observed Effect Concentration (NOEC) is the test concentration immediately below the LOEC.

ANNEX 2

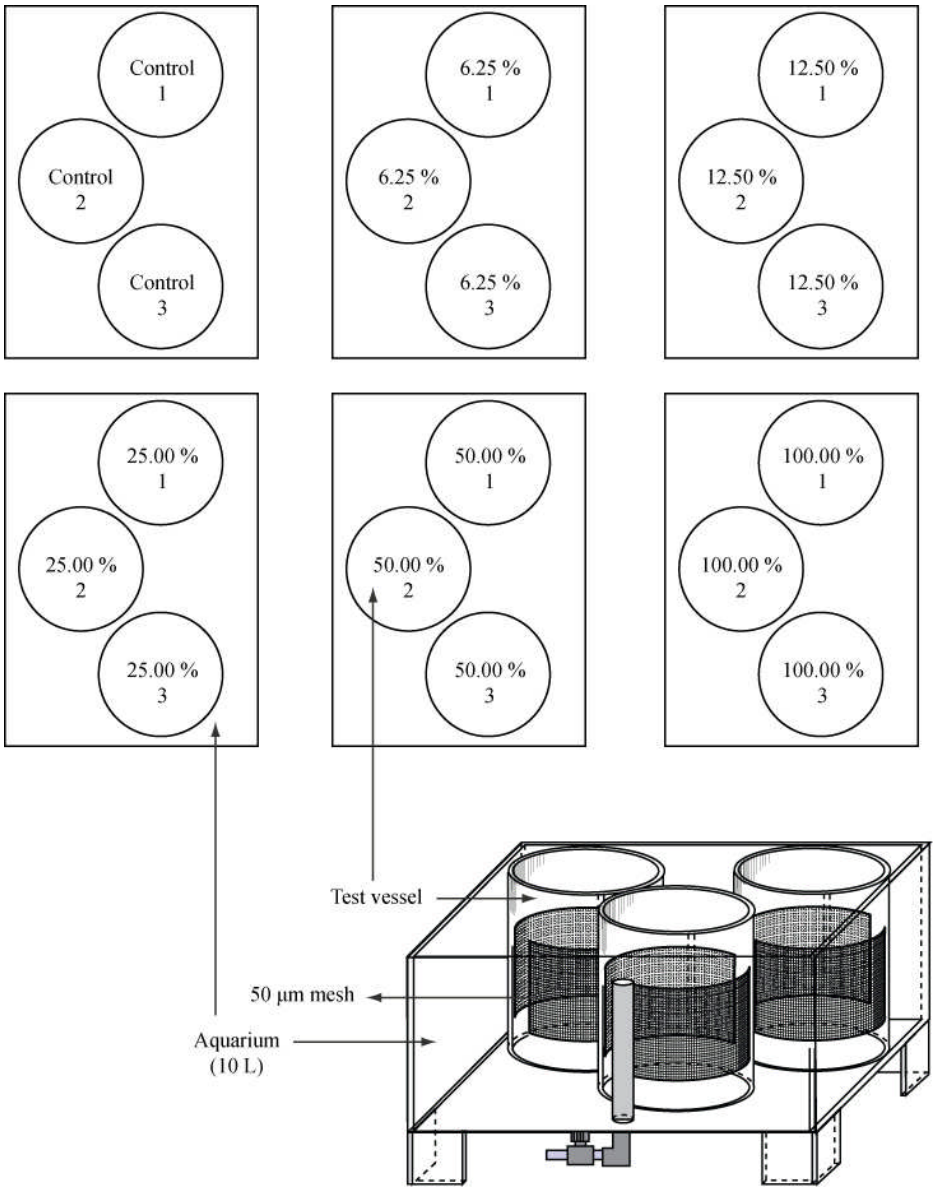
Experiment conditions and acceptability criteria for embryo and sac-fry stage of olive flounder, *Paralichthys olivaceus*, chronic mortality toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Semi-static
Temperature	20±1˚C
Light quality	“Cool white” fluorescent lighting
Light intensity	2,000 lux
Photoperiod	16 h light: 8 h dark
Test chamber size	1 L
Test solution volume	0.8 L
Renewal of test solutions	Every other day
Age of test organisms	Less than 8 h after fertilization
Number of individuals per test chamber	30
Replicate test chambers per concentration	3
Feeding regime	None
Cleaning	Exchange the test water
Aeration	To maintain DO (> 60 % of the air saturation value)
Dilution water	Filtered seawater (Control seawater) by 1μm CP Filter, Chisso Filter
Test concentration	5 including 100.00% effluents and a control
Dilution factor	Effluents: 0.5
Test duration	7 days
Endpoints	Survival
Test acceptability criteria	- The water temperature change: less than ± 1.5 ˚C - Overall survival of fertilized eggs in the controls: more than 70 %

ANNEX 3

Application Site

Application site for the chronic toxicity test with the fertilized eggs of olive flounder, *Paralichthys olivaceus* is shown as follow.



3. FINAL REPORT

3.1 Acute Toxicity

3.1.1 ANNEX 1

***Skeletonema costatum*, Growth Inhibition Test**

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
Tel +82-51-611-6200
Fax +82-51-611-0588

FINAL REPORT

Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- *Skeletonema costatum*, Growth Inhibition Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

GLP STATEMENT AND SIGN

Study title: Acute toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward algae growth inhibition

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

All procedure of this study was carried out following GLP regulation:

- "OECD Principles of Good Laboratory Practice"
  Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

This study was conducted on the basis of 'the Study Plan' complied and the procedure described in this report was carried out under 'the Responsibility of Study Director'. This report was built on the basis of raw data from the study.

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.

Study Director Myung-Baek Shon (signature)

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.

Test Facility Manager Min Ho Son (signature)

GUARANTEE OF ASSURANCE

Study title: Acute toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward algae growth inhibition

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Quality assurance personnel of Marine Eco-Technology Institute Co., Ltd., inspected as follows. Inspection was carried out on the basis of the Standard Operation Procedure and each inspection results were reported to Study Director and Test Facility Manager.

Types of inspection and the date inspection results were reported to the Test Facility Management and the Study Director as follow:

Type of Inspections	Date of Inspection	Date Reported to Test Facility Management and Study Director
Study Plan	2011. 04. 13	2011. 04. 13
Test organisms	2011. 06. 20 / 07. 15	2011. 06. 21 / 07. 15
Storage of Test substance	2011. 06. 29 / 07. 12	2011. 06. 29 / 07. 12
Preparation of Test substance	2011. 06. 29 / 07. 12	2011. 06. 29 / 07. 12
Exposure	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Observation	2011. 07. 01 / 07. 14	2011. 07. 01 / 07. 15
Record of raw data	2011. 07. 01 / 07. 14	2011. 07. 01 / 07. 15
Draft of final report	2011. 07. 19	2011. 07. 19
Final report	2011. 07. 20	2011. 07. 20

It is assurance that the procedure in this final report was appropriately carried out and the description of the results was reflected raw data from the study.

2011. 07. 20.

Quality Assurance personnel Jung-Im Park (signature)
Marine Eco-Technology Institute Co., Ltd.

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1. SUMMARY

The purpose of this study was to assess the acute toxic effects of the > 32 and 3-32 PSU seawater treated by the KTM-BWMS on the growth of diatom, *Skeletonema costatum*.

The test substances were divided into two different salinities, the one was > 32 PSU treated ballast water and the other was 3-32 PSU treated ballast water. Non-treated ballast water was used as control and dilution water. Diatom, *Skeletonema costatum* inoculated 3,757 cells/mL at > 32 PSU and 3,255 cells/mL at 3-32 PSU on the exponential phase was incubated at the various test concentrations (0.00 % (non-treated ballast water), 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % (treated ballast water)) for 72 hours. The cell density was counted daily by measuring chlorophyll *a* with 90% acetone using Tuner Designs AU-10 including the control. To obtain the regression equation between chlorophyll *a* and cell density, cell count in the control was done by manual cell counting used the microscope with counting chamber. The raw data of growth from this experiment was used for calculation of inhibition of growth rate and values of EC50, LOEC and NOEC.

For > 32 PSU treated ballast water, growth curves based on the cell density calculated from chlorophyll *a* value show that the growth at all test concentrations of the treated ballast water was similar to control except for 100.00 % during the experiment period (72 hours). But, percent inhibition in specific average growth rate at 50.00 % test concentration was slightly higher than other test concentrations at 72 hour and growth of *S. costatum* was inhibited at 100.00 % test concentration.

For 3-32 PSU treated ballast water, growth curves based on the cell density calculated from chlorophyll *a* value show that the growth at 6.25 % and 12.50 % test concentrations of the treated ballast water was similar to control during the experiment period (72 hours). And percent inhibitions in specific average growth rate at 25.00%, 50.00 % and 100.00 % test concentration were higher than other test concentrations at the end of experiment.

For >32 PSU treated ballast water, values of NOEC and LOEC from hypothesis test based on growth rate calculated from cell density were 25.00 % and 50.00 % at the end of the experiment, and EC50 value from point estimation using linear interpolation was 93.97 % treated ballast water. For 3-32 PSU treated ballast water, values of NOEC and LOEC from hypothesis test based on growth rate calculated from cell density were 12.50 %, 25.00 %, and EC50 value from point estimation using linear interpolation was 83.32 % treated ballast water.

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS had acute toxic effects on the growth of diatom, *Skeletonema costatum*.

2. OUTLINE OF STUDY

2.1 Purpose

The purpose of this study was to assess the acute toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS (or the >32 and 3-32 PSU de-ballast water from KTM-BWMS) on the growth of diatom, *Skeletonema costatum*.

2.2 Principle

The system response is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations of a treated ballast water. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicates, unexposed control cultures. For full expression of the system response to toxic effects, the cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate.

2.3 Good Laboratory Practice

All procedure of this study was complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

2.4 Guideline

This study was conducted on the basis of following standard method and applied for *Skeletonema costatum*:

- "OECD Guideline for Testing of Chemicals, 201, Freshwater alga and Cyanobacteria, Growth Inhibition test"

Organization for Economic Co-operation and Development, (Adopted: Mar. 23, 2006)

2.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

2.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax : + 82-51-611-0588

2.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.8 Study director

Name: Myung-Baek Shon

Department: BAT (Bio-assay Team for aquatic organisms)

2.9 Schedule timetable

2.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Batch culture of test organisms	2011. 04. 13
Acclimation	2011. 04. 13 ~ 06. 26
Pre-culture for test	2011. 06. 26
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation of cell density	2011. 06. 30 ~ 07. 03
Evaluation of chlorophyll *a*	2011. 07. 01 ~ 07. 04
Experimental completion	2011. 07. 04
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Batch culture of test organisms	2011. 04. 13
Acclimation	2011. 04. 13 ~ 07. 23
Pre-culture for test	2011. 07. 23
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation of cell density	2011. 07. 13 ~ 07. 16

Evaluation of chlorophyll *a*	2011. 07. 14 ~ 07. 17
Experimental completion	2011. 07. 17
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.10 Responsible Personnel

Laboratory manager (Test site manager) Statistical analysis Observation and record	Myung-Baek Shon
Culture of test organisms Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Check the chemical condition	Tae Won Kim

2.11 Retention of records and data

2.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

2.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

3. MATERIALS AND METHODS

3.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

3.1.1 Substance name

Treated water (KTM-S5D5-1)

3.1.2 Lot No.

None

3.1.3 Type

Liquid

3.1.4 Principal ingredients

Seawater

3.1.5 Temperature

23.2 ˚C

3.1.6 Salinity

33.6 PSU

3.1.7 pH

7.67

3.1.8 Custody condition

Constant room at 4 ˚C for 2 days

3.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.1.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.1.11 Disposal of the resting substances

Scrapped after test

3.1.12 Certificate of analysis

Client supplies to test organization

3.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

3.2.1 Substance name

Non-treated water (KTM-S3D5-1)

3.2.2 Lot No.

None

3.2.3 Type

Liquid

3.2.4 Principal ingredients

Seawater

3.2.5 Temperature

23.1 ˚C

3.2.6 Salinity

33.9 PSU

3.2.7 pH

7.50

3.2.8 Custody condition

Constant room at 4 ˚C for 2 days

3.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.2.11 Disposal of the resting substances

Scrapped after test

3.2.12 Certificate of analysis

Client supplies to test organization

3.3 Test substance-3 (Treated ballast water (3-32 PSU) -Treated)

3.3.1 Substance name

Treated water (KTM-S5D5-3)

3.3.2 Lot No.

None

3.3.3 Type

Liquid

3.3.4 Principal ingredients

Seawater

3.3.5 Temperature

24.6 ˚C

3.3.6 Salinity

21.7 PSU

3.3.7 pH

7.46

3.3.8 Custody condition

Constant room at 4 ˚C for 2 days

3.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.3.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.3.11 Disposal of the resting substances

Scrapped after test

3.3.12 Certificate of analysis

Client supplies to test organization

3.4 Test substance-4 (Non-treated ballast water (3-32 PSU) -Control)

3.4.1 Substance name

Non-treated water (KTM-S3D5-3)

3.4.2 Lot No.

None

3.4.3 Type

Liquid

3.4.4 Principal ingredients

Seawater

3.4.5 Temperature

24.8 ˚C

3.4.6 Salinity

22.7 PSU

3.4.7 pH

7.24

3.4.8 Custody condition

Constant room at 4 ˚C for 2 days

3.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.4.11 Disposal of the resting substances

Scrapped after test

3.4.12 Certificate of analysis

Client supplies to test organization

3.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance was diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid (see fig. 1 ~ 4 in the Annex 6). The six concentrations of test substance including control (0.00 %, only non-treated ballast water by the KTM-BWMS) and 100.00 % were arranged for the final definitive experiment.

3.6 Culture condition (SOP#TO-001)

3.6.1 Room

Incubator

3.6.2 Vessel type / volume

Glass flask / 250 mL

3.6.3 Medium volume / vessel volume & medium

100 mL/250 mL, f/2 (see Annex 2)

3.6.4 Inoculum density

5×10^3 cells/mL

3.6.5 Temperature

20 ˚C

3.6.6 Interval of inoculum

Every a week

3.6.7 L:D cycle

Continuous light

3.6.8 Intensity of light

3,000 lux

3.7 Test system

3.7.1 Test organisms (Strain code)

.1 Scientific name	*Skeletonema costatum* (VBA-007, see Fig.5 in the Annex 6)
.2 Producer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

3.7.2 Ground of selected test organisms

Skeletonema costatum is important as primary producer in trophic structure and widely distributed phytoplankton species (Phlylum Bacillariophyta) in estuarine and coastal areas.

3.7.3 Isolation of phytoplankton (SOP#TO-001)

Test organisms	*Skeletonema costatum*
Isolation method	Dilution techniques

3.7.4 Preparation of pre-culture and inoculation (SOP#TP-008)

A pre-culture was started 2 to 4 days before the beginning of the experiment. In order to maintain the exponential growth phase until the start of the experiment, a pre-culture was made to inoculate 1×10^4 cells/mL from the algal stock culture to the growth medium. The pre-culture was incubated under the same conditions as those in the experiment. And the pre-culture for the experiment with 3-32 PSU treated ballast water was carried out same way after acclimation of test organisms for more than 14 days.

3.7.5 Check the test organisms for the experiment (SOP#TP-001)

The cell density in the pre-culture was immediately measured before use, in order to calculate the required inoculation volume and appearance of cells.

3.7.6 Disposal of the resting test organisms (SOP#TO-006)

The resting test organisms were scrapped after sterilization by the autoclave.

3.8 Study design

3.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

3.8.2 Exposure condition (SOP#TP-001)

The final cell density (3×10^3 cells/mL) was added from the algal pre-culture to the each concentration in the test vessels. And then the test vessels were incubated for 72 hours at 22 ± 1˚C (see Fig.6 in the Annex 6).

Experiment conditions and criteria are shown Annex 3 and application site is shown Annex 5.

3.8.3 Observation item and measurement components

.1 Biomass (SOP#TP-009, SOP#TP-012)

Measurement of biomass was conducted by measuring chlorophyll *a* extracted with 90 % acetone using Tuner Designs AU-10. The measurement of the chlorophyll *a* was immediately done within 2 hours after

extraction of it (see Fig.7 in the Annex 6). To obtain the regression equation between chlorophyll *a* and cell density, cell count in the control was done by manual cell counting used the microscope (CKX 31, Olympus) with counting chamber.

Calculations of the growth and the inhibition growth rate are shown Annex 4.

.2 Other observations (SOP#TP-001)

Microscopic observation was performed to verify normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae at the end of the experiment.

.3 pH (SOP#TE-002)

pH of the solutions at the beginning and at the end of the experiment was measured.

3.9 Statistical procedures (SOP#SP-101, SOP#SP-102)

For satisfactory correlation with biomass, chlorophyll *a* and cell density in the control were analyzed by linear correlation analysis with Excel program and the greatest R^2 value was yielded. Cell density was calculated by equation from the linear correlation analysis.

Normal distribution of the data was tested by Shapiro-Wilk's Test ($p > 0.05$) and equal variance of the data was tested by Bartlett's Test ($p=0.62$ (>32 PSU), 0.15 (3-32 PSU)). Hypothesis test of the data was conducted by Dunnett's Test and differences were considered to be significant at $\alpha=0.05$. And then EC50 values of the end point were estimated by Linear Interpolation. TOXCALC 5.0 program (Tidepool scientific software, USA) was used program for above statistic analysis.

4. RESULTS

4.1 pH values

4.1.1 Treated ballast water - > 32 PSU (Table 1)

The initial pH value was ranged from 7.15 to 7.34 and the final one was ranged from 8.16 to 9.01.

4.1.2 Treated ballast water - 3-32 PSU (Table 2)

The initial pH value was ranged from 7.68 to 7.74 and the final one was ranged from 7.72 to 8.76.

4.2 Biomass

4.2.1 Treated ballast water - > 32 PSU (Table 3, 5, Figure 1)

The values of the chlorophyll *a* and cell density had functional relationship indicated by the equation $Y=0.93X+3.48$ ($R^2=0.99$, n=16, $P<0.001$). Therefore, cell density in the each test concentration including control was calculated from the equation. The initial cell density was ranged from 3,160 to 4,267 cells/mL. The mean cell density in the control at the end of the experiment was 527,553 cells/mL. The mean cell densities in the each test concentration at the end of the experiment, 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % were 511,026 cells/mL, 521,221 cells/mL, 488,738 cells/mL, 392,205 cells/mL and 34,291 cells/mL, respectively.

4.2.2 Treated ballast water - 3-32 PSU (Table 4, 6, Figure 2)

The values of the chlorophyll *a* and cell density had functional relationship indicated by the equation $Y=0.89X+3.65$ ($R^2=0.98$, n=16, $P<0.001$). Therefore, cell density in the each test concentration including control was calculated from the equation. The initial cell density was ranged from 1,932 to 5,757 cells/mL. The mean cell density in the control at the end of the experiment was 573,508 cells/mL. The mean cell densities in the each test concentration at the end of the experiment, 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % were 606,163 cells/mL, 572,085 cells/mL, 170,824 cells/mL, 60,502 cells/mL and 27,039 cells/mL, respectively.

4.3 Growth curve

4.3.1 Treated ballast water - > 32 PSU (Fig. 3)

For the > 32 PSU ballast water treated, growth curves show that the growth at all test concentrations of the treated ballast water is similar to control except for 100.00% during the experiment period (72 hours).

4.3.2 Treated ballast water - 3-32 PSU (Fig. 4)

For the 3-32 PSU ballast water treated, growth curves show that the growth at 6.25 % and 12.50 % test concentrations of the treated ballast water is similar to control during the experiment period (72 hours) and cell density at the end of the experiment is low at the higher test concentration.

4.4 Specific growth rate and coefficient of variation

4.4.1 Treated ballast water - > 32 PSU (Table 7, 8)

The mean specific growth rate in the control over the experiment duration was ranged from 1.63 to 2.17 day^{-1}.The mean specific growth rate in the each test concentration was ranged from 0.72 to 2.18 day^{-1}. The coefficient of variation for section-by-section specific growth rate in the control was ranged from 0.7 to 2.0% less than 35 % and their mean was 1.3 % less than 10% required validity of the experiment.

4.4.2 Treated ballast water - 3-32 PSU (Table 9, 10)

The mean specific growth rate in the control over the experiment duration was ranged from 1.65 to 3.03 day^{-1} was greater than 0.92 day^{-1} required the validity of experiment. The mean specific growth rate in the each test concentration was ranged from 0.76 to 3.50 day^{-1}. The coefficient of variation for section-by-section specific growth rate in the control was ranged from 6.5 to 10.8 % less than 35 % and their mean was 8.2 % less than 10 % required validity of the experiment.

4.5 Percent inhibition in average specific growth rate

4.5.1 Treated ballast water - > 32 PSU (Table 11)

The percent inhibition in average specific growth rate for the each test concentration was ranged from -6.16 to 55.81 %. The percent inhibition in average specific growth rate at the end of the experiment was ranged from -1.46 to 55.81 %.

4.5.2 Treated ballast water - 3-32 PSU (Table 12)

The percent inhibition in average specific growth rate for the each test concentration replicate was ranged from -15.39 to 54.14 %. The percent inhibition in average specific growth rate at the end of the experiment was ranged from -9.65 to 54.14 %.

4.6 Plotting of the concentration response curve (Figure 5, 6)

The cell density data on the each test concentration of > 32 and 3-32 PSU ballast water treated by the KTM-BWMS was plotted on the graph.

4.7 ECx, LOEC and NOEC (Table 13)

For the > 32 PSU ballast water treated, values of NOEC, LOEC and EC50 were 25.00 %, 50.00 % and 93.97 % at the end of the experiment. And for the 3-32 PSU ballast water treated, values of NOEC, LOEC and EC50 at the end of the experiment were 12.50 %, 25.00 % and 83.32 %.

4.8 Other observation

Any abnormal appearance of the algae during the experiment period was not observed.

5. DISCUSSION AND CONCLUSION

The inhibition growth test of *Skeletonema costatum* on > 32 and 3-32 PSU treated ballast water was carried out in a concentration series from 0.00 % (Control) to 100.00 % that were made by the treated ballast water diluted in non-treated ballast water.

For > 32 PSU treated ballast water, growth curves (Fig. 3) based on the cell density (Table 5) calculated from chlorophyll *a* value (Table 3) show that the growth at all test concentrations of the treated ballast water was similar to control except for 100.00 % during the experiment period (72 hours). But percent inhibition in specific average growth rate at 50.00 % test concentration was slightly higher than other test concentrations at 72 hour and growth of *S. costatum* was inhibited at 100.00 % test concentration (Table 11). Values of NOEC and LOEC from hypothesis test based on growth rate calculated from cell density were 25.00 % and 50.00 % at the end of the experiment (Table 13), and EC50 value from point estimation using linear interpolation was 93.97 % treated ballast water (Table 13).

For 3-32 PSU treated ballast water, growth curves (Fig. 4) based on the cell density (Table 6) calculated from chlorophyll *a* value (Table 4) show that the growth at 6.25 % and 12.50 % test concentrations of the treated ballast water was similar to control during the experiment period (72 hours). And percent inhibitions in specific average growth rate at 25.00%, 50.00 % and 100.00 % test concentration were higher than other test concentrations at the end of experiment (Table 12). Values of NOEC and LOEC from hypothesis test based on growth rate calculated from cell density were 12.50 %, 25.00 % (Table 13), and EC50 value from point estimation using linear interpolation was 83.32 % treated ballast water (Table 13).

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS had acute toxic effects on the growth of diatom, *Skeletonema costatum*.

6. REFERENCE

OECD guidelines for the testing of chemicals-201. 2006. Freshwater alga and Cyanobacteria, growth inhibition test. 25pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

TABLES

Table 1. pH values of > 32 PSU test concentration series at the beginning and the end of the experiment

Concentration (%)	Control	6.25	12.5	25	50	100
Initial	7.25	7.29	7.30	7.34	7.15	7.19
Final	8.94	8.96	8.98	9.01	8.94	8.16

Table 2. pH values of 3-32 PSU test concentration series at the beginning and the end of the experiment

Concentration (%)	Control	6.25	12.5	25	50	100
Initial	7.69	7.68	7.74	7.73	7.72	7.68
Final	8.66	8.76	8.76	8.54	7.94	7.72

Table 3. Chlorophyll *a* (μg/mL) of *Skeletonema costatum* at various concentrations of treated > 32 PSU ballast water during the experiment period

Elapsed time (h)	Control				6.25 %			
	1	2	3	Mean	1	2	3	Mean
0	1.34	1.34	1.36	1.35	1.15	1.05	1.45	1.22
24	10.30	10.10	11.10	10.50	12.50	8.64	11.20	10.78
48	147.00	150.00	136.00	144.33	163.00	104.00	120.00	129.00
72	248.00	255.00	270.00	257.67	241.00	243.00	263.00	249.00
Elapsed time (h)	**12.50 %**				**25.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	1.30	1.22	1.25	1.26	1.30	1.14	1.19	1.21
24	10.40	10.60	10.60	10.53	10.60	8.98	7.59	9.06
48	139.00	135.00	132.00	135.33	137.00	115.00	98.60	116.87
72	251.00	252.00	260.00	254.33	230.00	239.00	243.00	237.33
Elapsed time (h)	**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	1.26	1.23	1.17	1.22	1.36	1.43	1.23	1.34
24	8.90	9.98	9.23	9.37	7.93	9.73	10.00	9.22
48	108.00	109.00	107.00	108.00	23.20	24.20	23.50	23.63
72	179.00	187.00	196.00	187.33	13.50	14.00	13.40	13.63

Table 4. Chlorophyll *a* (μg/mL) of *Skeletonema costatum* at various concentrations of treated 3-32 PSU ballast water during the experiment period

Elapsed time (h)	**Control**				**6.25 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	1.15	0.64	1.02	0.94	0.57	0.57	0.56	0.57
24	26.80	29.40	26.10	27.43	22.20	24.70	18.00	21.63
48	196.00	192.00	147.00	178.33	173.00	165.00	125.00	154.33
72	235.00	249.00	218.00	234.00	239.00	261.00	247.00	249.00
Elapsed time (h)	**12.50 %**				**25.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	1.33	1.33	0.96	1.21	0.51	0.42	0.45	0.46
24	25.00	27.70	22.40	25.03	19.60	25.00	16.30	20.30
48	175.00	168.00	143.00	162.00	83.70	78.70	38.20	66.87
72	220.00	244.00	236.00	233.33	59.10	61.50	16.40	45.67
Elapsed time (h)	**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	0.44	0.60	0.35	0.46	0.49	0.60	0.70	0.60
24	20.10	20.60	19.40	20.03	4.97	4.81	4.95	4.91
48	39.60	31.40	30.90	33.97	12.60	11.20	9.99	11.26
72	19.70	20.00	18.80	19.50	6.48	7.90	8.32	7.57

Table 5. Cell density (cells/mL) of *Skeletonema costatum* calculated by equation from linear correlation analysis at various concentrations of treated > 32 PSU ballast water during the experiment period

Elapsed time (h)	Control				6.25 %			
	1	2	3	Mean	1	2	3	Mean
0	3,965	3,965	4,020	3,983	3,439	3,160	4,267	3,622
24	26,420	25,943	28,324	26,896	31,632	22,437	28,561	27,543
48	313,044	318,981	291,200	307,742	344,615	226,903	259,202	276,907
72	509,143	522,495	551,021	527,553	495,765	499,590	537,723	511,026
Elapsed time (h)	**12.50 %**				**25.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	3,854	3,633	3,716	3,735	3,854	3,411	3,550	3,605
24	26,659	27,135	27,135	26,976	27,135	23,257	19,890	23,427
48	297,169	289,208	283,226	289,868	293,191	249,143	215,926	252,753
72	514,869	516,776	532,017	521,221	474,687	491,938	499,590	488,738
Elapsed time (h)	**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	3,744	3,661	3,495	3,633	4,020	4,212	3,661	3,964
24	23,064	25,656	23,858	24,193	20,717	25,058	25,704	23,826
48	235,009	237,032	232,984	235,008	56,222	58,472	56,897	57,197
72	375,970	391,573	409,070	392,205	33,979	35,148	33,745	34,291

Table 6. Cell density (cells/mL) of *Skeletonema costatum* calculated by equation from linear correlation analysis at various concentrations of treated 3-32 PSU ballast water during the experiment period

Elapsed time (h)	Control				6.25 %			
	1	2	3	Mean	1	2	3	Mean
0	5,058	3,003	4,546	4,202	2,708	2,708	2,666	2,694
24	83,376	90,538	81,435	85,116	70,511	77,535	58,505	68,850
48	489,901	480,992	379,239	450,044	438,390	420,301	328,284	395,659
72	575,772	606,202	538,550	573,508	584,486	632,135	601,867	606,163
Elapsed time (h)	**12.50 %**				**25.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	5,757	5,757	4,307	5,274	2,453	2,064	1,932	2,150
24	78,373	85,863	71,076	78,437	63,111	78,373	71,358	70,947
48	442,898	427,096	370,040	413,345	229,734	217,479	186,952	211,388
72	542,945	595,356	577,952	572,085	168,544	174,622	169,305	170,824
Elapsed time (h)	**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	2,195	2,151	2,835	2,394	2,367	2,835	3,252	2,818
24	53,561	64,542	65,969	61,357	18,610	18,076	18,544	18,410
48	114,297	118,018	95,999	109,438	42,592	38,353	34,643	38,529
72	53,853	63,398	64,256	60,502	23,567	28,112	29,438	27,039

Table 7. Specific growth rate of *Skeletonema costatum* at various concentrations of treated > 32 PSU ballast water during the experiment period

Elapsed time (h)	Control				6.25%			
	1	2	3	Mean	1	2	3	Mean
24	1.90	1.88	1.95	1.91	2.22	1.96	1.90	2.03
48	2.18	2.19	2.14	2.17	2.30	2.14	2.05	2.16
72	1.62	1.63	1.64	1.63	1.66	1.69	1.61	1.65
Elapsed time (h)	**12.50%**				**25.00%**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
24	1.93	2.01	1.99	1.98	1.95	1.92	1.72	1.86
48	2.17	2.19	2.17	2.18	2.17	2.15	2.05	2.12
72	1.63	1.65	1.65	1.65	1.60	1.66	1.65	1.64
Elapsed time (h)	**50.00%**				**100.00%**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
24	1.82	1.95	1.92	1.90	1.64	1.78	1.95	1.79
48	2.07	2.09	2.10	2.08	1.32	1.32	1.37	1.34
72	1.54	1.56	1.59	1.56	0.71	0.71	0.74	0.72

Table 8. Coefficient of variation of specific growth rate at the each test concentration of treated > 32 PSU ballast water during the experiment period

Elapsed time (h)	Concentration (%)					
	Control	6.25	12.5	25.00	50.00	100.00
24	2.0	8.3	2.0	6.6	3.6	8.6
48	1.3	5.9	0.5	2.8	0.7	2.4
72	0.7	2.3	0.8	1.7	1.6	2.5
Mean	1.3	5.5	1.1	3.7	2.0	4.5

Table 9. Specific growth rate of *Skeletonema costatum* at various concentrations of treated 3-32 PSU ballast water during the experiment period

Elapsed time (h)	Control				6.25%			
	1	2	3	Mean	1	2	3	Mean
24	2.80	3.41	2.89	3.03	3.26	3.35	3.09	3.23
48	2.29	2.54	2.21	2.35	2.54	2.52	2.41	2.49
72	1.58	1.77	1.59	1.65	1.79	1.82	1.81	1.81
Elapsed time (h)	**12.50%**				**25.00%**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
24	2.61	2.70	2.80	2.71	3.25	3.64	3.61	3.50
48	2.17	2.15	2.23	2.18	2.27	2.33	2.29	2.29
72	1.52	1.55	1.63	1.56	1.41	1.48	1.49	1.46
Elapsed time (h)	**50.00%**				**100.00%**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
24	3.19	3.40	3.15	3.25	2.06	1.85	1.74	1.89
48	1.98	2.00	1.76	1.91	1.44	1.30	1.18	1.31
72	1.07	1.13	1.04	1.08	0.77	0.76	0.73	0.76

Table 10. Coefficient of variation of specific growth rate at the each test concentration of treated 3-32 PSU ballast water during the experiment period

Elapsed time (h)	Concentration (%)					
	Control	6.25	12.50	25.00	50.00	100.00
24	10.8	4.2	3.6	6.2	4.2	8.6
48	7.3	3.0	1.7	1.3	6.9	10.0
72	6.5	0.7	3.9	3.0	4.2	2.4
Mean	8.2	2.6	3.1	3.5	5.1	7.0

Table 11. Percent inhibition in average specific growth rate of *Skeletonema costatum* at the each test concentration of treated > 32 PSU ballast water during the experiment period

Elapsed time (h)	Concentration (%)				
	6.25	**12.50**	**25.00**	**50.00**	**100.00**
24	-6.16	-3.58	2.33	0.73	6.21
48	0.39	-0.12	2.37	4.06	38.55
72	-1.46	-1.08	-0.51	4.18	55.81

Table 12. Percent inhibition in average specific growth rate of *Skeletonema costatum* at the each test concentration of treated 3-32 PSU ballast water during the experiment period

Elapsed time (h)	Concentration (%)				
	6.25	**12.50**	**25.00**	**50.00**	**100.00**
24	-6.69	10.75	-15.39	-7.14	37.81
48	-6.19	6.90	2.16	18.43	44.15
72	-9.65	4.94	11.31	34.50	54.14

Table 13. NOEC, LOEC and EC50 value calculated from mean response cell density at the end of the experiment

Test substance	NOEC (%)	LOEC (%)	EC50 (%)	95 % confidence of limit (%)	
				lower	**upper**
> 32 PSU ballast water	25.00	50.00	93.97	92.08	95.77
3-32 PSU ballast water	12.50	25.00	83.32	72.13	92.16

FIGURES

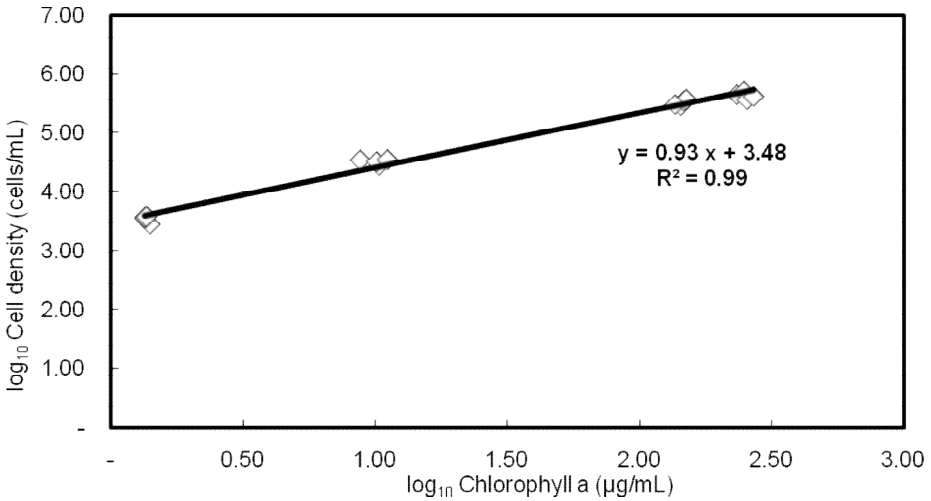


Fig. 1. Cell density in the control as function of chlorophyll *a* concentration (> 32 PSU ballast water, n=16, P<0.001).

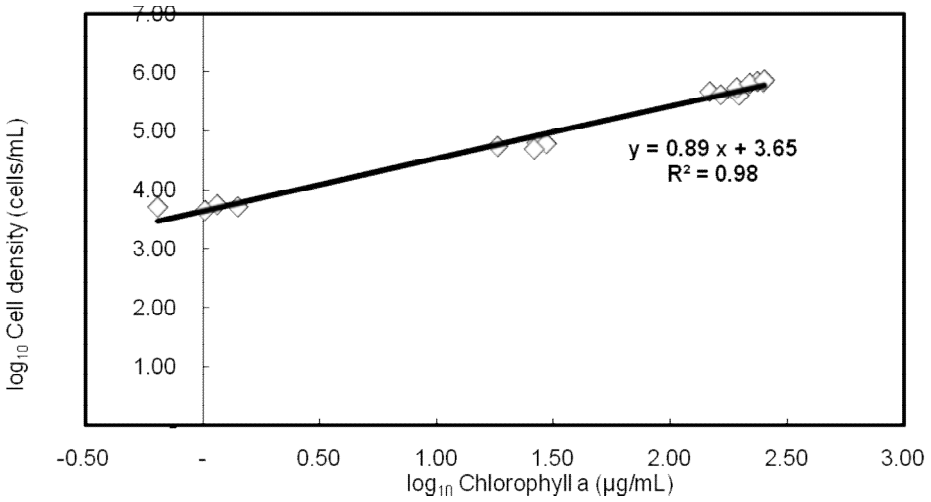


Fig. 2. Cell density in the control as function of chlorophyll *a* concentration (3-32 PSU ballast water, n=16, P<0.001).

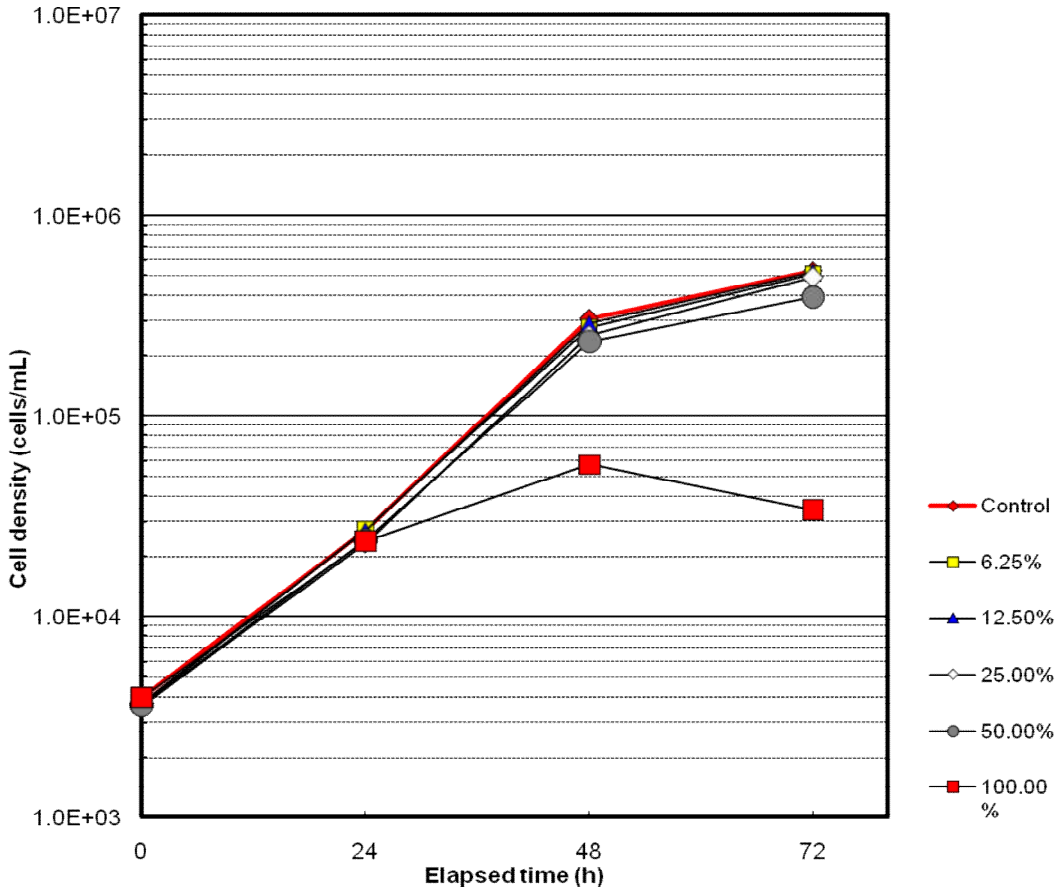


Fig. 3. Growth curves of *Skeletonema costatum* at various concentrations of treated > 32 PSU ballast water. The data of cell density was transformed to log value.

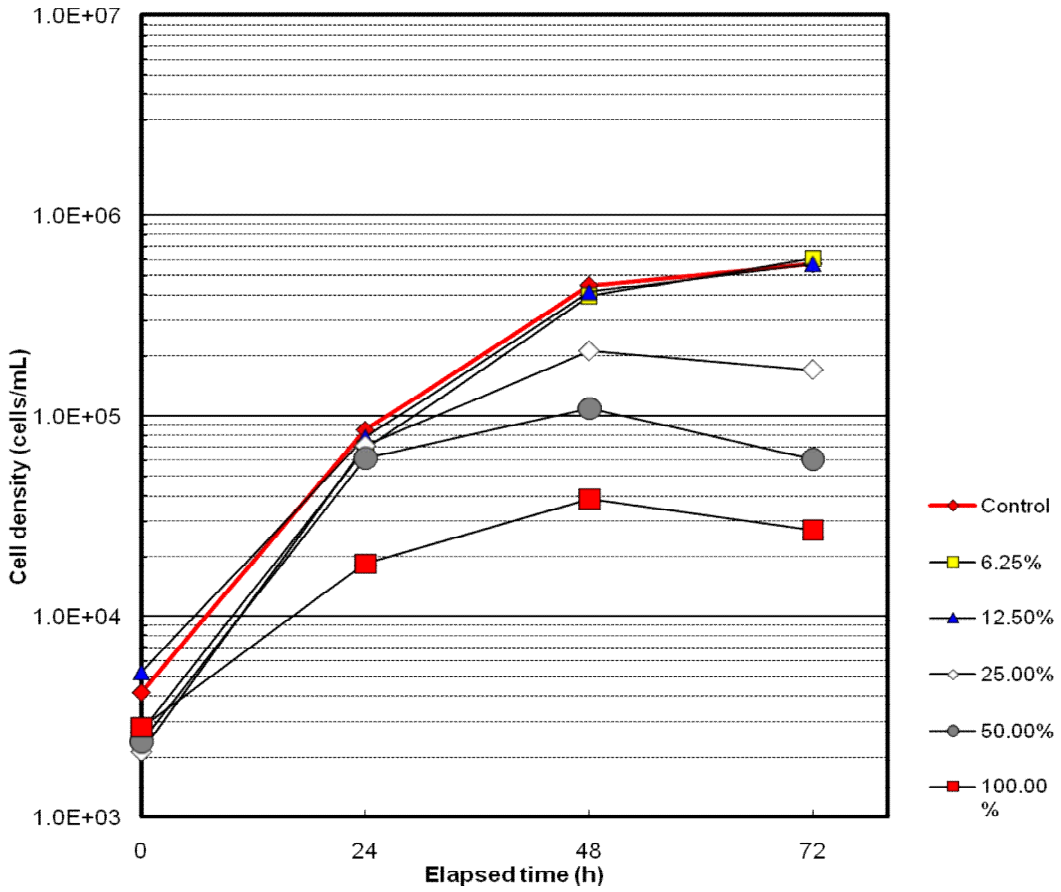


Fig. 4. Growth curves of *Skeletonema costatum* at various concentrations of treated 3-32 PSU ballast water. The data of cell density was transformed to log value.

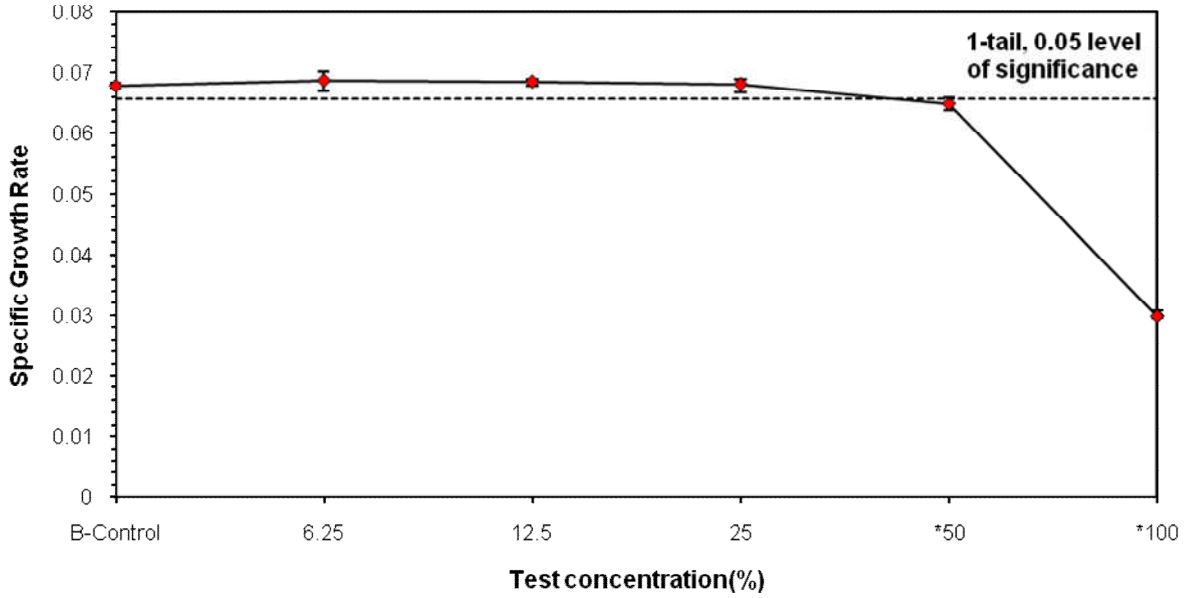


Fig. 5. Plot of the concentration-response data at the end of the experiment with *Skeletonema costatum* in > 32 PSU treated ballast water. The dotted line was represented as 0.05 level of significant and vertical bar was represented maximum and minimum.

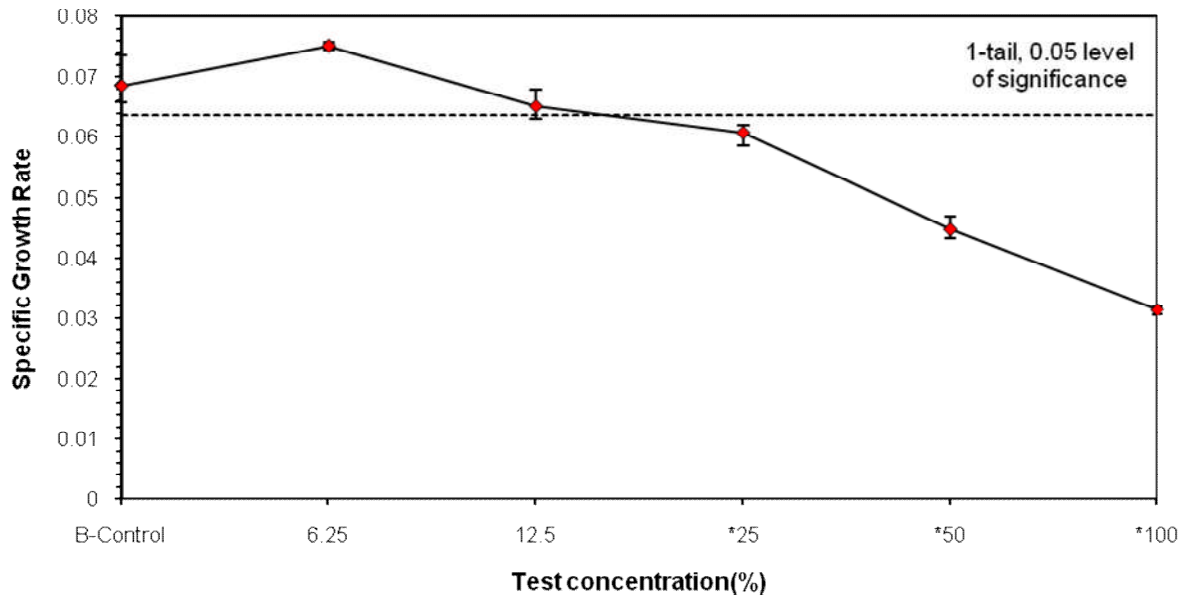


Fig. 6. Plot of the concentration-response data at the end of the experiment with *Skeletonema costatum* in 3-32 PSU treated ballast water. The dotted line was represented as 0.05 level of significant and vertical bar was represented maximum and minimum.

ANNEX 1

Definitions

1. Coefficient of variation is a dimensionless measure of the variability of a parameter, defined as the ratio of the standard deviation to the mean. This can also be expressed as a percent value. Mean coefficient of variation of average specific growth rate in replicate control cultures should be calculated as follows:

1. Calculate % CV of average specific growth rate out of the daily/section by section growth rates for the respective replicate;
2. Calculate the mean value out of all values calculated under point 1 to get the mean coefficient of variation of the daily/section by section specific growth rate in replicate control cultures.

2. EC_x is the concentration of the test substance dissolved in test medium that results in an x % (e.g. 50 %) reduction in growth of the test organisms within a stated exposure period.

3. Growth rate (average specific growth rate) is the logarithmic increase in biomass during the exposure period.

4. Lowest Observed Effect Concentration (LOEC) is the lowest tested concentration at which the substance is observed to have a statistically significant reducing effect on growth (at $p < 0.05$) when compared with the control, within a given exposure time.

5. No Observed Effect Concentration (NOEC) is the test concentration immediately below the LOEC.

6. Specific growth rate is a response variable defined as quotient of the difference of the natural logarithms of a parameter of observation and the respective time period.

7. Pre-culture is intended to give an amount of algae suitable for the inoculation of test cultures. The pre-culture is incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of 2 to 4 days.

8. Biomass is the dry weight of living matter present in a population expressed in terms of a given volume; e.g., mg algae/liter test solution. Usually “biomass” is defined as a mass, but in this study this word is used to refer to mass per volume. Also in this study, surrogates for biomass as cell density (cells/mL) from cell counts.

ANNEX 2

Composition of f/2 (Guillard & Ryther 1962, Guillard 1975)

Component	Stock solution	Quantity Used	Concentration in Final Medium (M)
$NaNO_3$	75.0 g/ℓdH_2O	1.0 mL	8.82×10^{-4}
$NaH_2PO\cdot4H_2O$	5.0 g/ℓdH_2O	1.0 mL	3.62×10^{-5}
$Na_2SiO_3\cdot9H_2O$	30.0 g/ℓdH_2O	2.0 mL	1.06×10^{-4}
*f/2 Trace metal solution	(see recipe below)	1.0 mL	-
**f/2 Vitamin solution	(see recipe below)	0.5 mL	-

*f/2 Trace metal solution

Component	Stock solution	Quantity Used	Concentration in Final Medium (M)
$FeCl_3\cdot6H_2O$	-	3.15 g	1.17×10^{-5}
$Na_2EDTA\cdot2H_2O$	-	4.36 g	1.17×10^{-5}
$CuSO_4\cdot5H_2O$	9.8 g/ℓdH_2O	1.0 mL	3.93×10^{-8}
$Na_2MoO_4\cdot2H_2O$	6.3 g/ℓdH_2O	1.0 mL	2.60×10^{-8}
$ZnSO_4\cdot7H_2O$	22.0 g/ℓdH_2O	1.0 mL	7.65×10^{-8}
$CoCl_2\cdot6H_2O$	10.0 g/ℓdH_2O	1.0 mL	4.20×10^{-8}
$MnCl_2\cdot4H_2O$	180.0 g/ℓdH_2O	1.0 mL	9.10×10^{-7}

**f/2 Vitamin solution

Component	Stock solution	Quantity Used	Concentration in Final Medium (M)
Cyanocobalamin (Vitamin B_{12})	1.0 g/ℓdH_2O	1.0 mL	3.69×10^{-10}
Biotin (Vitamin H)	0.1 g/ℓdH_2O	10.0 mL	2.05×10^{-9}
Thiamine HCl (vitamin B_1)	-	200.0 mL	2.96×10^{-7}

ANNEX 3

Experiment conditions and acceptability criteria for diatom, *Skeletonema costatum*, acute growth toxicity tests with > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Static
Temperature	22 ± 1 ˚C
Light quality	"Cool white" fluorescent lamp
Light intensity	3,000 lux
Photoperiod	Continuous illumination
Test chamber size	15 mL
Test solution volume	10 mL
Renewal of test solutions	None
Age of test organisms	2~3 days
Initial cell density in test chambers	3,000 cells/mL
No. replicate chambers per concentration	3
Shaking rate	Twice daily by hand
Aeration	None
Dilution water	Filtered seawater (Control seawater) by 0.2μm membrane filter
Test concentrations	Effluents: 5 including receiving water 100.00 % and a control
Test dilution factor	Effluents: 0.5
Test duration	72 hours
Endpoint	Growth (Cell counts)
Test acceptability criteria	Specific growth rate: ≥ 0.92 day^{-1} Variation coefficient of the control specific growth rate: ≤ 10 % Change of the control pH: ≤ 1.5

ANNEX 4

1.1. The average specific growth rate for a specific period was calculated from equation [1]:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} (\text{day}^{-1}) \qquad [1]$$

where:

μ_{i-j} is the average specific growth rate from time I to j;

X_i is the biomass at time i;

X_j is the biomass at time j;

1.2. The percent inhibition of growth rate for each treatment replicate was calculated from equation [2]:

$$\%I_r = \frac{\mu_C - \mu_T}{\mu_C} \times 100 \qquad [2]$$

where:

$\%I_r$ is percent inhibition in average specific growth rate;

μ_C is mean value for average specific growth rate (μ) in the control group ;

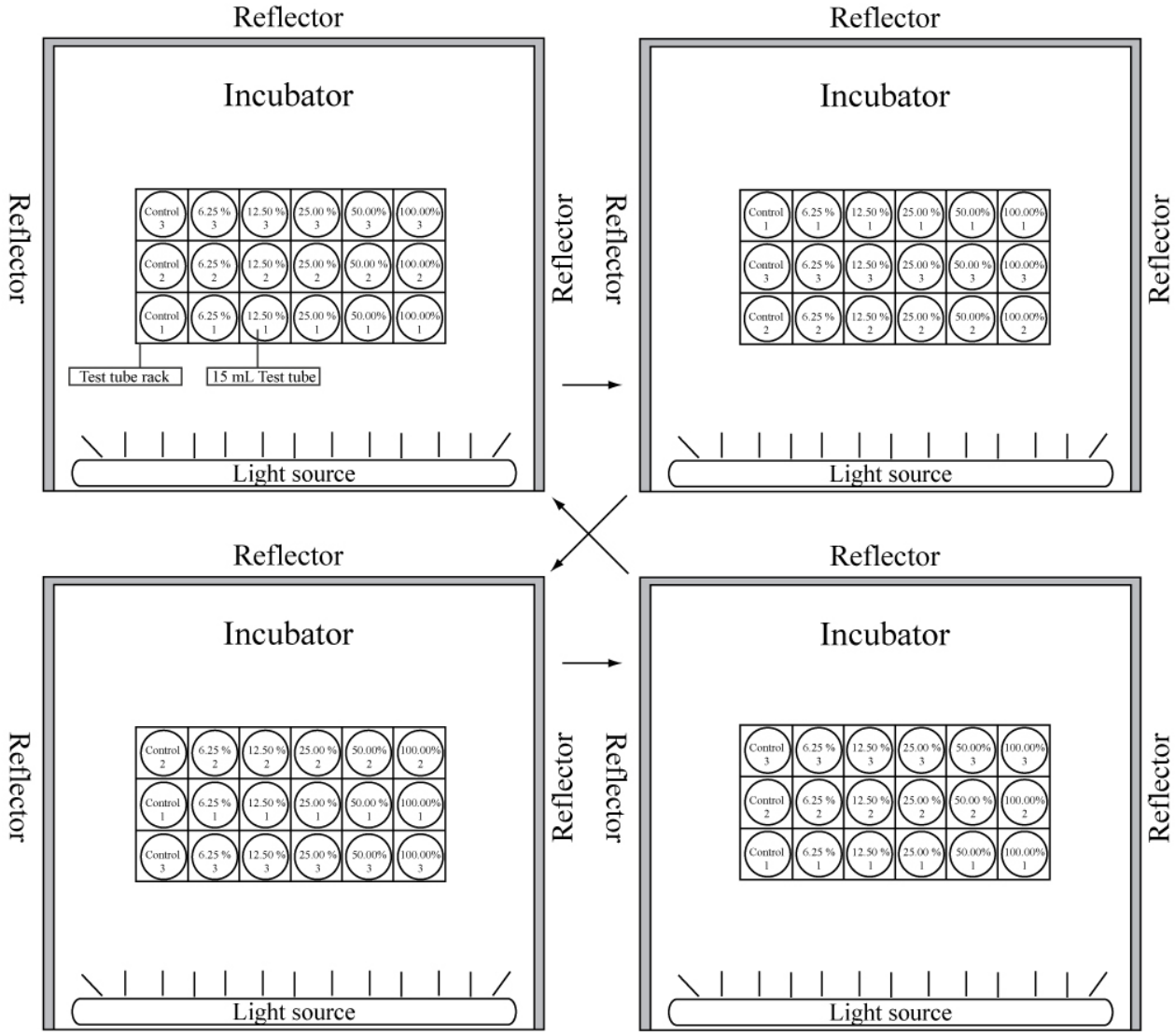
μ_T is average specific growth rate (μ) in treatment replicate

Each treatment group and control group were calculated a mean value for growth rate along with variance estimates.

ANNEX 5

Application Site

Application site for the acute toxicity test with the diatom, *Skeletonema costatum* is shown as follow. The arrowheads indicated the systematical rotation of the test vessels.



ANNEX 6

Preparation of test substance and procedures of the experiment

Fig. 1. For aquatic toxicity test, collection of the control (untreated) and treated de-ballasting water from the KTM-BWMS (left down: sampling equipments).

Fig. 2. Transfer collected samples to the constant room (4 ± 1 ˚C).

Fig. 3. Preparation of test concentration diluted with non-treated seawater on treated seawater through flow-meter.

Fig. 4. For the growth inhibition test using *Skeletonema costatum*, final preparation of test substance was filtered by 0.45 μm membrane filter to make the f/2 medium.

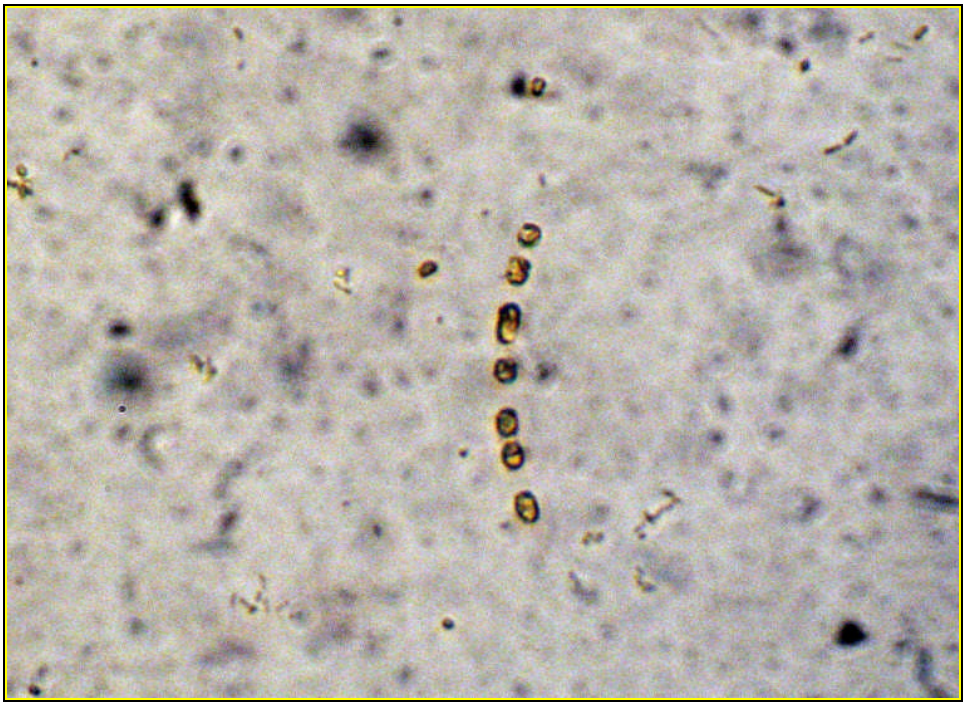


Fig. 5. Test organisms in this study: Aquatic plant (Diatom), *Skeletonema costatum*.

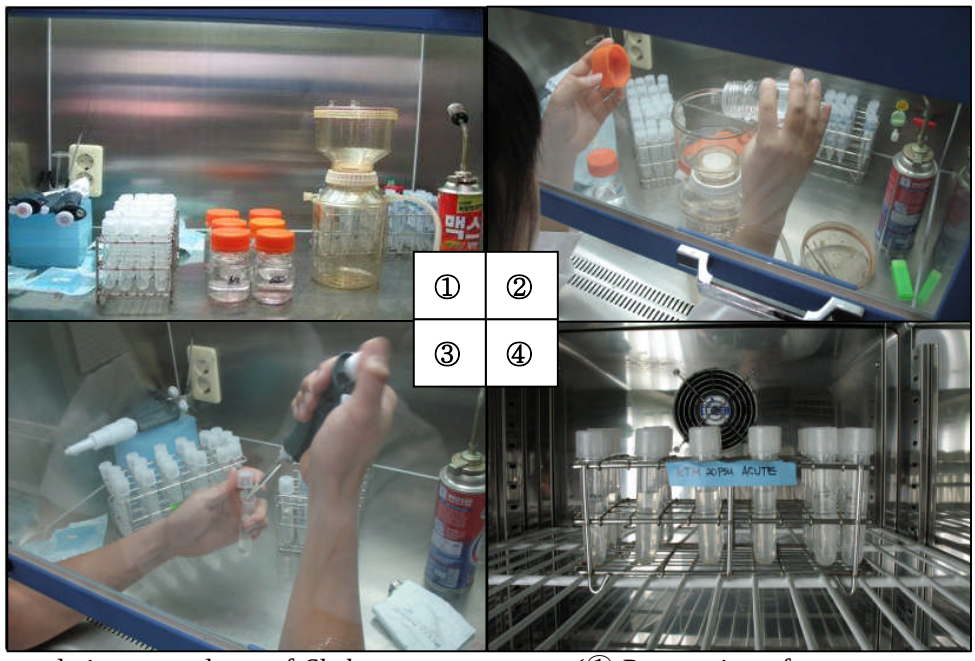


Fig. 6. The inoculation procedures of *Skeletonema costatum* (① Preparation of test apparatus; ② Sterilizing medium by filter (0.2 μm membrane filter); ③ Inoculation; ④ Incubation at 22 ˚C).

Fig. 7. The extraction of chlorophyll *a* at the each test concentration (① Collecting of the samples; ② Adding the aqueous acetone solution; ③ Centrifuging the extract after storage for a 24 hours in the 4˚C cold and dark room; ④ Estimating the chlorophyll *a*).

3.1.2 ANNEX 2

***Brachionus plicatilis*, Acute Toxicity Test**

Marine Eco-Technology Institute Co., Ltd.
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Tel +82-51-611-6200
Fax +82-51-611-0588

FINAL REPORT

Acute Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- Rotifer, *Brachionus plicatilis* Acute Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

GLP STATEMENT AND SIGN

Study title: Acute toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward survival of *Brachionus plicatilis*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

All procedure of this study was carried out following GLP regulation:

- "OECD Principles of Good Laboratory Practice"
  Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

This study was conducted on the basis of 'the Study Plan' complied and the procedure described in this report was carried out under 'the Responsibility of Study Director'. This report was built on the basis of raw data from the study.

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.
Study Director Jung-Im Park (signature)

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.
Test Facility Manager Min Ho Son (signature)

GUARANTEE OF ASSURANCE

Study title: Acute toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward survival of *Brachionus plicatilis*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Quality assurance personnel of Marine Eco-Technology Institute Co., Ltd., inspected as follows. Inspection was carried out on the basis of the Standard Operation Procedure and each inspection results were reported to Study Director and Test Facility Manager.

Types of inspection and the date inspection results were reported to the Test Facility Management and the Study Director as follow:

Type of Inspections	Date of Inspection	Date Reported to Test Facility Management and Study Director
Study Plan	2011. 04. 13	2011. 04. 13
Test organisms	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Storage of Test substance	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Preparation of Test substance	2011. 06. 29 / 07. 12	2011. 06. 29 / 07. 12
Exposure	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Observation	2011. 07. 01 / 07. 14	2011. 07. 01 / 07. 14
Record of raw data	2011. 07. 01 / 07. 14	2011. 07. 01 / 07. 14
Draft of final report	2011. 07. 19	2011. 07. 19
Final report	2011. 07. 20	2011. 07. 20

It is assurance that the procedure in this final report was appropriately carried out and the description of the results was reflected raw data from the study.

2011. 07. 20.

Quality Assurance personnel Jin Hee Kim (signature)
Marine Eco-Technology Institute Co., Ltd.

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1. SUMMARY

The purpose of this study was to assess the acute toxic effects of the > 32 and 3-32 PSU seawater treated by the KTM-BWMS on the survival of the rotifer, *Brachionus plicatilis*.

The test substances were divided into two different salinities, the one was > 32 PSU treated ballast water and the other was 3-32 PSU treated ballast water. Non-treated ballast water was used as control and dilution water. 5 individuals of the rotifers, *Brachionus plicatilis* were exposed at the various test concentrations (0.00 % (non-treated ballast water), 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % (treated ballast water)) for 24 hours. Dead rotifer was observed and recorded using a microscopy after 24 hours. The raw data of survival from this experiment was used for calculation of LCx values.

The survival of rotifer, *Brachionus plicatilis* on the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS system was assessed.

For the > 32 PSU treated ballast water, the test organisms survived in the all test concentrations including the control. For the 3-32 PSU treated ballast water, the test organisms survived in all test concentrations excluding 50.00 % and 100.00 % (the only 1 individual died in each test concentration).

Result of the statistical analysis was indicated that survival proportion of test organisms in the all test concentration (the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS) was not significantly different with control. So, LC50 values based on survival data at the end of the experiment with > 32 and 3-32 PSU treated ballast water were greater than 100.00 %.

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS did not have acute toxic effects on the survival of the rotifer, *Brachionus plicatilis*.

2. OUTLINE OF STUDY

2.1 Purpose

The purpose of this study was to assess the acute toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS (or the > 32 and 3-32 PSU de-ballast water from the KTM-BWMS) on the survival of *Brachionus plicatilis*.

2.2 Principle

Neonate of *Brachionus plicatilis* aged less than 2 hours at the start of test is exposed to the treated ballast water of the KTM-BWMS at a range of concentrations including 100.00 % effluent, for a period of 24 hours. Mortality are recorded at 24 hours and compared with control values. The results are analyzed in order to calculate the LC50 at 24 hours.

2.3 Good Laboratory Practice

All procedure of this study was complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

2.4 Guideline

This study using *Brachionus plicatilis* was complied with principle and conducted on the basis of following standard method:

-"Standard Guide for Acute Toxicity Test with the Rotifer *Brachionus*. E 1440-91"

American Society of Testing Materials, (Reapproved 2004)

2.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

2.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax : + 82-51-611-0588

2.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.8 Study director

Name: Jung-Im Park

Department: 2nd research team

2.9 Study timetable

2.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 06. 29
Separation of neonates for test	2011. 06. 30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation survival	2011. 07. 01
Experimental completion	2011. 07. 01
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 07. 25
Separation of neonates for test	2011. 07. 26
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation survival	2011. 07. 14
Experimental completion	2011. 07. 14
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.10 Responsible Personnel

Laboratory manager (Test site manager) Statistical analysis	Myung-Baek Shon
Receipt and culture of test organisms Observation and record Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Check the chemical condition	Tae Won Kim

2.11 Retention of records and data

2.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

2.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

3. MATERIALS AND METHODS

3.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

3.1.1 Substance name

Treated water (KTM-S5D5-1)

3.1.2 Lot No.

None

3.1.3 Type

Liquid

3.1.4 Principal ingredients

Seawater

3.1.5 Temperature

23.2 ˚C

3.1.6 Salinity

33.6 PSU

3.1.7 pH

7.67

3.1.8 Custody condition

Constant room at 4 ˚C for 2 days

3.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.1.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.1.11 Disposal of the resting substances

Scrapped after test

3.1.12 Certificate of analysis

Client supplies to test organization

3.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

3.2.1 Substance name

Non-treated water (KTM-S3D5-1)

3.2.2 Lot No.

None

3.2.3 Type

Liquid

3.2.4 Principal ingredients

Seawater

3.2.5 Temperature

23.1 ˚C

3.2.6 Salinity

33.9 PSU

3.2.7 pH

7.50

3.2.8 Custody condition

Constant room at 4 ˚C for 2 days

3.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.2.11 Disposal of the resting substances

Scrapped after test

3.2.12 Certificate of analysis

Client supplies to test organization

3.3 Test substance-3 (Treated ballast water (3 - 32 PSU) -Treated)

3.3.1 Substance name

Treated water (KTM-S5D5-3)

3.3.2 Lot No.

None

3.3.3 Type

Liquid

3.3.4 Principal ingredients

Seawater

3.3.5 Temperature

24.6 ˚C

3.3.6 Salinity

21.7 PSU

3.3.7 pH

7.46

3.3.8 Custody condition

Constant room at 4 ˚C for 2 days

3.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.3.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.3.11 Disposal of the resting substances

Scrapped after test

3.3.12 Certificate of analysis

Client supplies to test organization

3.4 Test substance-4 (Non-treated ballast water (3 - 32 PSU) -Control)

3.4.1 Substance name

Non-treated water (KTM-S3D5-3)

3.4.2 Lot No.

None

3.4.3 Type

Liquid

3.4.4 Principal ingredients

Seawater

3.4.5 Temperature

24.8 ˚C

3.4.6 Salinity

22.7 PSU

3.4.7 pH

7.24

3.4.8 Custody condition

Constant room at 4 ˚C for 2 days

3.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.4.11 Disposal of the resting substances

Scrapped after test

3.4.12 Certificate of analysis

Client supplies to test organization

3.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance was diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid (see fig. 1 ~ 4 in the Annex 4). The six concentrations of test substance including control (0.00 %, only non-treated ballast water by the KTM-BWMS) and 100.00 % were arranged for the final definitive experiment.

3.6 Culture condition

3.6.1 Hatching of test organisms (SOP#TO-007)

Test organisms	*Brachionus plicatilis*
Vessel	6 well plate
Medium	20 PSU filtered seawater (0.45μm membrane filter, Whatman)
Temperature	25 ˚C
Light source	"cool-white" fluorescent lamp
Light cycle	Continuous light
Light cycle	1,000 ~ 3,000 lux

The cysts were obtained from commercial supplier (See '3.7 Test system').

3.6.2 Feeding of the test organisms

None

3.7 Test system

3.7.1 Test organisms

.1 Scientific name	*Brachionus plicatilis*
.2 Producer	MicroBioTests Inc., Belgium
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

3.7.2 Ground of selected test organisms

Marine rotifer, *Brachionus plicatilis* have the advantages of cosmopolitan distribution, wide salinity adaptability, importance trophic roles as primary consumer in marine ecosystem, short generation time and easy culture in small spaces. For this reason *Brachionus plicatilis* is commonly used as a toxicity test.

3.7.3 Preparation of the test organisms (SOP#TO-007)

Hatching of rotifer cysts were initiated for approximately 24 hours before the start of the test, and had to be carried out in seawater of reduced salinity (20 PSU). They were incubated at 25 ˚C and 1,000 to 3,000 lux continuous illumination. They were checked for the hatching hourly after 24 hours incubation in order to

collect the test animals within 2 hours of their hatching. For the > 32 PSU ballast water treated, rotifer neonates were acclimated for approximately 1 hour to adapt to the eventual salinity change.

3.7.4 Check the test organisms for the test (SOP#TO-007)

Health of the test organisms such as the mortality, appearance and abnormal behavior was observed with a microscope (SZ51, Olympus).

3.7.5 Disposal of the resting test organisms (SOP#TO-006)

The resting test organisms were scrapped after sterilization by the autoclave.

3.8 Study design

3.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

3.8.2 Exposure condition (SOP#TP-013)

Brachionus plicatilis was placed in test vessels divided into five neonates each replication of concentration including the control. And then the test vessels were placed in incubator for 24 hours at 25 ± 1˚C.

Experiment conditions and criteria are shown Annex 2 and application site is shown Annex 3.

3.8.3 Observation item and measurement components

.1 Mortality (SOP#TP-013)

Each test vessel was checked for mortality of *Brachionus plicatilis* at 24 hours after the beginning of the experiment. Justification of mortality was that animals did not exhibit any internal or external movement in 5 seconds of observation.

.2 Other observations (SOP#TP-013)

In addition to mortality, any abnormal behavior and appearance were recorded.

.3 Temperature (SOP#TE-002)

Temperature of the solutions was measured at the beginning and end of the experiment.

.4 Dissolved oxygen (SOP#TE-002)

Dissolved oxygen (DO) of the solutions was measured at the beginning and end of the experiment.

.5 pH (SOP#TE-002)

pH of the solutions was measured at the beginning and end of the experiment.

3.9 Statistical procedures (SOP#SP-101)

For the >32 PSU treated ballast water, statistical analysis meant nothing because all test organisms survived in all test concentrations. For the 3-32 PSU treated ballast water, normal distribution of the data was tested by Kolmogorove D Test ($p \leq 0.05$) and equal variance of the data could not be confirmed. Therefore, hypothesis test of the data was conducted by Steel's Many-One Rank test and differences were considered to be significant at $\alpha=0.05$. TOXCALC 5.0 program (Tidepool scientific software, USA) was used program for above statistic analysis.

4. RESULTS

4.1 Dissolved oxygen, pH and Temperature

4.1.1 Treated ballast water - > 32 PSU (Table 1 ~ 3)

For the > 32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 4.16 to 4.64 mg/L at the beginning experiment and from 3.41 to 4.28 mg/L at the end of the experiment. pH value was ranged from 7.47 to 7.91 at the beginning experiment and from 7.58 to 7.83 at the end of the experiment. Mean temperature was 25.10 ± 0.47 ˚C (Mean ± S.D.).

4.1.2 Treated ballast water - 3-32 PSU (Table 4 ~ 6)

For the 3-32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 6.28 to 7.70 mg/L at the beginning experiment and from 5.88 to 6.54 mg/L at the end of the experiment. pH value was ranged from 7.43 to 7.61 at the beginning experiment and from 7.76 to 7.85 at the end of the experiment. Mean temperature was 25.13 ± 0.44 ˚C (Mean ± S.D.).

4.2 Survival proportion

4.2.1 Treated ballast water - > 32 PSU (Table 7, 8)

The rotifer, *Brachionus plicatilis* exposed > 32 PSU ballast water for 24 hours did not decease during the experiment period. The test organisms survived in all test concentrations including the control. Therefore, the mean survival proportion of the test organisms was 100.0 percent in all test concentrations.

4.2.2 Treated ballast water - 3-32 PSU (Table 9, 10)

The rotifer, *Brachionus plicatilis* exposed 3-32 PSU ballast water for 24 hours did not decease during the experiment period. The all test organisms survived in the control and 6.25 %, 12.50 % and 25.00 % of test concentrations including control. Therefore, the mean survival proportion of the test organisms was 100.0 percent in the 6.25 %, 12.50 % and 25.00 % of test concentrations including control. The only one individual died in 50.00 % and 100.00 % of test concentration, respectively. Therefore, the mean survival proportion of the test organisms was 98.3 ± 5.83 (Mean ± S.D.), respectively.

4.3 Plotting of the concentration response curve (Fig. 1, 2)

The raw data of proportion of survival from the each test concentration of the > 32 and 3-32 PSU treated ballast water was plotted on the graph.

4.4 LCx (Table 11)

LC50 values based on number of the living organisms at the end of the experiment both the > 32 and 3-32 PSU treated ballast water were greater than 100.00 %.

4.5 Other observation

Any abnormal appearance or behavior of the rotifer, *Brachionus plicatilis* during the experiment period was not observed.

5. DISCUSSION AND CONCLUSION

The survival of rotifer, *Brachionus plicatilis* on the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS system was assessed.

For the > 32 PSU treated ballast water, the test organisms survived in the all test concentrations including the control (Table 7). For the 3-32 PSU treated ballast water, the test organisms survived in all test concentrations excluding 50.00 % and 100.00 % (Table 9, the only 1 individual died in each test concentration).

Result of statistical analysis was indicated that survival proportion of test organisms in the all test concentration (the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS) was not significantly different with control ($p>0.05$). So, LC50 values based on survival data at the end of the experiment with > 32 and 3-32 PSU treated ballast water were greater than 100.00 %.

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS did not have acute toxic effects on the survival of the rotifer, *Brachionus plicatilis*.

6. REFERENCE

ASTM. 2004. Standard guide for acute toxicity test with the rotifer *Brachionus*. E-1440-91. 8pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

TABLES

Table 1. Dissolved oxygen value (mg/L) of > 32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	4.16	4.38	4.17	4.21	4.56	4.64
Final	3.77	3.41	3.54	4.04	4.28	3.94

Table 2. pH value of > 32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	7.47	7.74	7.73	7.85	7.91	7.71
Final	7.58	7.73	7.76	7.80	7.83	7.83

Table 3. Temperature (˚C) of > 32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	25.40	25.10	24.70	24.30	24.30	24.80
Final	25.20	25.30	25.60	25.50	25.50	25.50

Table 4. Dissolved oxygen value (mg/L) of 3-32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	7.70	7.31	7.10	6.63	6.28	6.66
Final	6.16	6.54	5.88	5.91	5.98	5.89

Table 5. pH value of 3-32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	7.61	7.57	7.54	7.47	7.43	7.44
Final	7.76	7.85	7.82	7.84	7.85	7.84

Table 6. Temperature (˚C) of 3-32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	24.30	24.50	25.60	25.00	25.10	25.30
Final	25.30	25.40	24.60	25.60	25.50	25.30

Table 7. Data of living and dead rotifer, *Brachionus plicatilis*, exposed to > 32 PSU treated ballast water for 24 hours

Concentration	**Control**		**6.25 %**		**12.50%**		**25.00%**		**50.00 %**		**100.00%**	
Elapsed time (h)	**24**		**24**		**24**		**24**		**24**		**24**	
Replication	**L**	**D**	**L**	**D**	**L**	**D**	**L**	**D**	**L**	**D**	**L**	**D**
1	5	0	5	0	5	0	5	0	5	0	5	0
2	5	0	5	0	5	0	5	0	5	0	5	0
3	5	0	5	0	5	0	5	0	5	0	5	0
4	5	0	5	0	5	0	5	0	5	0	5	0
5	5	0	5	0	5	0	5	0	5	0	5	0
6	5	0	5	0	5	0	5	0	5	0	5	0
7	5	0	5	0	5	0	5	0	5	0	5	0
8	5	0	5	0	5	0	5	0	5	0	5	0
9	5	0	5	0	5	0	5	0	5	0	5	0
10	5	0	5	0	5	0	5	0	5	0	5	0
11	5	0	5	0	5	0	5	0	5	0	5	0
12	5	0	5	0	5	0	5	0	5	0	5	0
Mean	5	0	5	0	5	0	5	0	5	0	5	0

*L: Living test organism; D: Dead test organism

Table 8. Survival proportion (%) for the rotifer, *Brachionus plicatilis*, exposed to > 32 PSU treated ballast water for 24 hours

Concentration	**Control**		**6.25 %**		**12.50 %**		**25.00 %**		**50.00 %**		**100.00 %**	
Elapsed time(h)	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**
Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
STDEV(±)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*STDEV: Standard deviation

Table 9. Data of living and dead rotifer, *Brachionus plicatilis*, exposed to 3-32 PSU treated ballast water for 24 hours

Concentration	**Control**		**6.25 %**		**12.50%**		**25.00%**		**50.00 %**		**100.00%**	
Elapsed time (h)	**24**		**24**		**24**		**24**		**24**		**24**	
Replication	**L**	**D**	**L**	**D**	**L**	**D**	**L**	**D**	**L**	**D**	**L**	**D**
1	5	0	5	0	5	0	5	0	5	0	5	0
2	5	0	5	0	5	0	5	0	5	0	5	0
3	5	0	5	0	5	0	5	0	5	0	5	0
4	5	0	5	0	5	0	5	0	5	0	5	0
5	5	0	5	0	5	0	5	0	5	0	5	0
6	5	0	5	0	5	0	5	0	5	0	5	0
7	5	0	5	0	5	0	5	0	5	0	5	0
8	5	0	5	0	5	0	5	0	4	1	5	0
9	5	0	5	0	5	0	5	0	5	0	5	0
10	5	0	5	0	5	0	5	0	5	0	5	0
11	5	0	5	0	5	0	5	0	5	0	4	1
12	5	0	5	0	5	0	5	0	5	0	5	0
Mean	5	0	5	0	5	0	5	0	5	0	5	0

*L: Living test organism; D: Dead test organism

Table 10. Survival proportion (%) for the rotifer, *Brachionus plicatilis*, exposed to 3-32 PSU treated ballast water for 24 hours

Concentration	**Control**		**6.25 %**		**12.50 %**		**25.00 %**		**50.00 %**		**100.00 %**	
Elapsed time(h)	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**	**0**	**24**
Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.3	100.0	98.3
STDEV(±)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.8	0.0	5.8

*STDEV: Standard deviation

Table 11. LC50 value calculated from survival data at the end of the experiment

Test substance	**LC50 (%)**	**95 % confidence of limit (%)**	
		lower	**upper**
> 32 PSU treated ballast water	> 100.00	-	-
3-32 PSU treated ballast water	> 100.00	-	-

* -: no data; > 100: greater than 100 %

FIGURES

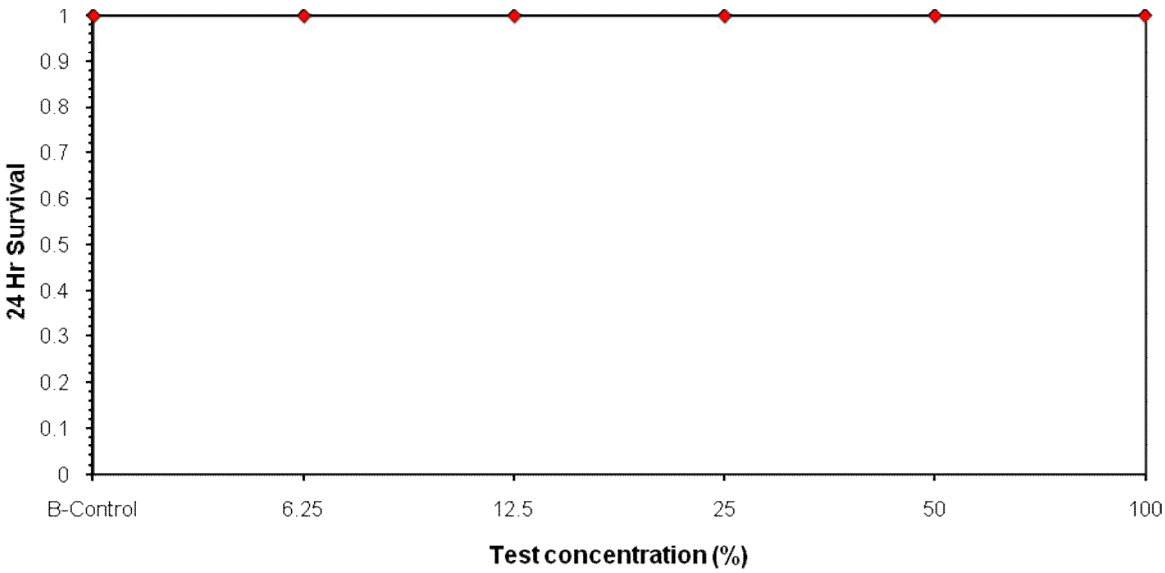


Fig. 1. Plot of the concentration-response (survival proportion) data from the each test concentration for the > 32 PSU treated ballast water.

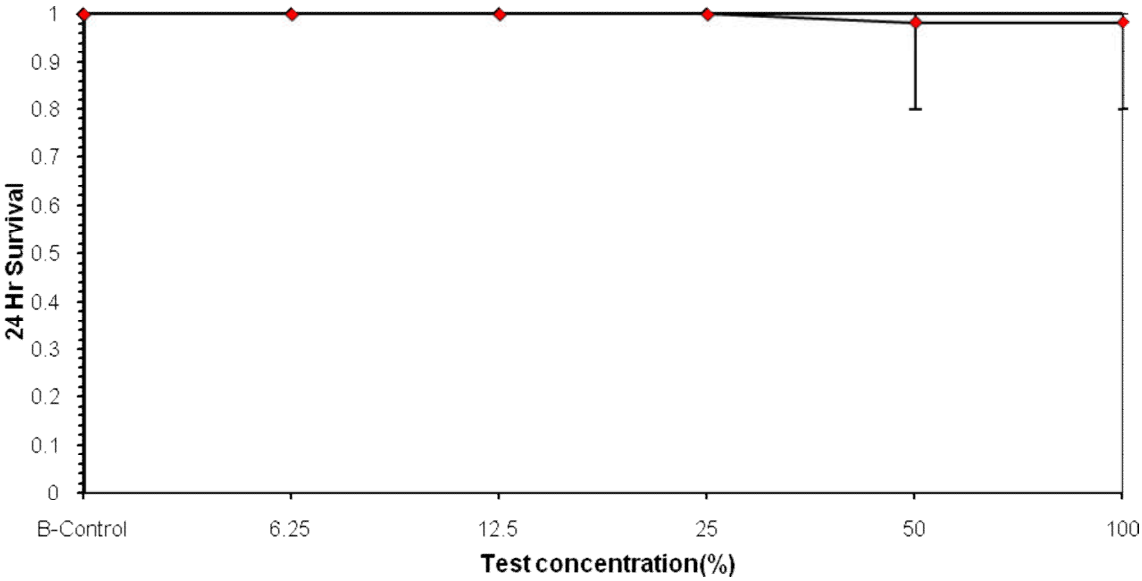


Fig. 2. Plot of the concentration-response (survival proportion) data from the each test concentration for the 3-32 PSU treated ballast water.

ANNEX 1

Definitions

1. Rotifer cyst is that a rotifer embryo arrested at an early stage in development, enclosed in an envelope and resistant to desiccation and temperature extremes. Rotifer cysts are often incorrectly referred to as resting eggs. Upon hydration, embryonic development resumes until a neonate female emerges from the cyst.

2. Rotifer neonate is a newly hatched, freely swimming rotifer. All neonates hatched from cysts are females.

3. LCx (Lethal concentration) is the concentration estimated to die x percent of the test organisms within a stated exposure period.

ANNEX 2

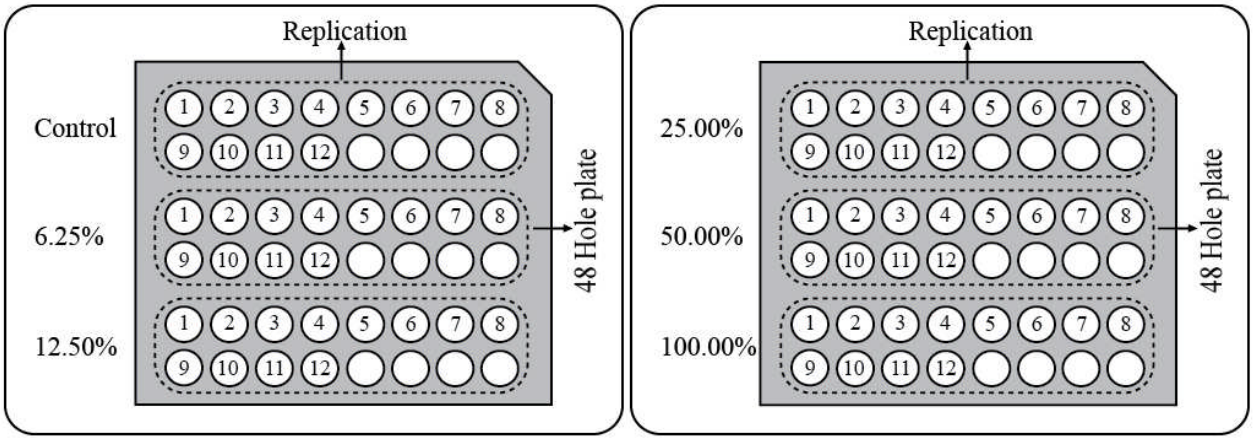
Experiment conditions and acceptability criteria for rotifer, *Brachionus plicatilis*, acute survival toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Static (non-renewal)
Temperature	25 ± 1˚C
Light quality	None
Light intensity	Darkness
Photoperiod	0 h light: 24 h dark
Test chamber size	12 mL (48 well plate)
Test solution volume	1 mL
Renewal of test solutions	None
Age of test organisms	Less than 2 hours
Number of individuals per test chamber	5 inds.
Replicate test chambers per concentration	12
Feeding regime	None
Cleaning	None
Aeration	None
Dilution water	Filtered seawater (Control seawater) by 0.45 µm membrane filter
Test concentration	5 including 100.00 % effluents and a control
Dilution factor	Effluents: 0.5
Test duration	24 hours
Endpoints	Survival
Test acceptability criteria	90% or greater survival of all control organisms

ANNEX 3

Application Site

Application site for the acute toxicity test with the rotifer, *Brachionus plicatilis* is shown as follow.



ANNEX 4

Preparation of test substance and procedures of the experiment

Fig. 1. For aquatic toxicity test, collection of the control (untreated) and treated de-ballasting water from KTM-BWMS (left down: sampling equipments).

Fig. 2. Transfer collected samples to the constant room (4 ± 1 ˚C).

Fig. 3. Preparation of test concentration diluted with non-treated seawater on treated seawater through flow-meter.

Fig. 4. For the survival test using the rotifer, *Brachionus plicatilis*, final preparation of test substance was filtered by 0.45 μm membrane filter.

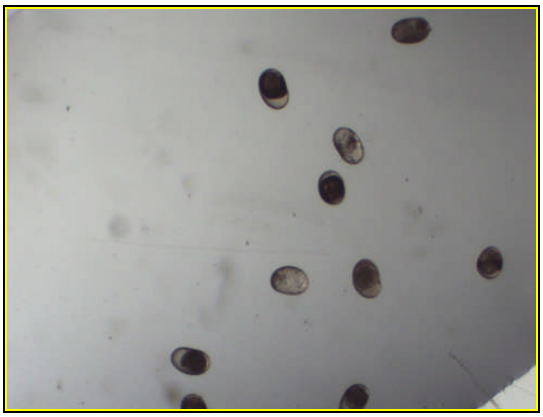


Fig. 5. Test organisms in this study: Cyst and neonates of rotifer, *Brachionus plicatilis*.

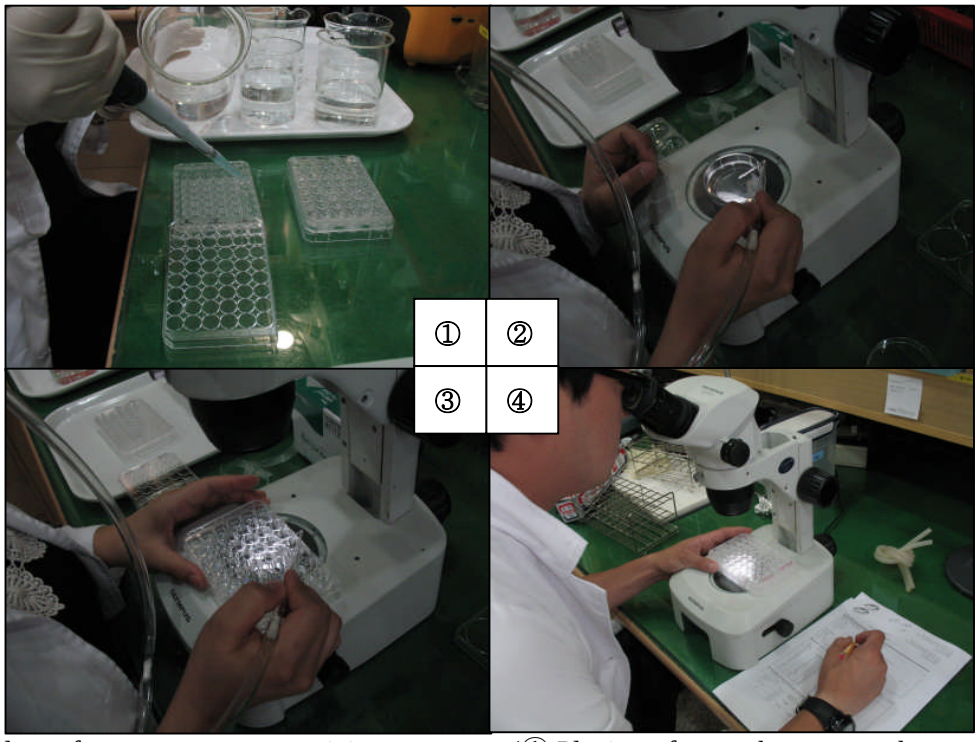


Fig. 6. Procedure of acute crustacean toxicity test set up (① Placing of test substance on the test vessel by pipette; ② Separation of test organisms from hatching chamber; ③ Placing of test organisms on the test vessel by modified pasteur pipette; ④ Observation of the test organisms).

3.1.3 ANNEX 3

The olive flounder fish, *Paralichthys olivaceus*, Acute Toxicity Test

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
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FINAL REPORT

Acute Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- The olive flounder fish, *Paralichthys olivaceus*, Acute Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

GLP STATEMENT AND SIGN

Study title: Acute toxicity to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward survival of olive flounder fish, *Paralichthys olivaceus*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

All procedure of this study was carried out following GLP regulation:

- "OECD Principles of Good Laboratory Practice"
  Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

This study was conducted on the basis of 'the Study Plan' complied and the procedure described in this report was carried out under 'the Responsibility of Study Director'. This report was built on the basis of raw data from the study.

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.
Study Director Jin Hee Kim (signature)

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.
Test Facility Manager Min Ho Son (signature)

GUARANTEE OF ASSURANCE

Study title: Acute toxicity to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward survival of olive flounder fish, *Paralichthys olivaceus*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Quality assurance personnel of Marine Eco-Technology Institute Co., Ltd., inspected as follows. Inspection was carried out on the basis of the Standard Operation Procedure and each inspection results were reported to Study Director and Test Facility Manager.

Types of inspection and the date inspection results were reported to the Test Facility Management and the Study Director as follow:

Type of Inspections	Date of Inspection	Date Reported to Test Facility Management and Study Director
Study Plan	2011. 04. 13	2011. 04. 13
Test organisms	2011. 06. 17	2011. 06. 17
Storage of Test substance	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Preparation of Test substance	2011. 06. 29 / 07. 12	2011. 06. 29 / 07. 13
Exposure	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 15
Observation	2011. 07. 01 / 07. 14	2011. 07. 01 / 07. 15
Record of raw data	2011. 07. 04 / 07. 15	2011. 07. 04 / 07. 15
Draft of final report	2011. 07. 19	2011. 07. 19
Final report	2011. 07. 20	2011. 07. 20

It is assurance that the procedure in this final report was appropriately carried out and the description of the results was reflected raw data from the study.

2011. 07. 20.

Quality Assurance personnel Jung-Im Park (signature)
Marine Eco-Technology Institute Co., Ltd.

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1. SUMMARY

The purpose of this study was to assess the acute toxic effects of the > 32 and 3-32 PSU seawater treated by the KTM-BWMS on the survival of olive flounder, *Paralichthys olivaceus*.

The test substance type was divided into two different salinities, one of them was > 32 PSU treated ballast water and the other was 3-32 PSU treated ballast water. Non-treated ballast water was used as control and dilution water. 20 individuals of olive flounder fish, *Paralichthys olivaceus* were exposed at the various test concentration (0.00 % (non-treated ballast water), 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % (treated ballast water)) for 96 hours. The mortality of the olive flounder fish, *Paralichthys olivaceus* test organisms was daily observed and recorded. The raw data of living test organisms from this experiment was used for calculation of LC25 and LC50 values.

The mortality on the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS using olive flounder fish, *Paralichthys olivaceus* was assessed for 96 hours.

For >32 PSU treated ballast water, Mean 19~20 of 20 test organisms in each test concentration (including control) were survived and mean mortality of test organism at the end of the experiment was ranged from 3.3 to 10.0 percent. The result of statistic analysis indicated that survival proportion of test organism in the control was not significantly different with other test concentrations.

For 3~32 PSU treated ballast water, all test organisms in all test concentration including control were survived during the test period. Therefore, mortality was 0.0 percent in all test concentrations.

LC25 and LC50 values based on the survival data were greater than 100.00 % in both > 32 and 3-32 PSU treated ballast water.

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS did not have acute toxic effects on the survival of the olive flounder, *Paralichthys olivaceus*.

2. OUTLINE OF STUDY

2.1 Purpose

The purpose of this study was to assess the acute toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS on the survival of juvenile olive flounder, *Paralichthys olivaceus.*

2.2 Principle

The juvenile olive flounder, *Paralichthys olivaceus* is exposed to the treated ballast water of the KTM-BWMS for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentration which kills 50 percent of the fish (LC50) are determined where possible.

2.3 Good Laboratory Practice

All procedure of this study was complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

2.4 Guideline

This study was complied with principle and conducted on the basis of following standard method and applied for *Paralichthys olivaceus:*

- "OECD Guideline for Testing of Chemicals, 203, Fish, Acute Toxicity Test"

Organization for Economic Co-operation and Development, (Adopted: July. 17, 1992)

2.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

2.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611- 6200 Fax: + 82-51-611- 0588

2.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.8 Study director

Name: Jin Hee Kim

Department: 3rd research team

2.9 Study timetable

2.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Receipt of test organisms	2011. 06. 16
Acclimatization on Lab Condition (> 32 PSU)	2011. 06. 16 ~ 06. 23
Settling in the test vessel	2011. 06. 23
Observation of mortality	2011. 06. 23 ~ 06.30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation of mortality	2011. 06. 30 ~ 07. 04
Experimental completion	2011. 07. 04
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2010. 05. 02
Receipt of test organisms	2011. 06. 16
Acclimatization on Lab Condition (20 PSU)	2011. 06. 16 ~ 07. 05
Settling in the test vessel	2011. 07. 05
Observation of mortality	2011. 07. 05 ~ 07. 12
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation of mortality	2011. 07. 13 ~ 07. 17
Experimental completion	2011. 07. 17
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.10 Responsible personnel

Laboratory manager (Test site manager) Statistical analysis	Myung-Baek Shon
Receipt and culture of test organisms Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Observation and record Check the chemical condition	Tae Won Kim

2.11 Retention of records and data

2.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

2.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

3. MATERIALS AND METHODS

3.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

3.1.1 Substance name

Treated water (KTM-S5D5-1)

3.1.2 Lot No.

None

3.1.3 Type

Liquid

3.1.4 Principal ingredients

Seawater

3.1.5 Temperature

23.2 ˚C

3.1.6 Salinity

33.6 PSU

3.1.7 pH

7.67

3.1.8 Custody condition

Constant room at 4 ˚C for 2 days

3.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.1.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.1.11 Disposal of the resting substances

Scrapped after test

3.1.12 Certificate of analysis

Client supplies to test organization

3.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

3.2.1 Substance name

Non-treated water (KTM-S3D5-1)

3.2.2 Lot No.

None

3.2.3 Type

Liquid

3.2.4 Principal ingredients

Seawater

3.2.5 Temperature

23.1 ˚C

3.2.6 Salinity

33.9 PSU

3.2.7 pH

7.50

3.2.8 Custody condition

Constant room at 4 ˚C for 2 days

3.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.2.11 Disposal of the resting substances

Scrapped after test

3.2.12 Certificate of analysis

Client supplies to test organization

3.3 Test substance-3 (Treated ballast water (3 - 32 PSU) -Treated)

3.3.1 Substance name

Treated water (KTM-S5D5-3)

3.3.2 Lot No.

None

3.3.3 Type

Liquid

3.3.4 Principal ingredients

Seawater

3.3.5 Temperature

24.6 ˚C

3.3.6 Salinity

21.7 PSU

3.3.7 pH

7.46

3.3.8 Custody condition

Constant room at 4 ˚C for 2 days

3.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.3.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.3.11 Disposal of the resting substances

Scrapped after test

3.3.12 Certificate of analysis

Client supplies to test organization

3.4 Test substance-4 (Non-treated ballast water (3 - 32 PSU) -Control)

3.4.1 Substance name

Non-treated water (KTM-S3D5-3)

3.4.2 Lot No.

None

3.4.3 Type

Liquid

3.4.4 Principal ingredients

Seawater

3.4.5 Temperature

24.8 ˚C

3.4.6 Salinity

22.7 PSU

3.4.7 pH

7.24

3.4.8 Custody condition

Constant room at 4 ˚C for 2 days

3.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.4.11 Disposal of the resting substances

Scrapped after test

3.4.12 Certificate of analysis

Client supplies to test organization

3.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance was diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid (see Fig. 1~3 in the Annex 4). The six concentrations of test substance including control (0.00 %, only non-treated ballast water by the KTM-BWMS) and 100.00 % were arrange for the final definitive experiment.

3.6 Culture condition (SOP#TO-005)

3.6.1 Holding of test organisms

Test organisms	*Paralichthys olivaceus*
Culture type	Semi- recycle filter system
Vessel	1,000 L cubic aquarium
Medium	600 L filtered seawater (1μm CP Filter, Chisso Filter)
Temperature	20 ± 1 ˚C
Light source	“cool-white” fluorescent lamp
Light cycle	L : D = 16 h :8 h
Light intensity	2,000 lux
Dissolved oxygen	6.0~8.0 mg/L

3.6.2 Feeding regime

Type of diet	Suhyup feed for fish 1-1S
Supplier	National Federation of Fisheries Cooperatives
Diet supply period	once a day

3.7 Test system

3.7.1 Test organisms

.1 Scientific name	*Paralichthys olivaceus* (see Fig. 4 in the Annex 4)
.2 Producer	KyeongYang Fisheries, Yo-Su city, Jeolla province, Republic of Korea
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

3.7.2 Ground of selected test organisms

Pleuronectiformes Paralichthyidae *Paralichthys olivaceus* called olive flounder is widely distributed in coastal water of Korea and is important as economic marine products. *P. olivaceus* plays in marine trophic structure as a top predator.

3.7.3 Preparation of the test organisms (SOP#TP-006)

Paralichthys olivaceus aged 30~50 days was acclimated in the laboratory for 2 ~ 3 weeks until 24 hours before the beginning of the experiment. Following a 48 hour settling-in period, mortalities were recorded and the following criteria applied:

.1 Mortalities of greater than 10 percent of population in seven days: rejection of entire batch.

.2 Mortalities of between 5 and 10 percent of population: acclimatization continued for seven additional days.

.3 Mortalities of less than 5 percent of population: acceptance of batch.

.4 The condition index of test organisms was shown Annex 5.

3.7.4 Check the test organisms for the experiment (SOP#TP-006)

Health of the test organisms such as the mortality, appearance and abnormal behavior was observed.

3.7.5 Disposal of the remaining test organisms (SOP#TO-006)

The resting test organisms were euthanized under tricaine methanesulfonate (MS-222).

3.8 Study design

3.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

3.8.2 Exposure condition (SOP#TP-006)

20 fishes on the > 32 PSU and 3-32 PSU treated ballast water were placed in the each replication of test vessel. The test vessels were placed in the constant room for 96 hours at 20 ± 1 ˚C (see Fig. 4~5 in the Annex 4).

Experiment condition and criteria are shown Annex 2 and application site was shown Annex 3.

3.8.3 Observation item and measurement components

.1 Mortality (SOP#TP-006)

Fish was considered dead if there was no visible movement and if touching of the caudal peduncle produces no reaction. Mortalities were recorded and then dead animals were removed.

.2 Other observations (SOP#TP-006)

Records were kept of visible abnormalities, such as loss of equilibrium, swimming behavior, respiratory function and pigmentation.

.3 Temperature (SOP#TE-002)

Temperature of the solutions on each vessel during the experiment period was measured daily.

.4 Dissolved oxygen (SOP#TE-002)

DO of the solutions on each vessel during the experiment period was measured daily.

.5 pH (SOP#TE-002)

pH of the solutions on each vessel during the experiment period was measured daily.

.6 Salinity (SOP#TE-002)

Salinity of the solutions on each vessel during the experiment period was measured daily.

3.9 Statistical procedures (SOP#SP-101)

For the > 32 treated ballast water, normal distribution and equal variances of the data were tested by Shapiro-Wilk's Test and Bartlett's Test. Hypothesis test was conducted by Dunnett's Test. Differences in all test were considered to be significant at α=0.05. For the > 32 treated ballast water, statistical analysis based on the survival data of test organisms meant nothing because all test organisms survived in all test concentrations. TOXCALC 5.0 program (Tidepool scientific software, USA) was used for above statistic analysis.

4. RESULTS

4.1 Dissolved oxygen, pH, Temperature and Salinity

4.1.1 Treated ballast water - > 32 PSU (Table 1~4)

For the > 32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 5.08 to 6.85 mg/L at the beginning experiment and from 5.26 to 6.43 mg/L at the end of the experiment. pH value was ranged from 7.71 to 7.88 at the beginning experiment and was ranged from 7.74 to 7.96 at the end of the experiment. Mean temperature was 19.95 ± 0.24 ˚C (Mean ± S.D.) and mean salinity was 33.24 ± 0.26 PSU (Mean ± S.D.).

4.1.2 Treated ballast water - 3-32 PSU (Table 5~8)

For the 3-32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 5.18 to 8.58 mg/L at the beginning experiment and from 6.02 to 7.09 mg/L at the end of the experiment. pH value was ranged from 7.63 to 7.77 at the beginning experiment and from 7.65 to 7.88 at the end of the experiment. Mean temperature was 20.02 ± 0.35 ˚C (Mean ± S.D.) and mean salinity was 21.81 ± 0.05 PSU (Mean ± S.D.).

4.2 Mortality

4.2.1 Treated ballast water - > 32 PSU (Table 9, 10)

Mean 19~20 of 20 test organisms in each test concentration were survived during the test period. Mean mortality of test organism at the end of the experiment was ranged from 3.3 to 10.0 percent.

4.2.2 Treated ballast water - 3-32 PSU (Table 11, 12)

All test organisms in all test concentration including control were survived during the test period. Therefore, mortality was 0.0 percent in all test concentrations.

4.3 Plotting of the concentration response curve (Fig. 1, 2)

The mortality data on the each test concentration of the > 32 and 3-32 PSU treated ballast water was plotted on the graph.

4.4 LC25 and LC50 (Table 13)

The LC25 and LC50 values based on number of the living organisms at the end of the experiment both the > 32 and 3-32 PSU treated ballast water were greater than 100.00 % in this study.

4.5 Other observation

Any abnormality, such as loss of equilibrium, swimming behavior, respiratory function, pigmentation of the olive flounder fish, *Paralichthys olivaceus* was not observed during the experiment period.

5. DISCUSSION AND CONCLUSION

The mortality on the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS using olive flounder fish, *Paralichthys olivaceus* was assessed for 96 hours.

For >32 PSU treated ballast water, mean 19~20 of 20 test organisms in each test concentration (including control) were survived and mean mortality of test organism at the end of the experiment was ranged from 3.3 to 10.0 percent (Table 9 and 10). The result of statistic analysis indicated that survival proportion of test organism in the control was not significantly different with other test concentrations (Dunnett's Test (1-tail), p>0.05).

For 3~32 PSU treated ballast water, all test organisms in all test concentration including control were survived during the test period (Table 11). Therefore, mortality was 0.0 percent in all test concentrations (Table 12).

LC25 and LC50 values based on the survival data were greater than 100.00 % in both > 32 and 3-32 PSU treated ballast water (Table 13, Fig. 1 and 2).

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS did not have acute toxic effects on the survival of the olive flounder, *Paralichthys olivaceus*.

6. REFERENCE

OECD guidelines for the testing of chemicals-203. Fish, Acute toxicity test. 9pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

TABLES

Table 1. Dissolved oxygen value (mg/L) of > 32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	5.57	5.08	5.53	**5.39**	5.08	5.71	5.26	**5.35**	5.09	5.18	5.90	**5.39**
24	5.73	5.92	5.66	**5.77**	5.95	5.43	5.67	**5.68**	5.51	5.03	5.76	**5.43**
48	5.98	5.92	5.85	**5.92**	5.89	5.98	6.00	**5.96**	5.96	6.92	6.01	**6.30**
72	6.24	6.02	6.54	**6.27**	7.02	7.00	6.96	**6.99**	6.76	6.41	6.48	**6.55**
96	6.06	5.73	6.13	**5.97**	5.50	5.62	5.89	**5.67**	6.20	5.40	5.93	**5.84**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	6.01	5.51	5.64	**5.72**	6.15	6.85	6.27	**6.42**	6.16	5.70	6.59	**6.15**
24	5.85	5.63	5.60	**5.69**	5.71	5.39	5.62	**5.57**	5.00	5.32	5.80	**5.37**
48	5.95	5.68	6.32	**5.98**	6.51	6.36	6.17	**6.35**	6.25	6.53	6.47	**6.42**
72	6.02	6.00	6.42	**6.15**	6.41	6.11	6.02	**6.18**	6.00	6.21	6.31	**6.17**
96	5.84	6.05	5.27	**5.72**	5.26	6.04	6.43	**5.91**	5.61	6.20	6.02	**5.94**

Table 2. pH value of > 32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	7.88	7.87	7.85	**7.87**	7.85	7.82	7.83	**7.83**	7.82	7.82	7.82	**7.82**
24	7.69	7.83	7.82	**7.78**	7.80	7.80	7.77	**7.79**	7.67	7.77	7.78	**7.74**
48	7.79	7.77	7.78	**7.78**	7.77	7.78	7.75	**7.77**	7.80	7.79	7.79	**7.79**
72	7.80	7.78	7.77	**7.78**	7.81	7.82	7.82	**7.82**	7.81	7.80	7.81	**7.81**
96	7.87	7.88	7.96	**7.90**	7.95	7.89	7.74	**7.86**	7.83	7.83	7.87	**7.84**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	7.78	7.73	7.77	**7.76**	7.71	7.73	7.73	**7.72**	7.75	7.75	7.74	**7.75**
24	7.74	7.61	7.43	**7.59**	7.71	7.78	7.78	**7.76**	7.72	7.73	7.74	**7.73**
48	7.75	7.75	7.74	**7.75**	7.78	7.78	7.79	**7.78**	7.76	7.77	7.75	**7.76**
72	7.80	7.79	7.77	**7.79**	7.80	7.79	7.77	**7.79**	7.78	7.75	7.74	**7.76**
96	7.84	7.88	7.83	**7.85**	7.84	7.88	7.83	**7.85**	7.74	7.83	7.82	**7.80**

Table 3. Temperature (˚C) of > 32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	19.70	20.10	20.40	**20.07**	20.40	20.50	20.10	**20.33**	20.20	20.30	20.20	**20.23**
24	19.70	20.10	20.10	**19.97**	20.20	20.30	20.20	**20.23**	20.00	20.10	20.10	**20.07**
48	20.00	20.50	20.40	**20.30**	20.10	20.30	20.20	**20.20**	20.30	20.20	20.20	**20.23**
72	19.70	19.80	19.80	**19.77**	20.00	20.00	19.90	**19.97**	19.70	19.80	19.70	**19.73**
96	19.60	19.80	20.00	**19.80**	20.10	20.00	20.10	**20.07**	19.90	19.90	19.70	**19.83**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	19.80	19.80	20.10	**19.90**	19.80	20.00	20.00	**19.93**	19.80	19.70	19.50	**19.67**
24	19.90	19.90	20.10	**19.97**	20.10	20.00	19.90	**20.00**	19.60	19.70	19.60	**19.63**
48	20.00	19.90	19.90	**19.93**	20.00	20.10	20.00	**20.03**	20.00	20.10	20.00	**20.03**
72	19.80	19.80	20.00	**19.87**	20.00	20.10	19.90	**20.00**	19.60	19.70	19.80	**19.70**
96	19.60	19.70	19.90	**19.73**	19.90	19.80	19.70	**19.80**	19.40	19.50	19.30	**19.40**

Table 4. Salinity (PSU) of > 32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	33.30	33.30	33.30	**33.30**	33.30	33.70	33.60	**33.53**	33.30	32.40	33.10	**32.93**
24	33.00	33.00	33.50	**33.17**	33.30	33.30	33.70	**33.43**	33.50	33.30	33.20	**33.33**
48	33.20	33.30	33.10	**33.20**	33.20	33.20	33.20	**33.20**	33.40	33.50	33.40	**33.43**
72	33.20	33.30	33.20	**33.23**	33.20	33.20	33.20	**33.20**	33.10	33.20	33.20	**33.17**
96	33.50	33.60	33.50	**33.53**	33.30	33.50	33.60	**33.47**	33.50	33.20	33.40	**33.37**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	33.40	33.00	32.50	**32.97**	33.40	33.30	32.50	**33.07**	32.70	33.00	33.10	**32.93**
24	32.90	33.30	33.20	**33.13**	32.50	33.40	33.40	**33.10**	32.80	33.20	33.00	**33.00**
48	33.50	33.40	33.50	**33.47**	33.50	33.50	33.50	**33.50**	33.00	33.30	33.00	**33.10**
72	33.20	33.20	33.20	**33.20**	33.10	33.20	33.10	**33.13**	33.20	33.20	33.10	**33.17**
96	33.10	33.40	33.40	**33.30**	33.50	33.60	33.00	**33.37**	33.50	33.00	33.40	**33.30**

Table 5. Dissolved oxygen value (mg/L) of 3-32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	8.58	7.78	7.18	**7.85**	6.95	6.50	6.21	**6.55**	5.59	6.05	6.03	**5.89**
24	6.28	6.06	5.94	**6.09**	6.46	5.71	6.15	**6.11**	5.49	5.90	5.56	**5.65**
48	5.17	5.81	5.96	**5.65**	5.63	5.09	5.73	**5.48**	5.48	5.66	5.16	**5.43**
72	6.21	6.42	6.43	**6.35**	6.21	6.71	6.93	**6.62**	6.34	6.27	6.41	**6.34**
96	7.09	6.02	6.12	**6.41**	6.41	6.92	6.71	**6.68**	6.42	6.41	6.71	**6.51**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	5.75	5.89	5.42	**5.69**	5.61	5.66	5.18	**5.48**	5.42	5.20	5.31	**5.31**
24	5.76	5.86	6.00	**5.87**	5.90	5.28	5.28	**5.49**	5.21	5.30	5.30	**5.27**
48	5.08	5.57	5.58	**5.41**	6.01	5.72	5.44	**5.72**	5.37	5.49	5.30	**5.39**
72	6.42	6.81	6.92	**6.72**	5.71	7.02	7.11	**6.61**	7.03	7.02	7.11	**7.05**
96	6.79	6.81	6.92	**6.84**	6.41	6.02	6.04	**6.16**	6.88	6.97	6.44	**6.76**

Table 6. pH value of 3-32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	7.67	7.63	7.74	**7.68**	7.77	7.76	7.73	**7.75**	7.65	7.66	7.71	**7.67**
24	7.88	7.88	7.88	**7.88**	7.87	7.88	7.88	**7.88**	7.85	7.82	7.84	**7.84**
48	7.58	7.54	7.53	**7.55**	7.54	7.55	7.57	**7.55**	7.55	7.51	7.54	**7.53**
72	7.81	7.83	7.82	**7.82**	7.79	7.78	7.76	**7.78**	7.78	7.79	7.64	**7.74**
96	7.78	7.77	7.79	**7.78**	7.78	7.76	7.75	**7.76**	7.70	7.76	7.65	**7.70**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	7.71	7.74	7.74	**7.73**	7.74	7.75	7.72	**7.74**	7.69	7.66	7.63	**7.66**
24	7.84	7.85	7.86	**7.85**	7.80	7.87	7.84	**7.84**	7.80	7.80	7.78	**7.79**
48	7.54	7.61	7.62	**7.59**	7.64	7.59	7.53	**7.59**	7.43	7.43	7.40	**7.42**
72	7.81	7.92	7.42	**7.72**	7.83	7.87	7.88	**7.86**	7.84	7.88	7.88	**7.87**
96	7.78	7.87	7.88	**7.84**	7.86	7.88	7.80	**7.85**	7.72	7.73	7.74	**7.73**

Table 7. Temperature (˚C) of 3-32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	19.60	20.60	20.90	**20.37**	20.20	20.50	20.10	**20.27**	20.30	20.50	20.50	**20.43**
24	19.90	20.00	20.20	**20.03**	20.20	20.30	20.40	**20.50**	20.40	20.30	20.20	**20.30**
48	20.00	20.00	20.20	**20.07**	20.30	20.30	20.40	**20.33**	20.00	20.30	20.30	**20.20**
72	19.90	20.00	20.00	**19.97**	20.20	20.10	20.20	**20.17**	20.00	20.00	20.00	**20.00**
96	19.40	20.00	20.00	**19.80**	20.20	20.10	20.20	**20.17**	19.70	20.00	20.00	**19.90**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	20.20	20.00	20.30	**20.17**	20.50	20.40	20.20	**20.37**	20.40	20.20	20.00	**20.20**
24	20.00	20.00	20.20	**20.07**	20.30	20.30	20.10	**20.23**	19.90	19.70	19.60	**19.73**
48	20.10	20.10	20.30	**20.17**	20.30	20.20	20.10	**20.20**	19.80	19.80	19.70	**19.77**
72	19.40	19.20	19.30	**19.30**	19.30	19.20	19.40	**19.30**	19.10	20.00	20.10	**19.73**
96	19.60	19.90	19.80	**19.77**	19.60	20.00	20.00	**19.87**	19.10	19.80	19.80	**19.57**

Table 8. Salinity (PSU) of 3-32 PSU test concentration series during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	21.70	21.80	21.80	**21.77**	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**
24	21.90	21.90	21.90	**21.90**	21.90	21.80	21.80	**21.83**	21.80	21.80	21.80	**21.80**
48	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**	21.90	21.80	21.80	**21.83**
72	21.80	21.90	21.90	**21.87**	21.90	21.80	21.80	**21.83**	21.80	21.90	21.80	**21.83**
96	21.90	21.80	21.80	**21.83**	21.90	21.80	21.80	**21.83**	21.80	21.90	21.80	**21.83**
Elapsed time (h)	**25.00 %**				**50.00 %**				**100.00 %**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	21.90	21.80	21.80	**21.83**	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**
24	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**
48	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**	21.80	21.70	21.70	**21.73**
72	21.80	21.80	21.80	**21.80**	21.70	21.80	21.70	**21.73**	21.90	21.70	21.90	**21.83**
96	21.80	21.80	21.80	**21.80**	21.70	21.70	21.70	**21.70**	21.90	21.80	21.80	**21.83**

Table 9. Live (L) and dead (D) data of *Paralichthys olivaceus*, exposed to > 32 PSU treated ballast water during the experiment period

E. T* (h)	Control								6.25%								12.50%							
	1		2		3		Mean		1		2		3		Mean		1		2		3		Mean	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
24	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
48	20	0	20	0	20	0	20	0	19	1	20	0	20	0	20	0	20	0	19	1	20	0	20	0
72	20	0	20	0	20	0	20	0	18	2	20	0	20	0	19	1	20	0	18	2	20	0	19	1
96	20	0	19	1	18	2	19	1	17	3	20	0	20	0	19	1	20	0	18	2	20	0	19	1
E. T (h)	25.00%								50.00%								100.00%							
	1		2		3		Mean		1		2		3		Mean		1		2		3		Mean	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
24	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
48	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
72	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
96	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0

* E.T. : Elapsed time; L: living test organism; D: dead test organism

Table 10. Mortality of *Paralichthys olivaceus*, exposed to > 32 PSU treated ballast water during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	5.0	0.0	0.0	1.7	0.0	5.0	0.0	1.7
72	0.0	0.0	0.0	0.0	10.0	0.0	0.0	3.3	0.0	10.0	0.0	3.3
96	0.0	5.0	10.0	5.0	15.0	0.0	0.0	5.0	0.0	10.0	0.0	3.3
Elapsed time (h)	25.00 %				50.00 %				100.00 %			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	5.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	5.0	0.0	0.0	1.7	5.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0
72	10.0	0.0	0.0	3.3	5.0	5.0	0.0	3.3	0.0	0.0	0.0	0.0
96	15.0	0.0	0.0	5.0	15.0	5.0	10.0	10.0	0.0	10.0	0.0	3.3

Table 11. Live (L) and dead (D) data of *Paralichthys olivaceus*, exposed to 3-32 PSU treated ballast water during the experiment period

E. T* (h)	Control								6.25%								12.50%							
	1		2		3		Mean		1		2		3		Mean		1		2		3		Mean	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
24	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
48	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
72	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
96	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
E. T (h)	25.00%								50.00%								100.00%							
	1		2		3		Mean		1		2		3		Mean		1		2		3		Mean	
	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D
0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
24	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
48	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
72	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0
96	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0

* E.T. : Elapsed time; L: living test organism; D: dead test organism

Table 12. Mortality of *Paralichthys olivaceus*, exposed to 3-32 PSU treated ballast water during the experiment period

Elapsed time (h)	Control				6.25 %				12.50 %			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elapsed time (h)	25.00 %				50.00 %				100.00 %			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 13. LC25 and LC50 value calculated from survival data at the end of the experiment

Test substance	LC25 (%)	95 % confidence of limit (%)		LC50 (%)	95 % confidence of limit (%)	
		lower	upper		lower	upper
> 32 PSU treated ballast water	> 100.0	-	-	> 100.0	-	-
	> 100.0	-	-	> 100.0	-	-
3-32 PSU treated ballast water	> 100.0	-	-	> 100.0	-	-
	> 100.0	-	-	> 100.0	-	-

* -: no data; > 100.0: greater than 100.0 %.

FIGURES

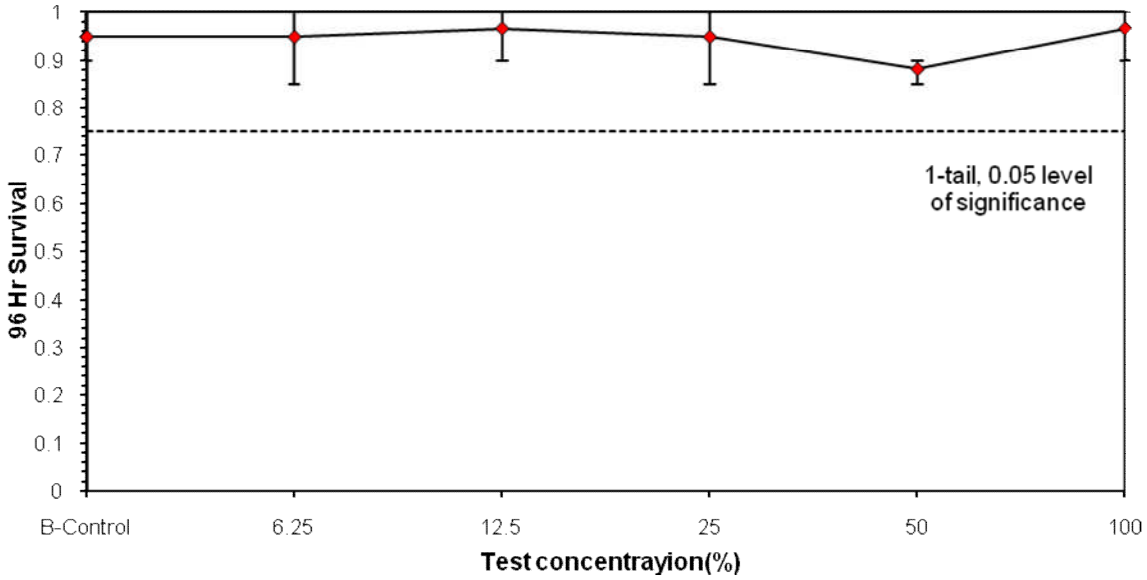


Fig. 1. Plot of the concentration-response data from the each test concentration of the > 32 PSU treated ballast water. The dotted line was represented as 0.05 level of significant and vertical bar was represented maximum and minimum.

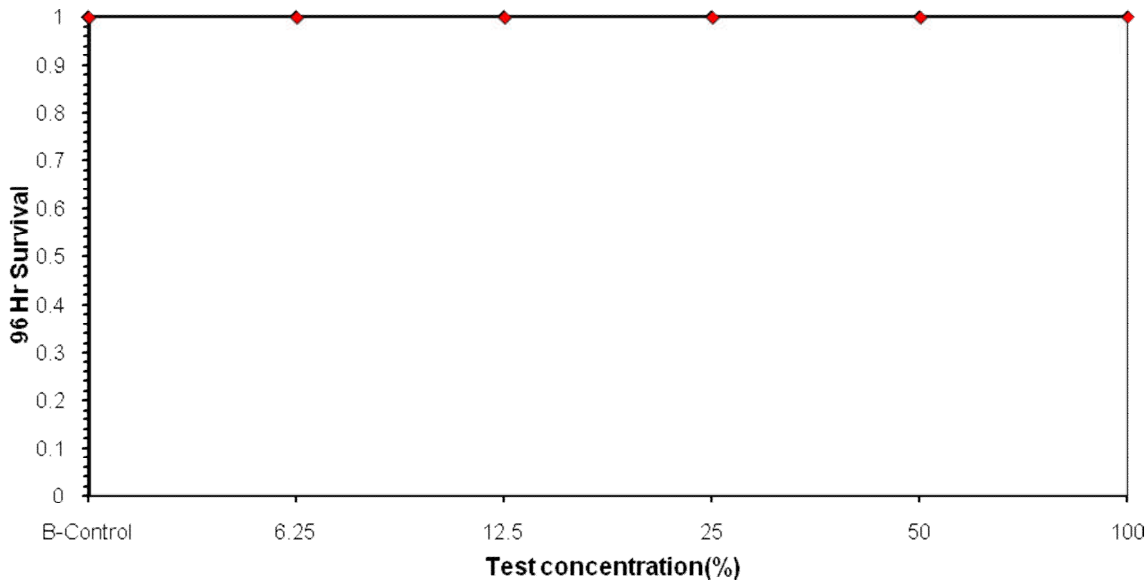


Fig. 2. Plot of the concentration-response data from the each test concentration of the 3-32 PSU treated ballast water. The dotted line was represented as 0.05 level of significant and vertical bar was represented maximum and minimum.

ANNEX 1

Definitions

1. Semi-static test is a test without flow of solution, but with occasional batch wise renewal of the test solution after prolonged periods.

2. LCx in this study is the median lethal concentration, i.e. that concentration of the test substance in water which kills **x** percent of a test batch of fish within a particular period of exposure.

ANNEX 2

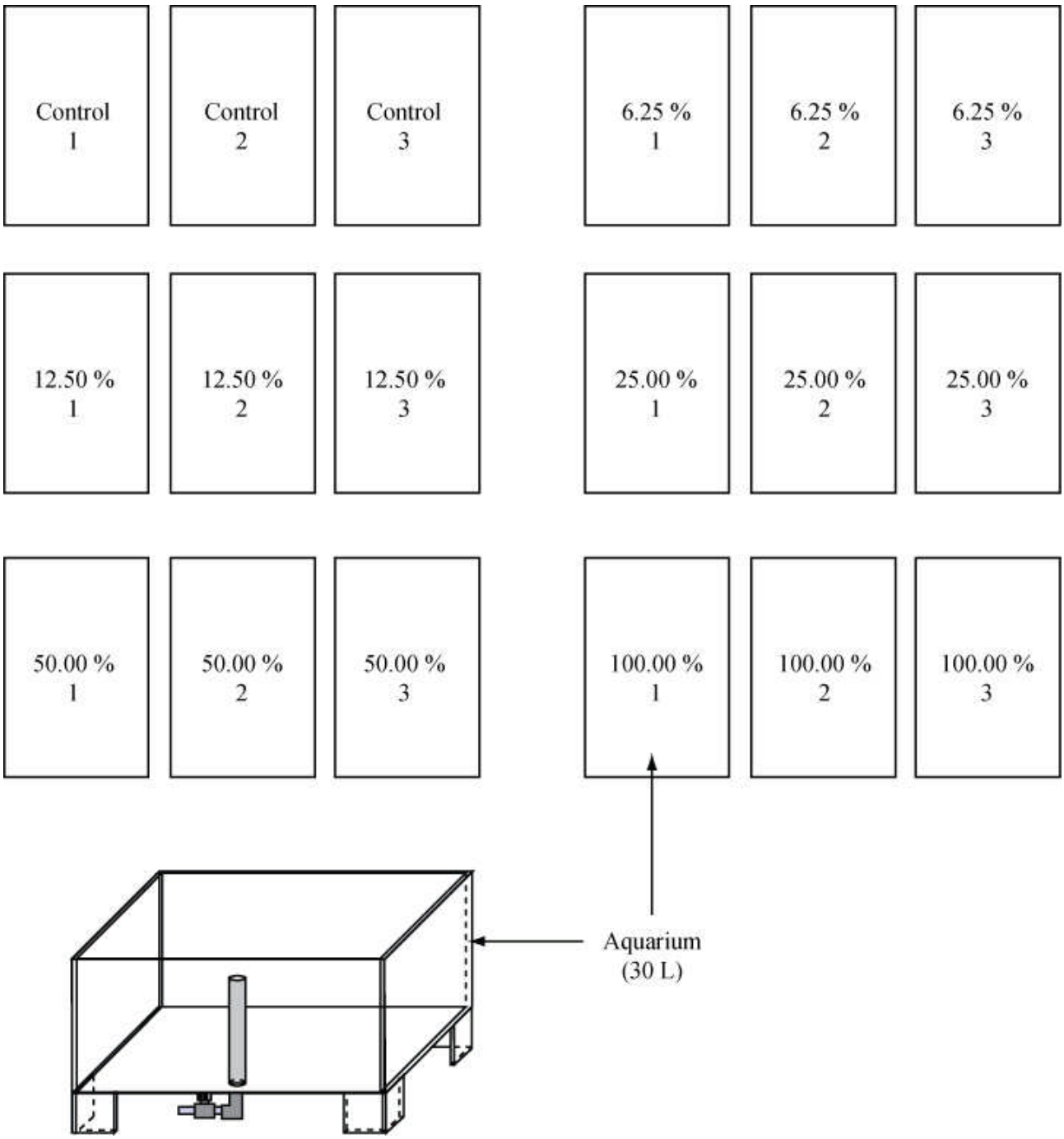
Experiment conditions and acceptability criteria for olive flounder, *Paralichthys olivaceus*, acute mortality toxicity tests with > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Semi-static
Temperature	20±1˚C
Light quality	Cool white fluorescent lighting
Light intensity	2,000 lux
Photoperiod	16 h light : 8 h dark
Test chamber size	30 L
Test solution volume	20 L
Renewal of test solutions	Every other day
Age of test organisms	30~50 days old
Number of individuals per test chamber	20
Replicate test chambers per concentration	3
Feeding regime	None
Cleaning	None
Aeration	To maintain DO (> 60 % of the air saturation value)
Dilution water	Filtered seawater (Control seawater) by 1μm CP Filter, Chisso Filter
Test concentration	5 including 100.00 % effluents and a control
Dilution factor	Effluents: 0.5
Test duration	96 hours
Endpoints	Survival
Test acceptability criteria	90 % or greater survival of all control organisms

ANNEX 3

Application Site

Application site for the acute toxicity test with olive flounder, *Paralichthys olivaceus* is shown as follow.



ANNEX 4

Preparation of test substance and procedures of the experiment

Fig. 1. For aquatic toxicity test, collection of the control (untreated) and treated de-ballasting water from KTM-BWMS (left down: sampling equipments).

Fig. 2. Transfer collected samples to the constant room (4 ± 1 ˚C).

Fig. 3. Preparation of test concentration diluted with non-treated seawater on treated seawater through flow-meter.

Fig. 4. Test organisms in this study: Olive flounder, *Paralichthys olivaceus*.

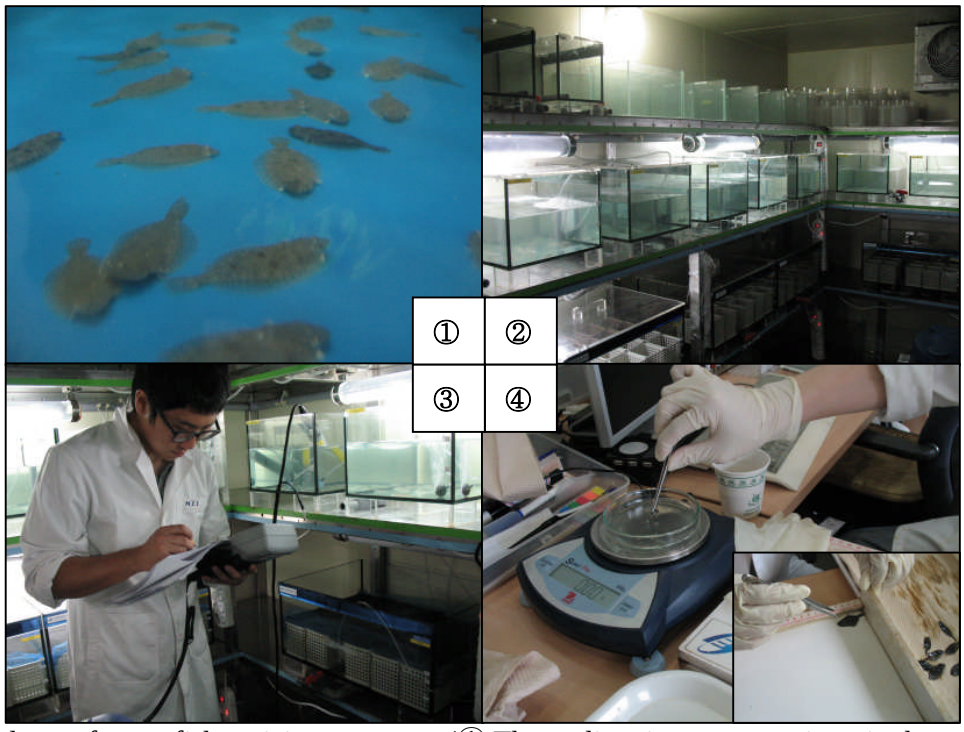


Fig. 5. Procedures of acute fish toxicity test set up (① The acclimating test organisms in the aquarium placed on test facility; ② Test vessels set up; ③ Observation and recording of test organisms and the water quality; ④ Measurement of length and wet weight of the samples).

ANNEX 5

Condition Index

▶ Total length and wet weight of olive flounder juvenile, *Paralichthys olivaceus*, for the > 32 PSU seawater treated by the KTM-BWMS

▷ **Concentration :** Control						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	4.7	0.52	4.9	0.73	5.0	0.63
2	5.4	0.86	5.0	0.74	5.2	0.82
3	4.8	0.66	5.1	0.80	4.5	0.50
4	5.3	0.98	5.1	0.75	4.8	0.67
5	4.4	0.53	4.7	0.63	5.0	0.82
6	4.5	0.60	5.1	0.75	5.0	0.89
7	4.7	0.57	5.1	0.81	5.1	0.92
8	5.4	0.98	4.8	0.65	5.1	0.88
9	4.5	0.56	4.7	0.74	5.0	0.87
10	4.2	0.52	4.6	0.65	4.9	0.75
11	4.6	0.61	4.8	0.84	4.8	0.76
12	4.8	0.64	5.0	0.87	4.8	0.74
13	4.7	0.70	4.9	0.67	4.7	0.65
14	4.9	0.61	4.8	0.69	4.9	0.74
15	5.1	0.80	4.7	0.82	4.8	0.85
16	5.2	0.82	4.6	0.69	4.8	0.54
17	5.3	0.86	4.2	0.70	5.2	0.64
18	4.9	0.69	4.8	0.95	5.3	0.65
19	4.5	0.61	4.8	0.87	5.0	0.85
20	4.4	0.63	4.5	0.65	5.1	0.80
Mean	4.8	0.69	4.8	0.75	5.0	0.75

▷ **Concentration :** 6.25 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	5.0	0.85	4.6	0.66	5.0	0.87
2	4.5	0.66	5.1	0.79	4.8	0.66
3	4.7	0.68	4.5	0.60	4.8	0.67
4	4.9	0.69	5.5	0.90	5.0	0.71
5	5.5	1.00	5.1	0.80	4.5	0.62
6	5.0	0.95	4.7	0.57	4.9	0.72
7	5.1	0.95	5.0	0.64	4.8	0.62
8	4.8	0.96	5.0	0.73	4.8	0.68
9	4.9	0.84	5.1	0.95	4.7	0.95
10	5.0	0.87	4.8	0.85	4.9	0.85
11	5.0	0.95	4.9	0.85	4.5	0.54
12	5.1	0.95	4.2	0.74	4.8	0.87
13	4.7	0.96	5.2	0.65	4.8	0.68
14	4.8	0.98	5.8	0.85	4.9	0.69
15	4.8	0.85	4.7	0.82	5.0	0.95
16	4.9	0.65	4.9	0.91	5.0	0.85
17	4.9	0.84	4.8	0.63	5.1	0.82
18	4.8	0.75	4.9	0.45	5.0	0.83
19	4.6	0.72	4.8	0.85	4.9	0.95
20	4.7	0.73	5.0	0.90	4.8	0.84
Mean	4.9	0.84	4.9	0.76	4.9	0.77

▷ **Concentration :** 12.50 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	5.4	0.82	5.0	0.69	4.3	0.57
2	5.4	0.92	4.6	0.70	5.5	1.01
3	4.8	0.68	4.8	0.62	4.5	0.62
4	4.7	0.74	5.4	0.90	4.9	0.77
5	4.9	0.59	5.1	0.79	4.9	0.76
6	5.1	0.65	5.6	1.02	4.8	0.86
7	4.9	0.76	5.0	0.90	5.0	0.80
8	5.0	0.73	4.9	0.81	5.1	0.75
9	4.9	0.82	4.8	0.79	5.3	0.74
10	4.8	0.75	4.9	0.78	5.4	0.62
11	4.7	0.68	5.0	0.76	5.2	0.69
12	4.9	0.95	5.3	0.65	5.0	0.85
13	5.1	0.84	5.2	0.74	4.9	0.65
14	5.2	0.95	4.9	0.73	4.8	0.72
15	5.0	0.84	4.6	0.70	4.7	0.75
16	5.3	0.75	5.1	0.95	4.6	0.78
17	4.9	0.64	4.9	0.86	5.0	0.95
18	4.7	0.68	4.8	0.85	5.1	0.99
19	4.8	0.69	4.9	0.85	5.0	0.85
20	4.6	0.70	5.0	0.92	4.8	0.86
Mean	5.0	0.76	5.0	0.80	4.9	0.78

▷ **Concentration :** 25.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	5.2	0.89	5.1	0.79	4.3	0.57
2	4.3	0.60	5.2	0.83	4.5	0.60
3	5.0	0.77	4.9	0.70	4.5	0.58
4	5.2	0.83	5.5	1.01	5.3	0.88
5	5.3	0.89	4.7	0.63	4.7	0.63
6	5.6	1.01	4.7	0.65	4.6	0.60
7	5.0	0.72	5.2	0.88	5.0	0.75
8	5.0	0.95	5.6	1.01	4.6	0.85
9	4.9	0.85	5.2	0.81	5.0	0.84
10	4.9	0.86	5.1	0.74	5.2	0.68
11	4.8	0.75	5.0	0.95	5.0	0.69
12	4.9	0.76	4.9	0.85	4.9	0.65
13	4.6	0.85	4.8	0.75	4.8	0.75
14	4.6	0.65	4.8	0.65	4.7	0.85
15	5.2	0.70	4.9	0.84	4.6	0.68
16	4.9	0.59	4.9	0.75	4.8	0.75
17	4.9	0.69	5.0	0.65	4.9	0.88
18	4.8	0.67	5.3	0.65	4.6	0.95
19	4.5	0.56	4.8	0.95	5.0	0.85
20	4.7	0.85	4.9	0.85	5.0	0.86
Mean	4.9	0.77	5.0	0.80	4.8	0.74

▷ **Concentration :** 50.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	5.6	1.03	5.2	0.86	4.1	0.50
2	5.4	0.99	4.5	0.58	4.5	0.59
3	5.5	1.00	4.8	0.86	4.6	0.61
4	5.2	0.83	4.9	0.75	4.6	0.77
5	5.0	0.85	5.0	0.98	5.0	0.75
6	5.0	0.65	5.0	0.85	4.9	0.65
7	4.9	0.85	5.0	0.84	5.0	0.95
8	4.8	0.84	5.0	0.65	4.6	0.65
9	4.9	0.75	5.1	0.95	4.9	0.85
10	5.0	0.76	4.9	0.84	4.8	0.65
11	5.1	0.85	4.9	0.75	4.8	0.65
12	5.2	0.84	4.8	0.84	4.8	0.69
13	4.8	0.82	4.7	0.78	4.7	0.75
14	4.9	0.76	4.5	0.65	4.6	0.85
15	4.5	0.75	5.3	1.02	4.9	0.65
16	4.7	0.86	5.9	1.05	5.0	0.95
17	5.0	0.75	5.8	1.09	5.0	0.65
18	5.2	0.86	5.0	0.74	5.4	0.99
19	5.2	0.99	4.5	0.68	5.1	0.85
20	5.1	0.89	4.8	0.95	5.0	0.95
Mean	5.1	0.85	5.0	0.84	4.8	0.75

▷ **Concentration :** 100.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	5.1	0.97	4.4	0.58	4.4	0.58
2	5.4	1.02	4.2	0.50	4.5	0.68
3	5.6	0.98	4.3	0.60	4.4	0.63
4	5.1	1.10	4.4	0.68	4.3	0.60
5	5.0	0.90	4.5	0.65	5.2	0.92
6	5.5	0.77	4.6	0.64	5.1	0.91
7	4.9	1.02	4.9	0.72	5.0	0.77
8	5.5	0.85	4.9	0.85	5.4	0.92
9	5.5	1.03	4.8	0.65	5.0	0.85
10	5.4	1.02	5.0	0.68	5.1	0.86
11	5.2	0.95	4.0	0.95	5.2	0.98
12	4.9	0.85	4.9	0.84	4.9	0.86
13	4.8	0.69	4.8	0.82	5.6	1.03
14	4..8	0.75	5.0	0.86	4.8	0.75
15	4.6	0.85	4.9	0.87	4.6	0.69
16	4.9	0.75	4.6	0.68	4.8	0.98
17	4.8	0.59	5.0	0.85	4.7	0.68
18	5.0	0.68	4.9	0.75	4.9	0.79
19	4.9	0.85	4.8	0.78	4.6	0.78
20	4.8	0.85	4.9	0.65	4.8	0.69
Mean	5.1	0.87	4.7	0.73	4.9	0.80

▶ Total length and wet weight of juvenile olive flounder, *Paralichthys olivaceus*, for the 3-32 PSU seawater treated by the KTM-BWMS

▷ **Concentration :** Control						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	4.6	0.93	4.5	0.97	4.6	0.85
2	4.2	0.62	4.2	0.89	4.7	0.91
3	3.8	0.41	4.6	1.20	5.0	1.05
4	4.2	0.85	4.5	1.13	4.7	0.86
5	4.6	1.00	4.4	1.10	4.5	0.88
6	4.4	0.71	4.6	1.18	4.2	0.76
7	3.8	0.45	4.5	0.99	4.7	0.97
8	4.6	1.01	4.4	1.09	4.8	0.95
9	4.5	0.69	4.2	1.07	4.5	0.80
10	4.8	0.80	4.5	1.06	4.6	0.92
11	4.5	0.88	4.1	0.96	4.5	0.65
12	4.4	0.89	4.2	0.72	4.5	0.83
13	4.4	0.90	4.3	1.02	4.6	0.91
14	4.3	0.54	4.5	1.03	4.3	0.80
15	4.8	1.00	4.3	0.98	4.5	0.88
16	4.7	0.89	3.8	0.76	4.5	0.82
17	4.5	0.82	3.7	0.75	4.6	0.92
18	4.5	0.74	4.3	0.98	4.7	0.91
19	4.7	0.99	4.4	0.99	4.7	0.94
20	4.5	0.74	4.5	1.12	4.8	0.95
Mean	4.4	0.79	4.3	1.00	4.6	0.88

▷ **Concentration :** 6.25 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	4.9	1.08	4.0	0.63	4.0	0.71
2	4.5	0.90	4.3	0.76	4.0	0.47
3	4.3	0.82	4.5	0.91	4.6	0.82
4	4.3	0.72	4.1	0.66	4.0	0.51
5	4.6	0.92	4.0	0.53	4.1	0.62
6	4.3	0.88	3.8	0.51	4.3	0.70
7	4.5	0.94	4.1	0.82	4.2	0.80
8	4.4	0.79	4.2	0.80	4.2	0.74
9	4.6	0.88	3.8	0.45	4.3	0.84
10	4.8	0.99	3.9	0.53	4.0	0.55
11	4.9	1.02	4.5	0.82	4.4	0.76
12	4.8	1.00	4.3	0.92	3.8	0.52
13	4.4	0.78	4.4	0.94	3.9	0.54
14	4.9	1.00	3.7	0.48	3.9	0.60
15	4.5	0.92	4.1	0.69	3.9	0.60
16	4.3	0.90	4.2	0.84	4.0	0.62
17	4.5	0.88	4.4	0.90	4.0	0.59
18	4.8	0.92	4.1	0.80	4.4	0.68
19	4.9	1.04	4.2	0.82	4.3	0.73
20	4.8	1.10	4.5	0.92	4.0	0.65
Mean	4.6	0.92	4.2	0.74	4.1	0.65

▷ **Concentration :** 12.50 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	5.0	0.98	4.0	0.56	4.6	1.00
2	4.7	0.74	3.9	0.58	4.1	0.72
3	5.0	0.92	4.1	0.51	4.6	0.79
4	4.6	0.90	3.7	0.59	4.5	0.92
5	4.4	0.83	3.9	0.52	4.2	0.69
6	4.8	0.90	4.2	0.62	4.6	0.98
7	4.8	0.92	4.4	0.80	4.7	0.89
8	4.4	0.79	3.8	0.50	4.7	0.92
9	4.3	0.83	4.0	0.60	5.0	1.02
10	4.8	0.92	4.1	0.66	5.0	0.98
11	4.7	0.90	3.6	0.41	4.5	0.77
12	4.4	0.81	3.9	0.55	4.5	0.82
13	4.7	1.00	4.3	0.64	4.9	0.93
14	4.7	0.95	4.2	0.76	4.9	1.00
15	4.4	0.79	4.5	0.69	4.2	0.79
16	4.4	0.87	4.5	0.82	4.5	0.79
17	4.7	0.98	3.8	0.55	4.4	0.82
18	5.0	0.90	3.8	0.56	4.3	0.68
19	4.9	1.00	4.5	0.78	4.2	0.70
20	4.7	0.99	4.0	0.57	4.7	0.92
Mean	4.7	0.90	4.1	0.61	4.6	0.86

▷ **Concentration :** 25.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	4.4	0.66	4.6	0.93	4.4	0.63
2	4.6	0.97	4.4	0.72	4.5	0.91
3	4.3	0.62	4.4	0.83	4.8	1.02
4	4.4	0.74	5.0	1.00	4.8	0.99
5	4.5	1.00	4.5	0.90	4.5	0.76
6	4.8	1.16	4.9	0.88	4.9	1.03
7	4.0	0.53	4.6	0.92	4.9	1.10
8	4.5	0.87	5.0	1.03	4.0	0.68
9	4.5	0.83	4.6	0.82	4.6	0.92
10	4.3	0.73	4.7	0.84	4.5	0.67
11	4.4	0.74	3.9	0.63	4.6	0.93
12	4.4	0.58	4.7	1.00	4.7	1.00
13	4.4	0.83	4.3	0.78	4.7	0.98
14	4.0	0.62	4.3	0.83	4.8	1.02
15	4.6	0.86	4.5	0.84	4.3	0.62
16	4.1	0.70	4.5	0.89	4.6	0.89
17	4.1	0.80	4.6	0.92	4.7	1.00
18	4.3	0.84	4.4	0.77	4.8	1.05
19	4.4	0.90	4.8	1.02	4.7	0.93
20	4.7	0.89	4.4	0.79	4.0	0.69
Mean	4.4	0.79	4.6	0.87	4.6	0.89

▷ **Concentration :** 50.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	4.6	0.83	4.9	0.94	5.1	1.00
2	4.5	0.92	5.0	1.00	4.5	0.92
3	5.1	1.10	4.1	0.69	5.0	1.00
4	4.8	0.88	4.6	0.92	4.5	0.92
5	4.9	1.00	3.9	0.65	5.0	0.88
6	4.9	1.04	4.2	0.67	4.5	0.83
7	4.3	0.78	3.8	0.60	4.6	0.91
8	4.7	0.84	4.6	0.92	4.7	0.93
9	4.3	0.85	4.4	0.83	4.9	1.00
10	5.0	0.92	4.7	1.00	5.1	1.05
11	4.5	0.90	5.1	1.10	4.6	0.88
12	4.6	0.70	4.3	0.67	4.7	0.89
13	4.1	0.65	4.9	1.02	4.4	0.78
14	5.1	0.84	4.8	0.83	5.0	1.04
15	5.0	0.91	4.5	0.81	5.1	1.10
16	4.7	0.84	3.9	0.50	4.7	0.93
17	4.6	0.94	4.3	0.64	4.6	0.83
18	4.9	1.00	4.7	0.84	4.2	0.69
19	4.5	0.94	4.4	0.72	4.9	1.04
20	5.0	1.05	4.7	0.90	5.0	0.99
Mean	4.7	0.90	4.5	0.81	4.8	0.93

▷ **Concentration :** 100.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**	**Length (cm)**	**Wet weight (g)**
1	5.1	1.00	4.8	0.83	4.9	0.92
2	4.5	0.88	5.0	1.00	5.2	1.05
3	4.8	1.00	4.1	0.83	5.0	1.00
4	4.7	0.92	4.7	0.93	5.0	1.02
5	4.5	0.68	5.0	1.05	4.2	0.71
6	5.2	1.20	4.7	0.84	5.0	0.92
7	4.7	0.92	5.4	1.15	5.1	0.88
8	5.0	1.00	5.1	0.89	5.1	1.02
9	4.6	0.84	4.7	0.88	4.6	0.78
10	5.2	1.08	5.0	1.00	4.9	0.83
11	5.0	1.05	4.9	0.86	5.0	1.03
12	5.0	1.04	5.1	1.15	4.9	0.92
13	5.1	1.10	4.7	1.02	5.0	1.05
14	5.1	1.11	4.6	0.86	4.9	0.92
15	5.0	1.10	5.0	1.00	5.0	1.00
16	4.9	1.02	5.0	1.05	4.8	0.79
17	5.0	0.98	4.7	0.97	5.0	0.99
18	4.8	0.95	5.0	1.08	5.0	1.03
19	4.8	0.91	4.8	0.97	4.8	0.84
20	4.7	0.92	4.7	0.88	5.0	1.05
Mean	4.9	0.99	4.9	0.96	4.9	0.94

3.2 Chronic Toxicity

3.2.1 ANNEX 1

***Brachionus plicatilis*, Chronic Toxicity Test**

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea
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FINAL REPORT

Chronic Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- Rotifer, *Brachionus plicatilis* Chronic Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

GLP STATEMENT AND SIGN

Study title: Chronic toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward population growth rate of *Brachionus plicatilis*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

All procedure of this study was carried out following GLP regulation:

- "OECD Principles of Good Laboratory Practice"
  Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

This study was conducted on the basis of 'the Study Plan' complied and the procedure described in this report was carried out under 'the Responsibility of Study Director'. This report was built on the basis of raw data from the study.

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.
Study Director Jung-Im Park (signature)

2011. 07. 20.

Marine Eco-Technology Institute Co., Ltd.
Test Facility Manager Min Ho Son (signature)

GUARANTEE OF ASSURANCE

Study title: Chronic toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward population growth rate of *Brachionus plicatilis*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Quality assurance personnel of Marine Eco-Technology Institute Co., Ltd., inspected as follows. Inspection was carried out on the basis of the Standard Operation Procedure and each inspection results were reported to Study Director and Test Facility Manager.

Types of inspection and the date inspection results were reported to the Test Facility Management and the Study Director as follow:

Type of Inspections	Date of Inspection	Date Reported to Test Facility Management and Study Director
Study Plan	2011. 04. 13	2011. 04. 13
Test organisms	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Storage of Test substance	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Preparation of Test substance	2011. 06. 29 / 07. 12	2011. 06. 29 / 07. 12
Exposure	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Observation	2011. 07. 04 / 07. 16	2011. 07. 04 / 07. 16
Record of raw data	2011. 07. 04 / 07. 16	2011. 07. 04 / 07. 16
Draft of final report	2011. 07. 19	2011. 07. 19
Final report	2011. 07. 20	2011. 07. 20

It is assurance that the procedure in this final report was appropriately carried out and the description of the results was reflected raw data from the study.

2011. 07. 20.

Quality Assurance personnel Jin Hee Kim (signature)
Marine Eco-Technology Institute Co., Ltd.

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1. SUMMARY

The purpose of this study was to assess the chronic toxic effects of the > 32 and 3-32 PSU seawater treated by the KTM-BWMS on the population growth rate of rotifer, *Brachionus plicatilis*.

The test substances were divided into two different salinities, the one was > 32 PSU treated ballast water and the other was 3-32 PSU treated ballast water. Non-treated ballast water was used as control and dilution water. 5 individuals of the rotifers, *Brachionus plicatilis* were exposed at the various test concentrations (0.00 % (non-treated ballast water), 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % (treated ballast water)) for 96 hours. The rotifer was observed daily using microscopy. Dead rotifers were removed after record. The raw data of population growth from this experiment was used for calculation of NOEC, LOEC and ECx values.

The population growth rate of the rotifer, *Brachionus plicatilis* on the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS was assessed.

For the > 32 PSU treated ballast water, the mean number of the test organisms in the control was 47 individuals and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 41, 37, 40, 47 and 45 individuals, respectively. The population growth rate of the test organisms in the control was 0.56 and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 0.53, 0.50, 0.51, 0.56 and 0.55, respectively. The percent inhibition in average growth rate of the test organisms in the 6.25 %, 12.50 % and 25.00% of the test concentration at the end of the experiment was higher than 50.00 % and 100.00 % of the test concentration. However, the result of statistic analysis was indicated that growth rate in the 50.00 % and 100.00 % of the test concentration was not significantly different with growth rate in the control.

For the 3-32 PSU treated ballast water, the mean number of the test organisms in the control was 56 individuals and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00% of the test concentration were 51, 54, 56, 53 and 54 individuals, respectively. The population growth rate of the test organisms in the control was 0.60 and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 0.58, 0.59, 0.60, 0.59 and 0.59, respectively. The percent inhibition in average growth rate of the test organisms in the 6.25 % of the test concentration at the end of the experiment was higher than the other test concentrations. However, the result of statistic analysis was indicated that growth rate in all the test concentration was not significantly different with growth rate in the control.

NOEC, LOEC and EC50 values based on reproduction data at the end of the experiment with the > 32 and 3-32 PSU treated ballast water were equal to or greater than 100.00 %.

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS did not have chronic toxic effects on the population growth of the rotifer, *Brachionus plicatilis*.

2. OUTLINE OF STUDY

2.1 Purpose

The purpose of this study was to assess the chronic toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS (or the > 32 and 3-32 PSU de-ballast water from the KTM-BWMS) on the population growth rate of *Brachionus plicatilis*.

2.2 Principle

Neonate of *Brachionus plicatilis* aged less than 2 hours at the start of test is exposed to the treated ballast water of the KTM-BWMS at a range of concentrations including 100.00% effluent, for a period of 96 hours. The rotifer density is counted every 24 hours until 96 hours. At the end of the experiment, the population growth rate (*r*) is calculated that count the number of live rotifers in each test concentration compared with control values. And the percentage inhibition (*I*%) of the rotifer growth for each test concentration is evaluated. The results are analyzed in order to calculate the NOEC, LOEC and EC50 at 96 hour.

2.3 Good Laboratory Practice

All procedure of this study was complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

2.4 Guideline

This study using *Brachionus plicatilis* was complied with principle and conducted on the basis of following:

- "Cyst-based toxicity tests. VIII. Short-chronic toxicity tests with the freshwater rotifer *Brachionus calyciflorus*" (Janssen et al., 1994)

The test preparation in this study was complied on the basis of following standard method:

- "Standard Guide for Acute Toxicity Test with the Rotifer *Brachionus*. E 1440-91"

American Society of Testing Materials, (Reapproved 2004)

2.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

2.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax : + 82-51-611-0588

2.7 Test site

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.8 Study director

Name: Jung-Im Park

Department: 2nd research team

2.9 Study timetable

2.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 06. 29
Separation of neonates for test	2011. 06. 30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation population growth rate	2011. 06. 30 ~ 07. 04
Experimental completion	2011. 07. 04
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Induced to hatch for cysts	2011. 07. 12
Separation of neonates for test	2011. 07. 13
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation population growth rate	2011. 07. 13 ~ 07. 17

Experimental completion	2011. 07. 17
Draft of final report	2011. 07. 19
Final Report	2011. 07. 20
Study completion	2013. 07. 31

2.10 Responsible Personnel

Laboratory manager (Test site manager)	Myung-Baek Shon
Statistical analysis	
Receipt and culture of test organisms	So Eun Jo
Observation and record	
Disposal of the resting test organisms	
Sampling, transit and custody of test substance or solution	Tae Won Kim
Disposal of the resting substance	
Check the chemical condition	

2.11 Retention of records and data

2.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

2.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.11.3 Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

3. MATERIALS AND METHODS

3.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

3.1.1 Substance name

Treated water (KTM-S5D5-1)

3.1.2 Lot No.

None

3.1.3 Type

Liquid

3.1.4 Principal ingredients

Seawater

3.1.5 Temperature

23.2 ˚C

3.1.6 Salinity

33.6 PSU

3.1.7 pH

7.67

3.1.8 Custody condition

Constant room at 4 ˚C for 2 days

3.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.1.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.1.11 Disposal of the resting substances

Scrapped after test

3.1.12 Certificate of analysis

Client supplies to test organization

3.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

3.2.1 Substance name

Non-treated water (KTM-S3D5-1)

3.2.2 Lot No.

None

3.2.3 Type

Liquid

3.2.4 Principal ingredients

Seawater

3.2.5 Temperature

23.1 ˚C

3.2.6 Salinity

33.9 PSU

3.2.7 pH

7.50

3.2.8 Custody condition

Constant room at 4 ˚C for 2 days

3.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.2.11 Disposal of the resting substances

Scrapped after test

3.2.12 Certificate of analysis

Client supplies to test organization

3.3 Test substance-3 (Treated ballast water (3 - 32 PSU) -Treated)

3.3.1 Substance name

Treated water (KTM-S5D5-3)

3.3.2 Lot No.

None

3.3.3 Type

Liquid

3.3.4 Principal ingredients

Seawater

3.3.5 Temperature

24.6 ˚C

3.3.6 Salinity

21.7 PSU

3.3.7 pH

7.46

3.3.8 Custody condition

Constant room at 4 ˚C for 2 days

3.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.3.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.3.11 Disposal of the resting substances

Scrapped after test

3.3.12 Certificate of analysis

Client supplies to test organization

3.4 Test substance-4 (Non-treated ballast water (3 – 32 PSU) -Control)

3.4.1 Substance name

Non-treated water (KTM-S3D5-3)

3.4.2 Lot No.

None

3.4.3 Type

Liquid

3.4.4 Principal ingredients

Seawater

3.4.5 Temperature

24.8 ˚C

3.4.6 Salinity

22.7 PSU

3.4.7 pH

7.24

3.4.8 Custody condition

Constant room at 4 ˚C for 2 days

3.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.4.11 Disposal of the resting substances

Scrapped after test

3.4.12 Certificate of analysis

Client supplies to test organization

3.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance was diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid (see Fig. 1 ~ 4 in the Annex 5). The six concentrations of test substance including control (0.00 %, only non-treated ballast water by the KTM-BWMS) and 100.00 % were arranged for the final definitive experiment.

3.6 Culture condition

3.6.1 Hatching of test organisms (SOP#TO-007)

Test organisms	*Brachionus plicatilis*
Vessel	6 well plate
Medium	20 PSU filtered seawater (0.45μm membrane filter, Whatman)
Temperature	25 ˚C
Light source	“cool-white” fluorescent lamp
Light cycle	Continuous light
Light cycle	1,000 ~ 3,000 lux

The cysts were obtained from commercial supplier (See ‘3.7 Test system’).

3.6.2 Feeding of the test organisms

None

3.7 Test system

3.7.1 Test organisms

.1 Scientific name	*Brachionus plicatilis*
.2 Producer	MicroBioTests Inc., Belgium
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

3.7.2 Ground of selected test organisms

Marine rotifer, *Brachionus plicatilis* have the advantages of cosmopolitan distribution, wide salinity adaptability, importance trophic roles as primary consumer in marine ecosystem, short generation time and easy culture in small spaces. For this reason *Brachionus plicatilis* is commonly used as a toxicity test.

3.7.3 Preparation of the test organisms (SOP#TO-007)

Hatching of rotifer cysts were initiated for approximately 24 hours before the start of the test, and had to be carried out in seawater of reduced salinity (20 PSU). They were incubated at 25 ˚C and 1,000 to 3,000 lux continuous illumination. They were checked for the hatching hourly after 24 hours incubation in order to

collect the test animals within 2 hours of their hatching. For the > 32 PSU ballast water treated, rotifer neonates were acclimated for approximately 1 hour to adapt to the eventual salinity change.

3.7.4 Check the test organisms for the test (SOP#TO-007)

Health of the test organisms such as the mortality, appearance and abnormal behavior was observed with a microscope (SZ51, Olympus).

3.7.5 Disposal of the resting test organisms (SOP#TO-006)

The resting test organisms were scrapped after sterilization by the autoclave.

3.8 Study design

3.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

3.8.2 Exposure conditions (SOP#TP-014, see Fig. 6 in the Annex 5)

Brachionus plicatilis were placed in test vessels containing food (*Chlorella vulgaris*, 1×10^6 cells/mL) divided into five neonates each replication of concentration including the control. And then the test vessels were placed in incubator for 96 hours at 25 ± 1˚C.

Experiment conditions and criteria are shown Annex 2 and application site is shown Annex 4.

3.8.3 Observation item and measurement components

.1 Population growth rate (SOP#TP-014)

Each test vessel was checked for density of *Brachionus plicatilis* every 24 hours after the beginning of the experiment. Mortalities were recorded and then dead animals were removed.

Calculations of the population growth rate and the percentage inhibition growth are shown Annex 3.

.2 Other observations (SOP#TP-014)

In addition to mortality, any abnormal behavior and appearance were recorded.

.3 Temperature (SOP#TE-002)

Temperature of the solutions was measured at the beginning and end of the experiment.

.4 Dissolved oxygen (SOP#TE-002)

Dissolved oxygen (DO) of the solutions was measured at the beginning of the experiment.

.5 pH (SOP#TE-002)

pH of the solutions was measured at the beginning and end of the experiment.

3.9 Statistical procedures (SOP#SP-101)

For the > 32 PSU treated ballast water, non-normal distribution of the data was tested by Kolmogorov D Test ($p>0.05$) and Bartlett's Test indicated unequal variances ($p=4.72E-04$). Therefore hypothesis test was conducted by Steel's Many-One Rank Test. Differences were considered to be significant at $\alpha=0.05$. For the 3-32 PSU ballast water treated, normal distribution of the data was tested by Kolmogorov D Test ($p\leq0.05$) and equal variance of the data was tested by Bartlett's Test ($p=0.17$). Hypothesis test was conducted by Steel's Many-One Rank Test. Differences were considered to be significant at $\alpha=0.05$.

TOXCALC 5.0 program (Tidepool scientific software, USA) was used for above statistic analysis.

4. RESULTS

4.1 Dissolved oxygen, pH and Temperature

4.1.1 Treated ballast water - > 32 PSU (Table 1 ~ 3)

For the > 32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 4.43 to 4.58 mg/L at the beginning of the experiment and from 3.95 to 4.28 mg/L at the end of the experiment. pH value was ranged from 8.00 to 8.03 at the beginning of the experiment and from 7.64 to 7.69 at the end of the experiment. And mean temperature was 25.59 ± 0.13 ˚C (Mean ± S.D.).

4.1.2 Treated ballast water - 3-32 PSU (Table 4 ~ 6)

For the 3-32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 4.01 to 4.86 mg/L at the beginning of the experiment and from 4.40 to 5.18 mg/L at the end of the experiment. pH value was ranged from 7.65 to 7.72 at the beginning of the experiment and from 7.72 to 7.78 at the end of the experiment. And mean temperature was 24.93 ± 0.46 ˚C (Mean ± S.D.).

4.2 Population growth rate

4.2.1 Treated ballast water - > 32 PSU (Table 7, 8)

The mean number of the rotifer, *Brachionus plicatilis* exposed the > 32 PSU treated ballast water for 96 hours in the control was 47 individuals and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 41, 37, 40, 47 and 45 individuals, respectively. The population growth rate of the test organisms in the control was 0.56 and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 0.53, 0.50, 0.51, 0.56 and 0.55, respectively.

4.2.2 Treated ballast water - 3-32 PSU (Table 10, 11)

The mean number of the rotifer, *Brachionus plicatilis* exposed the 3-32 PSU treated ballast water for 96 hours in the control was 56 individuals and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00% of the test concentration were 51, 54, 56, 53 and 54 individuals, respectively. The population growth rate of the test organisms in the control was 0.60 and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 0.58, 0.59, 0.60, 0.59 and 0.59, respectively.

4.3 Percentage inhibition growth

4.3.1 Treated ballast water - > 32 PSU (Table 9)

The percent inhibition in average growth rate of the test organisms in the 6.25 % of the test concentration at the end of the experiment was 12.57% and those that in 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 21.06 %, 14.34 %, -0.18 % and 4.78 %, respectively.

4.3.2 Treated ballast water - 3-32 PSU (Table 12)

The percent inhibition in average growth rate of the test organisms in the 6.25 % of the test concentration at the end of the experiment was 8.40 % and those that in the 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 2.85 %, -0.90 %, 4.65 % and 3.00 %, respectively.

4.4 Plotting of the concentration response curve (Fig. 1, 2)

The raw data of population growth rate from the each test concentration of the > 32 and 3-32 PSU treated ballast water was plotted on the graph.

4.5 NOEC, LOEC and EC50 (Table 13)

For the > 32 and 3-32 PSU ballast water treated, values of NOEC, LOEC and EC50 were equal to or greater than 100.00% at the end of the experiment.

4.6 Other observation

Any abnormal appearance or behavior of the rotifer, *Brachionus plicatilis* during the experiment period was not observed.

5. DISCUSSION AND CONCLUSION

The population growth rate of the rotifer, *Brachionus plicatilis* on the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS was assessed.

For the > 32 PSU treated ballast water, the mean number of the test organisms in the control was 47 individuals and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 41, 37, 40, 47 and 45 individuals, respectively (Table 7). The population growth rate of the test organisms in the control was 0.56 and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 0.53, 0.50, 0.51, 0.56 and 0.55, respectively (Table 8). The percent inhibition in average growth rate of the test organisms in the 6.25 %, 12.50 % and 25.00% of the test concentration at the end of the experiment was higher than 50.00 % and 100.00 % of the test concentration (Table 9). However, the result of statistic analysis was indicated that growth rate in the 50.00 % and 100.00% of the test concentration was not significantly different with growth rate in the control (Steel's Many-One Rank Test, $p>0.05$).

For the 3-32 PSU treated ballast water, the mean number of the test organisms in the control was 56 individuals and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00% of the test concentration were 51, 54, 56, 53 and 54 individuals, respectively (Table 10). The population growth rate of the test organisms in the control was 0.60 and those that in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration were 0.58, 0.59, 0.60, 0.59 and 0.59, respectively (Table 11). The percent inhibition in average growth rate of the test organisms in the 6.25 % of the test concentration at the end of the experiment was higher than the other test concentrations (Table 12). However, the result of statistic analysis was indicated that growth rate in all the test concentration was not significantly different with growth rate in the control (Steel's Many-One Rank Test, $p>0.05$).

NOEC, LOEC and EC50 values based on reproduction data at the end of the experiment with the > 32 and 3-32 PSU treated ballast water were equal to or greater than 100.00 % (Table 13).

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS did not have chronic toxic effects on the population growth of the rotifer, *Brachionus plicatilis*.

6. REFERENCE

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TABLES

Table 1. Dissolved oxygen value (mg/L) of > 32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	4.48	4.50	4.58	4.43	4.52	4.53
Final	4.04	3.95	4.05	4.07	4.28	4.03

Table 2. pH value of > 32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	8.00	8.02	8.03	8.00	8.00	8.01
Final	7.66	7.65	7.68	7.64	7.69	7.66

Table 3. Temperature (˚C) of > 32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	25.70	25.60	25.60	25.70	25.80	25.80
Final	25.50	25.50	25.40	25.50	25.50	25.50

Table 4. Dissolved oxygen value (mg/L) of 3-32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	4.37	4.68	4.86	4.41	4.01	4.78
Final	5.18	4.67	4.73	4.71	4.98	4.40

Table 5. pH value of 3-32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	7.72	7.67	7.69	7.68	7.67	7.65
Final	7.76	7.75	7.78	7.76	7.74	7.72

Table 6. Temperature (˚C) of 3-32 PSU test concentration series at the beginning and the end of the test using *Brachionus plicatilis*

Concentration	Control	6.25 %	12.50 %	25.00 %	50.00 %	100.00 %
Initial	25.20	25.00	25.20	25.10	25.60	25.70
Final	24.60	24.60	24.50	25.00	24.50	24.20

Table 7. Mean number of the *Brachionus plicatilis*, exposed to > 32 PSU treated ballast water for 96 hours

Concentration	**Control**					**6.25%**				
Elapsed time (day)	**0**	**24**	**48**	**72**	**96**	**0**	**7**	**14**	**21**	**28**
Mean	5	5	11	22	47	5	5	9	18	41
STDEV(±)	0	0	2	5	3	0	0	2	4	5
Concentration	**12.50%**					**25.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Mean	5	5	9	16	37	5	5	9	18	40
STDEV(±)	0	0	2	4	8	0	0	1	3	10
Concentration	**50.00%**					**100.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Mean	5	5	9	17	47	5	5	8	16	45
STDEV(±)	0	0	2	4	5	0	0	1	3	8

*STDEV: Standard deviation

Table 8. Mean population growth rate for the *Brachionus plicatilis*, exposed to > 32 PSU treated ballast water for 96 hours

Concentration	**Control**					**6.25%**				
Elapsed time (day)	**0**	**24**	**48**	**72**	**96**	**0**	**7**	**14**	**21**	**28**
Growth rate	0.00	-0.02	0.38	0.49	0.56	0.00	0.00	0.31	0.41	0.53
STDEV(±)	0.00	0.06	0.09	0.07	0.02	0.00	0.00	0.09	0.07	0.03
Concentration	**12.50%**					**25.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Growth rate	0.00	0.00	0.30	0.39	0.50	0.00	0.00	0.30	0.43	0.51
STDEV(±)	0.00	0.00	0.11	0.08	0.05	0.00	0.00	0.06	0.05	0.07
Concentration	**50.00%**					**100.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Growth rate	0.00	0.00	0.28	0.40	0.56	0.00	0.00	0.24	0.39	0.55
STDEV(±)	0.00	0.00	0.12	0.09	0.03	0.00	0.00	0.08	0.06	0.04

*STDEV: Standard deviation

Table 9. Percent inhibition in average growth rate of *Brachionus plicatilis*, at the each test concentration of treated > 32 PSU ballast water during the experiment period

Elapsed time (h)	**Concentration (%)**				
	6.25	**12.50**	**25.00**	**50.00**	**100.00**
24	-1.69	-1.69	-1.69	-1.69	-1.69
48	14.50	15.27	16.03	16.79	24.43
72	20.75	26.04	16.98	23.77	26.04
96	12.57	21.06	14.34	-0.18	4.78

Table 10. Number of the *Brachionus plicatilis*, exposed to 3-32 PSU treated ballast water for 96 hours

Concentration	**Control**					**6.25%**				
Elapsed time (day)	**0**	**24**	**48**	**72**	**96**	**0**	**7**	**14**	**21**	**28**
Mean	5	5	17	41	56	5	5	15	39	51
STDEV(±)	0	0	3	5	5	0	0	2	6	5
Concentration	**12.50%**					**25.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Mean	5	5	14	34	54	5	5	14	32	56
STDEV(±)	0	0	1	6	4	0	0	3	4	3
Concentration	**50.00%**					**100.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Mean	5	5	12	31	53	5	5	15	31	54
STDEV(±)	0	0	2	5	5	0	0	3	5	5

*STDEV: Standard deviation

Table 11. Population growth rate for the *Brachionus plicatilis*, exposed to 3-32 PSU treated ballast water for 96 hours

Concentration	**Control**					**6.25%**				
Elapsed time (day)	**0**	**24**	**48**	**72**	**96**	**0**	**7**	**14**	**21**	**28**
Growth rate	0.00	0.00	0.60	0.70	0.60	0.00	0.00	0.53	0.68	0.58
STDEV(±)	0.00	0.00	0.11	0.04	0.02	0.00	0.00	0.08	0.06	0.02
Concentration	**12.50%**					**25.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Growth rate	0.00	0.00	0.51	0.63	0.59	0.00	0.00	0.52	0.62	0.60
STDEV(±)	0.00	0.00	0.05	0.05	0.02	0.00	0.00	0.08	0.04	0.01
Concentration	**50.00%**					**100.00%**				
Elapsed time (day)	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Growth rate	0.00	0.02	0.42	0.60	0.59	0.00	0.02	0.53	0.61	0.59
STDEV(±)	0.00	0.05	0.08	0.05	0.03	0.00	0.05	0.09	0.06	0.02

*STDEV: Standard deviation

Table 12. Percent inhibition in average growth rate of *Brachionus plicatilis*, at the each test concentration of treated 3-32 PSU ballast water during the experiment period

Elapsed time (h)	**Concentration (%)**				
	6.25	**12.50**	**25.00**	**50.00**	**100.00**
24	0.00	0.00	0.00	-1.67	-1.67
48	13.79	17.24	15.76	31.03	13.30
72	5.27	17.65	22.11	25.15	23.53
96	8.40	2.85	-0.90	4.65	3.00

Table 13. NOEC, LOEC and EC50 values calculated from population growth data of animal at the end of the experiment

Test substance	**NOEC (%)**	**LOEC (%)**	**EC50 (%)**	**95 % confidence of limit (%)**	
				lower	**upper**
> 32 PSU treated ballast water	100.0	> 100.0	> 100.0	-	-
3-32 PSU treated ballast water	100.0	> 100.0	> 100.0	-	-

* -: no data; > 100.0: greater than 100.0 %

FIGURES

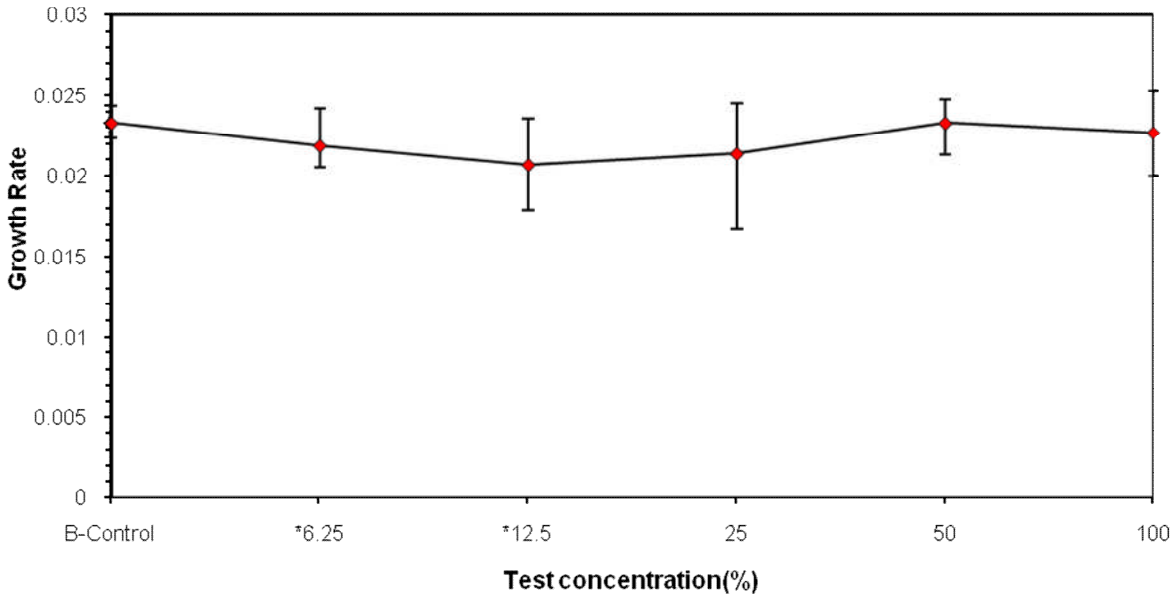


Fig. 1. Plot of the concentration-response (population growth) data from the each test concentration for > 32 PSU treated ballast water. The dotted line was represented as 0.05 level of significant and vertical bar was represented maximum and minimum.

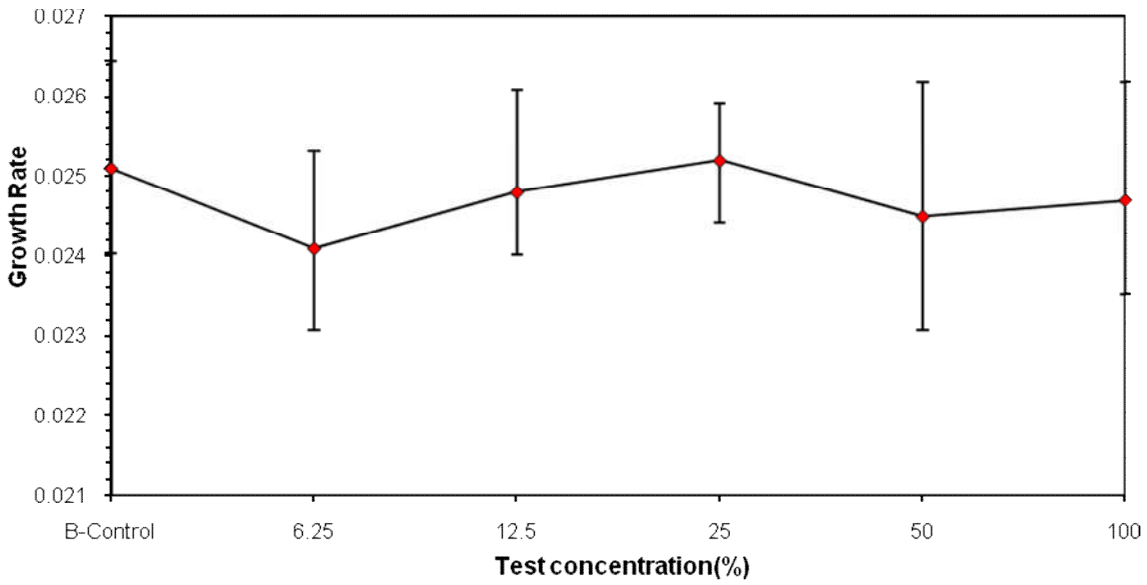


Fig. 2. Plot of the concentration-response (population growth) data from the each test concentration for 3-32 PSU treated ballast water. And vertical bar was represented maximum and minimum.

ANNEX 1

Definitions

1. Rotifer cyst is that a rotifer embryo arrested at an early stage in development, enclosed in an envelope and resistant to desiccation and temperature extremes. Rotifer cysts are often incorrectly referred to as resting eggs. Upon hydration, embryonic development resumes until a neonate female emerges from the cyst.

2. Rotifer neonate is a newly hatched, freely swimming rotifer. All neonates hatched from cysts are females.

3. ECx is the concentration of the test substance dissolved in water that results in a x percent reduction in reproduction of *Brachionus plicatilis* within a stated exposure period.

4. Lowest Observed Effect Concentration (LOEC) is the lowest tested concentration at which the substance is observed to have a statistically significant effect on reproduction (at $p < 0.05$) when compared with the control, within a stated exposure period.

5. No Observed Effect Concentration (NOEC) is the test concentration immediately below the LOEC.

ANNEX 2

Experiment conditions and acceptability criteria for rotifer, *Brachionus plicatilis* chronic population growth rate toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Static (non-renewal)
Temperature	25 ± 1˚C
Light quality	None
Light intensity	Darkness
Photoperiod	0h light: 24 h dark
Test chamber size	1.5 mL (48 well plate)
Test solution volume	1mL
Renewal of test solutions	None
Age of test organisms	Less than 2 hours
Number of individuals per test chamber	5 inds.
Replicate test chambers per concentration	12
Feeding regime	Once at the beginning of the experiment (*Chlorella vulgaris* , 1×10^6 cells/mL)
Cleaning	None
Aeration	None
Dilution water	Filtered seawater (Control seawater) by 0.45 µm membrane filter
Test concentration	5 including 100.00% effluents and a control
Dilution factor	Effluents: 0.5
Test duration	96 hours
Endpoints	Population growth rate
Test acceptability criteria	- Population growth rate in the control : ≥ 0.55 - Percentage growth inhibition in the lowest toxicant concentration: < 50%

ANNEX 3

1. The population growth rate for a specific period is calculated from equation [1]:

$$r_m = \frac{\ln N_t - \ln N_0}{t} \qquad [1]$$

where:

r_m is the intrinsic rate of natural increase in the population growth experiment.

N_0 and N_t are represented the population size at t=0 and t=4, respectively.

2. The percentage growth inhibition for each treatment replicate is calculated from equation [2]:

$$I\% = \frac{N_{control} - N_{toxicant}}{N_{control}} \times 100 \qquad [2]$$

where:

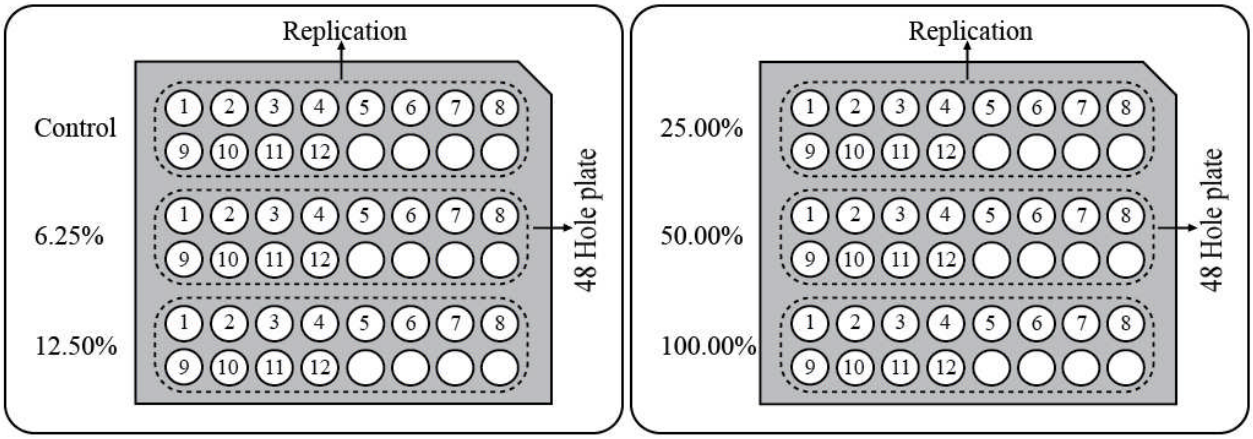
$I\%$ is percent inhibition in average growth rate.

N are represented the population in each test concentrations.

ANNEX 4

Application Site

Application site for the population growth test with the rotifer, *Brachionus plicatilis* is shown as follow.



ANNEX 5

Preparation of test substance and procedures of the experiment

Fig. 1. For aquatic toxicity test, collection of the control (untreated) and treated de-ballasting water from KTM-BWMS (left down: sampling equipments).

Fig. 2. Transfer collected samples to the constant room (4 ± 1 ˚C).

Fig. 3. Preparation of test concentration diluted with non-treated seawater on treated seawater through flow-meter.

Fig. 4. For the population growth test using the rotifer, *Brachionus plicatilis*, final preparation of test substance was filtered by 0.45 μm membrane filter.

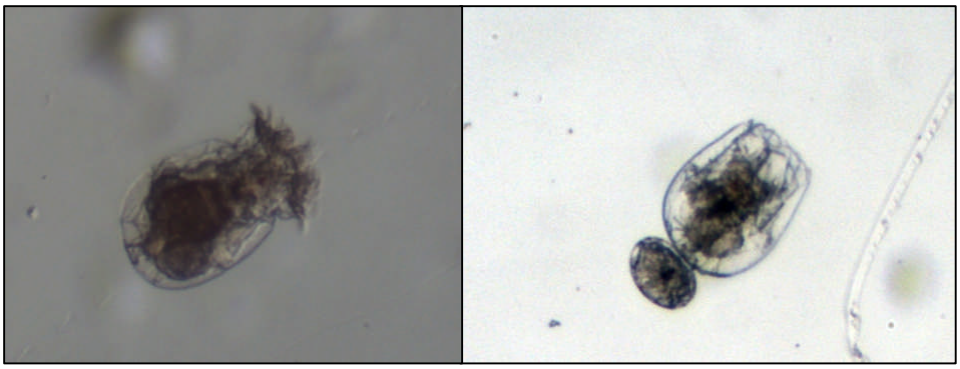


Fig. 5. Test organisms in this study: Neonates (left) and adult of female (right) of rotifer, *Brachionus plicatilis*.

Fig. 6. Procedure of chronic crustacean toxicity test set up (① Addition of *Chlorella vulgaris* (1×10^6 cells/mL) on the test substance; ② Separation of test organisms from hatching chamber; ③ Placing of test organisms on the test vessel by modified Pasteur pipette; ④ Observation and counting of test organisms).

3.2.2 ANNEX 2

The olive flounder fish, *Paralichthys olivaceus*, Chronic Toxicity Test

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FINAL REPORT

Chronic Aquatic Toxicity For > 32 & 3-32 PSU Treated Ballast Water of KTM-BWMS
- The olive flounder fish, *Paralichthys olivaceus*, Chronic Toxicity Test

Study Name: KTM-BWMS

Study No: BW- DBWT1104-KT

Marine Eco-Technology Institute Co., Ltd.

485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

GLP STATEMENT AND SIGN

Study title: Chronic toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward embryo and sac-fry stages of olive flounder fish, *Paralichthys olivaceus*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

All procedure of this study was carried out following GLP regulation:

- "OECD Principles of Good Laboratory Practice"
  Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

This study was conducted on the basis of 'the Study Plan' complied and the procedure described in this report was carried out under 'the Responsibility of Study Director'. This report was built on the basis of raw data from the study.

2011. 07. 22.

Marine Eco-technology Institute Co., Ltd.
Study Director Jin Hee Kim (signature)

2011. 07. 22.

Marine Eco-technology Institute Co., Ltd.
Test Facility Manager Min Ho Son (signature)

GUARANTEE OF ASSURANCE

Study title: Chronic toxicity test to assess the toxic effects of the > 32 & 3-32 PSU of ballast water treated by the KTM-BWMS toward embryo and sac-fry stages of olive flounder fish, *Paralichthys olivaceus*

Study name: KTM-BWMS Study No: BW-DBWT1104-KT

Quality assurance personnel of Marine Eco-technology Institute Co., Ltd. inspected as follows. Inspection was carried out on basis of the Standard Operation Procedure and each Inspection results were reported to Study Director and Test Facility Manager.

Types of inspections and the date inspection results were reported to the Test Facility Management and the Study Director as follow:

Type of Inspections	Date of inspection	Date Reported to Test Facility Management and Study Director
Study Plan	2011. 04. 13	2011. 04. 13
Test organisms	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Storage of Test substance	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Preparation of Test substance	2011. 06. 29 / 07. 12	2011. 06. 30 / 07. 13
Exposure	2011. 06. 30 / 07. 13	2011. 06. 30 / 07. 13
Observation	2011. 07. 06 / 07. 19	2011. 07. 07 / 07. 20
Record of raw data	2011. 07. 07 / 07. 20	2011. 07. 07 / 07. 20
Draft of final report	2011. 07. 21	2011. 07. 21
Final report	2011. 07. 22	2011. 07. 22

It is assurance that the procedure in this final report was appropriately carried out and the description of the results was reflected raw data from the study.

2011. 07. 22.

Quality Assurance personnel Jung-Im Park (signature)
Marine Eco-technology Institute Co., Ltd.

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1. SUMMARY

The purpose of this study was to assess the chronic toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS on the embryo and sac-fry stages of olive flounder, *Paralichthys olivaceus.*

The test substance type was divided into two different salinities, the one was > 32 PSU treated ballast water and the other was 3-32 PSU treated ballast water. Non-treated ballast water was used as control and dilution water. 30 fertilized eggs of olive flounder fish, *Paralichthys olivaceus* were exposed at the various test concentrations (0.00 % (non-treated ballast water), 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % (treated ballast water)) for 7 days. The mortality of the test organisms was daily observed and recorded. The raw data of living test organisms from this experiment was used for calculation of NOEC, LOEC, LC25 and LC50 values.

For the > 32 PSU treated ballast water, mean number of the living eggs on the embryo stages was 29 individuals in the control and those were 30, 29, 29, 29 and 29 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively. Mean number of the survival larvae was 26 individuals in the control and those were 27, 25, 24, 19 and 26 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively. Mean survival proportion in the 50.00 % of test concentration was lower than other test concentration at the end of the experiment. However, result of statistic analysis indicated that survival proportions of olive flounder in the all test concentrations were not significantly different with control.

For the 3-32 PSU treated ballast water, mean number of the living eggs on the embryo stages was 28 individuals in the control and those were 28, 27, 28, 29 and 29 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively. Mean number of the survival larvae at the end of the experiment was 22 individuals in the control and those were 23, 19, 24, 25 and 22 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively. Mean survival proportions in the 12.00 % of test concentration was lower than other test concentration at the end of the experiment. However, result of statistic analysis indicated that survival proportions of olive flounder in the all test concentrations were not significantly different with control.

For the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS, all values of NOEC, LOEC, LC25 and LC50 at the end of experiment was greater than 100.00 %.

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS had chronic toxic effects on the survival of the olive flounder, *Paralichthys olivaceus*.

2. OUTLINE OF STUDY

2.1 Purpose

The purpose of this study was to assess the chronic toxic effects of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS on the survival concerning early life stage of olive flounder, *Paralichthys olivaceus.*

2.2 Principle

The embryo and sac-fry stages of olive flounder fish, *Paralichthys olivaceus* are exposed to the treated ballast water of the KTM-BWMS for 7 days. The test is begun by placing fertilized eggs in the test chambers and terminated just before complete absorption of the yolk-sac of any larvae or mortalities by starvation start in any of the test chambers in the control. Lethal and sub-lethal effects are assessed and compared with the control.

2.3 Good Laboratory Practice

All procedure of this study was complied with following GLP regulation:

- "OECD Principles of Good Laboratory Practice"

Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17 (as revised in 1997)

2.4 Guideline

This study was complied with principle and conducted on the basis of following standard method and applied for *Paralichthys olivaceus*:

- "OECD Guideline for Testing of Chemicals, 212, Fish, Short-term Toxicity Test on the Embryo and Sac-fry Stages"

Organization for Economic Co-operation and Development, (Adopted: September. 21, 1998)

2.5 Sponsor

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea.

Tel: +82-51-441-0692~3 Fax: +82-51-441-0695

2.6 Test facility

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

Tel: + 82-51-611-6200 Fax: + 82-51-611-0588

2.7 Test site

Name	Marine Eco-technology Institute Co., Ltd.
Address	485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.8 Study director

Name	Jin Hee Kim
Department	3rd research team

2.9 Study timetable

2.9.1 Treated ballast water (> 32 PSU) and non-Treated ballast water (> 32 PSU)

Study initiation	2011. 04. 13
Receipt of test organisms	2011. 06. 30
Collect of sample	2011. 06. 29
Experimental start	2011. 06. 30
Exposure date	2011. 06. 30
Evaluation of mortality	2011. 06. 30 ~ 07. 07
Experimental completion	2011. 07. 07
Draft of final report	2011. 07. 21
Final Report	2011. 07. 22
Study completion	2013. 07. 31

2.9.2 Treated ballast water (3-32 PSU) and non-Treated ballast water (3-32 PSU)

Study initiation	2011. 04. 13
Receipt of test organisms	2011. 07. 13
Collect of sample	2011. 07. 12
Experimental start	2011. 07. 13
Exposure date	2011. 07. 13
Evaluation of mortality	2011. 07. 13 ~ 07. 20
Experimental completion	2011. 07. 20
Draft of final report	2011. 07. 21
Final Report	2011. 07. 22
Study completion	2013. 07. 31

2.10 Responsible Personnel

Laboratory manager (Test site manager) Statistical analysis	Myung-Baek Shon
Receipt of test organisms Disposal of the resting test organisms	So Eun Jo
Sampling, transit and custody of test substance or solution Disposal of the resting substance Observation and record Check the chemical condition	Tae Won Kim

2.11 Retention of records and data

2.11.1 Duration

Duration of storage is 5 years after end of the study. It will be decided that storage of data after 5 years should be consulted with the client.

2.11.2 Place of storage

Name: Archives of Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

2.11.3. Retention of records and data

Study plan, final report, raw data, documents related with the study, communication documents, and etc.

3. MATERIALS AND METHODS

3.1 Test substance-1 (Treated ballast water (> 32 PSU) -Treated)

3.1.1 Substance name

Treated water (KTM-S5D5-1, 2)

3.1.2 Lot No.

None

3.1.3 Type

Liquid

3.1.4 Principal ingredients

Seawater

3.1.5 Temperature

23.2 ~ 25.2 ˚C

3.1.6 Salinity

33.6 ~ 34.7 PSU

3.1.7 pH

7.67

3.1.8 Custody condition

Constant room at 4 ˚C for 2 days

3.1.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.1.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.1.11 Disposal of the resting substances

Scrapped after test

3.1.12 Certificate of analysis

Client supplies to test organization

3.2 Test substance-2 (Non-treated ballast water (> 32 PSU) -Control)

3.2.1 Substance name

Non-treated water (KTM-S3D5-1, 2)

3.2.2 Lot No.

None

3.2.3 Type

Liquid

3.2.4 Principal ingredients

Seawater

3.2.5 Temperature

23.1 ~ 25.6 ˚C

3.2.6 Salinity

33.9 ~ 34.8 PSU

3.2.7 pH

7.50 ~ 7.60

3.2.8 Custody condition

Constant room at 4 ˚C for 2 days

3.2.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.2.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.2.11 Disposal of the resting substances

Scrapped after test

3.2.12 Certificate of analysis

Client supplies to test organization

3.3 Test substance-3 (Treated ballast water (3 - 32 PSU) -Treated)

3.3.1 Substance name

Treated water (KTM-S5D5-3)

3.3.2 Lot No.

None

3.3.3 Type

Liquid

3.3.4 Principal ingredients

Seawater

3.3.5 Temperature

24.6 ˚C

3.3.6 Salinity

21.7 PSU

3.3.7 pH

7.46

3.3.8 Custody condition

Constant room at 4 ˚C for 2 days

3.3.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.3.10 Responsible for Sampling, Handling and Custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.3.11 Disposal of the resting substances

Scrapped after test

3.3.12 Certificate of analysis

Client supplies to test organization

3.4 Test substance-4 (Non-treated ballast water (3 - 32 PSU) -Control)

3.4.1 Substance name

Non-treated water (KTM-S3D5-3)

3.4.2 Lot No.

None

3.4.3 Type

Liquid

3.4.4 Principal ingredients

Seawater

3.4.5 Temperature

24.8 ˚C

3.4.6 Salinity

22.7 PSU

3.4.7 pH

7.24

3.4.8 Custody condition

Constant room at 4 ˚C for 2 days

3.4.9 Supplier

Name: KT Marine Co., Ltd.

Address: Centum IS Tower Rm. 1801 #1209 Jaesong-dong, Haeundae-gu, Busan, Korea

3.4.10 Responsible for sampling, handling and custody

Name: Marine Eco-Technology Institute Co., Ltd.

Address: 485-1 Yongdang, Namgu, Busan, 608-830, Republic of Korea

3.4.11 Disposal of the resting substances

Scrapped after test

3.4.12 Certificate of analysis

Client supplies to test organization

3.5 Preparation of the test substance (SOP#TP-011)

The concentration of test substance was diluted on the control seawater (non-treated ballast water by the KTM-BWMS) as crude liquid (see Fig. 1~3 in the Annex 4). The six concentrations of test substance including control (0.00 %, only non-treated ballast water by the KTM-BWMS) and 100.00 % were arranged for the final definitive experiment.

3.6 Culture condition

3.6.1 Holding of test organisms

Test organisms	*Paralichthys olivaceus*
Culture type	-
Vessel	-
Medium	-
Temperature	-
Light source	-
Light cycle	-
Light intensity	-
Dissolved oxygen	-

The eggs were obtained from commercial supplier (see 3.7 Test system).

3.6.2 Feeding regime

None

3.7 Test system

3.7.1 Test organisms

.1 Scientific name	*Paralichthys olivaceus* (see Fig. 4 in the Annex 4)
.2 Producer	KyeongYang Fisheries, Yo-Su city, Jeolla province, Republic of Korea
.3 Dealer	Marine Eco-Technology Institute Co., Ltd., Republic of Korea

3.7.2 Ground of selected test organisms

Pleuronectiformes Paralichthyidae *Paralichthys olivaceus* called olive flounder is widely distributed in coastal water of Korea and is important as economic marine products. *P. olivaceus* plays in marine trophic structure as a top predator.

3.7.3 Preparation of the test organisms (SOP#TP-007)

The fertilized eggs are obtained from commercial supplier. As the sensitivity of the experiment could be

seriously influenced by delaying the start of the test, the experiment was initiated within 8 hour after fertilization.

3.7.4 Check the test organisms for the experiment (SOP#TP-007)

Health of the eggs such as the color and appearance was observed.

3.7.5 Disposal of the remaining test organisms (SOP#TO-006)

The resting test organisms were scrapped after test.

3.8 Study design

3.8.1 Test concentration (SOP#TP-011)

Control	Control seawater	100.00 %: Seawater treated by KTM-BWMS	0.00 %
6.25 %	Control seawater	93.75 %: Seawater treated by KTM-BWMS	6.25 %
12.50 %	Control seawater	87.50 %: Seawater treated by KTM-BWMS	12.50 %
25.00 %	Control seawater	75.00 %: Seawater treated by KTM-BWMS	25.00 %
50.00 %	Control seawater	50.00 %: Seawater treated by KTM-BWMS	50.00 %
100.00 %	Control seawater	0.00 %: Seawater treated by KTM-BWMS	100.00 %

3.8.2 Exposure condition (SOP#TP-007)

30 fertilized eggs were placed in the each replication of test vessel for the > 32 and 3-32 PSU treated ballast water. And then the test chambers were placed in the constant room for 7 days at 20 ± 1˚C (see Fig. 4 ~ 6 in the Annex 4).

Experiment condition and test criteria are shown Annex 2 and application site is shown Annex 3.

3.8.3 Observation item and measurement components

.1 Hatching and survival (SOP#TP-007)

Fertilized eggs were considered dead if they were a marked loss of translucency and change in coloration. Embryos were considered dead if they were absence of body movement and/or absence of heart-beat and/or opaque discoloration. Larvae were considered if they had immobility and/or absence of respiration movement and/or absence of heart-beat and/or opaque coloration of central nervous system and/or lack of reaction mechanical stimulus. Dead eggs, embryos and larvae were removed after record.

.2 Length and weight (SOP#TP-010)

Total length and dry weights were measured at the end of the experiment.

.3 Stage of embryonic development (SOP#TP-007)

Embryonic development was observed and recorded as remark.

.4 Temperature (SOP#TE-002)

Temperature of the test concentrations during the experiment period was measured daily.

.5 Dissolved oxygen (SOP#TE-002)

DO of the solutions on each vessel during the experiment period was measured daily.

.6 pH (SOP#TE-002)

pH of the solutions during the experiment period was measured daily.

.7 Salinity (SOP#TE-002)

Salinity of the solutions during the experiment period was measured daily.

3.9 Statistical procedures (SOP#SP-101)

For > 32 and 3-32 PSU treated ballast water, normal distribution of the data was tested by Shapiro-Wilk's Test ($p>0.05$) and equal variance of the data was tested by Bartlett's Test ($p=0.27$ (>32 PSU), $p=0.41$ (3-32 PSU). Hypothesis test was conducted by Dunnett's Test. Differences in all test were considered to be significant at $\alpha=0.05$.

TOXCALC 5.0 program (Tidepool scientific software, USA) was used for above statistic analysis.

4. RESULTS

4.1 Dissolved oxygen, pH, Temperature and Salinity

4.1.1 Treated ballast water - > 32 PSU (Table 1~4)

For the > 32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 5.39 to 6.41 mg/L at the beginning of the experiment and from 5.40 to 6.33 mg/L at the end of the experiment. pH value was ranged 7.67 to 7.85 at the beginning of the experiment and was ranged from 7.81 to 7.84 at the end of the experiment. Mean temperature was 19.67 ± 0.22 ˚C (Mean ± S.D. ˚C) and mean salinity was 33.40 ± 0.22 PSU (Mean ± S.D. PSU).

4.1.2 Treated ballast water - 3-32 PSU (Table 5~8)

For the 3-32 PSU ballast water treated by the KTM-BWMS, DO value was ranged from 5.48 to 6.14 mg/L at the beginning of the experiment and from 5.31 to 6.03 mg/L at the end of the experiment. pH value was ranged from 7.72 to 7.77 at beginning of the experiment and from 7.78 to 7.82 at the end of the experiment. Mean temperature was 19.70 ± 0.34 ˚C (Mean ± S.D. ˚C) and Mean salinity was 20.81 ± 0.06 PSU (Mean ± S.D. PSU).

4.2 Mortality / Survival

4.2.1 Embryo stages (Table 9, 10)

For the > 32 PSU treated ballast water, mean number of the living eggs on the embryo stages was 29 individuals in the control and those were 30, 29, 29, 29 and 29 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively. Numbers hatched was equal with numbers of the living eggs. The all living eggs hatched for 3 days after the beginning of the experiment.

For the 3-32 PSU treated ballast water, mean number of the living eggs on the embryo stages was 28 individuals in the control and those were 28, 27, 28, 29 and 29 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively. Also numbers hatched was equal with numbers of the living eggs. The all living eggs hatched for 3 days after the beginning of the experiment.

4.2.2 Larval stages (Table 11, 12)

For the > 32 PSU treated ballast water, mean number of the survival larvae at the end of the experiment was 26 individuals in the control and those were 27, 25, 24, 19 and 26 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively.

For the 3-32 PSU treated ballast water, mean number of the survival larvae at the end of the experiment was 22 individuals in the control and those were 23, 19, 24, 25 and 22 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively.

4.2.3 Overall survival proportions (Table 13, 14)

For the > 32 PSU treated ballast water, mean survival proportions of olive flounder, *Paralichthys olivaceus* at the end of the experiment was 86.7 percent in the control and those were 90.0, 83.3, 81.1, 64.4 and 85.6 percent in the 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively.

For the 3-32 PSU treated ballast water, mean survival proportions of olive flounder, *Paralichthys olivaceus* at the end of the experiment was 72.2 percent in the control and those were 75.6, 63.3, 80.0, 84.4 and 72.2 percent in 6.25 %, 12.50 %, 25.00 %, 50.00 %, 100.00 % of the test concentration, respectively.

4.3 Length and weight

Total length and dry weight is shown in the Annex 5. For the > 32 PSU treated ballast water, mean total length and dry weight of the larval *Paralichthys olivaceus* was 2.77 ± 0.09 mm (Mean ± S.D. mm) and 0.1 mg.

For 3-32 PSU treated ballast water, mean total length and wet weight of the larval *Paralichthys olivaceus* was 2.80 ± 0.10 mm (Mean ± S.D. mm) and 0.1 mg.

4.4 Plotting of the concentration response curve (Fig. 1, 2)

The survival data on the each test concentration for the > 32 and 3-32 PSU treated ballast water was plotted on the graph.

4.5 NOEC, LOEC, LC25 and LC50 (Table 15)

NOEC and LOEC values from result of hypothesis test at the end of the experiment of the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS were 100.00 % and >100.00 %, respectively. The LC25 and LC50 values based on number of the living organisms at the end of the experiment of the > 32 and 3-32PSU ballast water were greater than 100.00 %, both of them.

4.6 Other observation

Abnormality of body form and/or pigmentation was not observed. Abnormal behavior and abnormalities, such as hyperventilation, uncoordinated swimming, and atypical quiescence was not observed. Embryonic development of all survival test organisms was normal.

5. DISCUSSION AND CONCLUSION

The mortality of embryo and sac-fry stages of olive founder, *Paralichthys olivaceus* on the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS was tested for 7 days.

For the > 32 PSU treated ballast water, mean number of the living eggs on the embryo stages was 29 individuals in the control and those were 30, 29, 29, 29 and 29 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively (Table 9). Mean number of the survival larvae was 26 individuals in the control and those were 27, 25, 24, 19 and 26 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively (Table 11). Mean survival proportions in the 50.00 % of test concentration was lower than other test concentration at the end of the experiment (Table 13). However, result of statistic analysis indicated that survival proportions of olive flounder in the all test concentrations were not significantly different with control (Dunnett's Test (1-tail), p>0.05).

For the 3-32 PSU treated ballast water, mean number of the living eggs on the embryo stages was 28 individuals in the control and those were 28, 27, 28, 29 and 29 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively (Table 10). Mean number of the survival larvae at the end of the experiment was 22 individuals in the control and those were 23, 19, 24, 25 and 22 individuals in 6.25 %, 12.50 %, 25.00 %, 50.00 % and 100.00 % of the test concentration, respectively (Table 12). Mean survival proportions in the 12.00 % of test concentration was lower than other test concentration at the end of the experiment (Table 14). However, result of statistic analysis indicated that survival proportions of olive flounder in the all test concentrations were not significantly different with control (Dunnett's Test (1-tail), p>0.05)

For the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS, all values of NOEC, LOEC, LC25 and LC50 at the end of experiment was greater than 100.00 %.

Therefore, it was assessed that the > 32 and 3-32 PSU ballast water treated by the KTM-BWMS had chronic toxic effects on the survival of the olive flounder, *Paralichthys olivaceus*.

6. REFERENCE

OECD guidelines for the testing of chemicals-212. Fish, Short-term toxicity test on the embryo and sac-fry stages. 20pp.

U.S. EPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 1200 Pennsylvania Avenue NW Washington, DC 20460.EPA-821-R-02-013.

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TABLES

Table 1. Dissolved oxygen values (mg/L) of > 32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	6.41	-	6.10	-	6.20	-	6.17	-	5.39	-	5.96	-
1	-	5.32	-	5.16	-	5.72	-	5.48	-	5.78	-	5.77
2	5.01	5.24	5.27	5.88	5.94	5.11	5.80	5.61	6.00	6.10	5.02	6.00
3	-	5.47	-	5.44	-	5.01	-	5.07	-	5.77	-	5.93
4	6.01	5.11	5.70	5.57	5.32	5.09	5.92	5.14	5.72	5.62	5.72	5.30
5	-	7.03	-	6.53	-	6.10	-	5.80	-	5.56	-	5.32
6	6.58	5.35	6.38	5.11	6.08	5.09	5.96	5.93	5.85	5.84	5.68	5.82
7	-	6.33	-	6.06	-	5.40	-	5.82	-	5.71	-	5.73

Table 2. pH values of > 32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	7.85	-	7.75	-	7.74	-	7.67	-	7.71	-	7.67	-
1	-	7.82	-	7.84	-	7.83	-	7.81	-	7.81	-	7.67
2	7.84	7.87	7.86	7.87	7.84	7.87	7.81	7.86	7.81	7.87	7.82	7.85
3	-	7.89	-	7.88	-	7.89	-	7.88	-	7.86	-	7.83
4	7.70	7.96	7.80	7.90	7.82	7.89	7.74	7.89	7.79	7.82	7.75	7.82
5	-	7.63	-	7.74	-	7.74	-	7.75	-	7.75	-	7.72
6	7.56	7.78	7.59	7.78	7.61	7.90	7.61	7.78	7.59	7.77	7.54	7.76
7	-	7.82	-	7.84	-	7.84	-	7.83	-	7.82	-	7.81

Table 3. Temperature (˚C) of > 32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	20.10	-	20.00	-	19.90	-	19.80	-	19.70	-	19.50	-
1	-	19.60	-	19.50	-	19.50	-	19.40	-	19.30	-	19.40
2	19.60	19.80	19.60	19.60	19.60	19.50	19.60	19.50	19.50	19.40	19.60	19.40
3	-	19.90	-	19.70	-	19.70	-	19.70	-	19.60	-	19.60
4	19.50	20.10	19.70	19.70	19.70	19.60	19.60	19.50	19.00	19.40	19.30	19.40
5	-	20.20	-	19.90	-	19.80	-	19.80	-	19.70	-	19.60
6	20.10	19.90	20.00	19.80	20.00	19.80	19.70	19.50	19.70	19.60	19.60	19.60
7	-	20.00	-	19.90	-	19.90	-	19.80	-	19.70	-	19.60

Table 4. Salinity (PSU) of > 32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	33.30	-	33.30	-	33.40	-	33.20	-	33.20	-	33.20	-
1	-	33.20	-	33.10	-	33.30	-	33.20	-	33.20	-	33.20
2	33.20	33.10	33.30	33.30	33.30	33.30	33.20	33.20	33.40	33.30	33.30	33.20
3	-	33.00	-	33.10	-	33.30	-	33.30	-	33.40	-	33.10
4	33.50	33.40	33.40	33.80	33.60	33.70	33.50	33.60	33.40	33.70	33.50	33.60
5	-	33.30	-	33.30	-	33.30	-	33.20	-	33.30	-	33.20
6	33.90	33.40	33.80	33.40	33.70	33.30	33.70	33.30	33.80	33.30	33.70	33.20
7	-	33.80	-	33.80	-	33.70	-	33.60	-	33.70	-	33.70

Table 5. Dissolved oxygen values (mg/L) of 3-32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	5.78	-	5.69	-	5.60	-	6.14	-	5.56	-	5.48	-
1	-	5.99	-	6.04	-	6.00	-	6.13	-	6.40	-	6.17
2	6.25	6.04	5.57	5.97	6.03	5.77	5.73	5.65	5.47	6.45	5.77	6.26
3	-	6.13	-	6.02	-	6.71	-	6.24	-	6.82	-	6.02
4	6.00	6.75	6.72	5.97	6.14	6.02	5.97	6.52	6.92	6.21	6.24	6.81
5	-	6.21	-	6.36	-	5.77	-	6.93	-	6.75	-	6.48
6	6.53	5.51	6.24	5.97	5.79	6.17	5.74	6.18	5.63	6.09	5.47	5.94
7	-	5.49	-	5.31	-	5.36	-	6.03	-	5.85	-	5.81

Table 6. pH values of 3-32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	7.72	-	7.74	-	7.76	-	7.77	-	7.77	-	7.76	-
1	-	7.77	-	7.79	-	7.80	-	7.81	-	7.82	-	7.82
2	7.35	7.73	7.48	7.73	7.44	7.73	7.49	7.73	7.50	7.72	7.41	7.71
3	-	7.73	-	7.73	-	7.72	-	7.70	-	7.74	-	7.69
4	7.67	7.77	7.69	7.78	7.73	7.76	7.70	7.73	7.71	7.73	7.65	7.73
5	-	7.76	-	7.75	-	7.74	-	7.75	-	7.75	-	7.72
6	7.82	7.79	7.81	7.77	7.78	7.76	7.79	7.77	7.79	7.76	7.77	7.72
7	-	7.82	-	7.82	-	7.80	-	7.81	-	7.80	-	7.78

Table 7. Temperature (˚C) of 3-32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	20.60	-	20.60	-	20.60	-	20.40	-	20.40	-	20.00	-
1	-	20.20	-	19.90	-	19.70	-	19.60	-	19.60	-	19.50
2	20.10	19.90	19.90	19.50	20.00	19.30	19.70	19.30	19.60	19.20	19.60	19.20
3	-	19.70	-	19.80	-	19.50	-	19.90	-	19.90	-	19.90
4	19.90	19.70	19.90	19.80	19.90	19.90	20.00	19.90	20.00	20.00	19.60	19.40
5	-	19.80	-	19.60	-	19.40	-	19.40	-	19.30	-	19.30
6	19.60	19.60	19.60	19.50	19.50	19.40	19.50	19.40	19.40	19.40	19.30	19.40
7	-	19.60	-	19.50	-	19.40	-	19.40	-	19.40	-	19.30

Table 8. Salinity (PSU) of 3-32 PSU test concentration series during the experiment period

Elapsed time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	20.80	-	20.80	-	20.80	-	20.80	-	20.80	-	20.80	-
1	-	20.80	-	20.90	-	20.80	-	20.90	-	20.90	-	20.80
2	20.70	20.90	20.80	20.90	20.80	20.80	20.80	20.80	20.80	20.90	20.80	20.80
3	-	20.80	-	20.90	-	20.90	-	20.80	-	20.90	-	20.80
4	20.80	20.70	20.80	20.90	20.90	20.90	20.80	20.80	20.70	20.70	20.70	20.90
5	-	20.70	-	20.80	-	20.80	-	20.90	-	20.80	-	20.80
6	20.80	20.80	20.80	20.80	20.80	20.90	20.80	20.80	20.80	20.80	20.70	20.80
7	-	20.80	-	20.80	-	20.80	-	20.80	-	20.80	-	20.80

Table 9. Number of living eggs on embryo stages of *Paralichthys olivaceus* exposed to > 32 PSU treated ballast water

Elapsed time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	30	29	30	30	30	30	30	30	30	30	28	29
2	30	27	30	29	30	30	30	30	30	29	27	29
3	30	27	30	29	30	30	30	30	30	29	27	29
Elapsed time (day)	**25.00%**				**50.00%**				**100.00%**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	30	30	30	30	30	30	30	30	30	30	30	30
1	29	29	30	29	30	29	28	29	29	30	29	29
2	29	29	29	29	30	29	28	29	29	30	29	29
3	29	29	29	29	30	29	28	29	29	30	29	29

Table 10. Number of living eggs on the embryo stages of *Paralichthys olivaceus* exposed to 3-32 PSU treated ballast water

Elapsed time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	28	29	27	28	29	27	29	28	28	26	28	27
2	28	29	27	28	29	27	29	28	28	26	28	27
3	28	29	27	28	29	26	29	28	28	25	28	27
Elapsed time (day)	**25.00%**				**50.00%**				**100.00%**			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	28	28	29	28	28	30	30	29	29	30	29	29
2	28	28	29	28	28	30	30	29	29	30	29	29
3	28	28	29	28	28	30	30	29	29	30	29	29

Table 11. Number of survival larvae of *Paralichthys olivaceus* exposed to > 32 PSU treated ballast water

Elapsed time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
4	29	26	29	28	30	28	25	28	29	29	23	27
5	29	24	28	27	30	28	25	28	29	28	23	27
6	29	22	28	26	30	27	25	27	29	28	23	27
7	29	22	27	26	30	26	25	27	29	26	20	25
Elapsed time (day)	**25.00%**				**50.00%**				**100.00%**			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
4	27	26	26	26	23	21	19	21	27	28	28	28
5	27	26	26	26	23	20	18	20	26	28	27	27
6	27	26	26	26	22	19	18	20	26	28	27	27
7	27	23	23	24	22	18	18	19	26	26	25	26

Table 12. Number of survival larvae of *Paralichthys olivaceus* exposed to 3-32 PSU treated ballast water

Elapsed time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
4	23	24	21	23	21	23	27	24	23	21	24	23
5	23	22	21	22	21	22	27	23	22	21	23	22
6	22	22	21	22	21	22	26	23	17	18	22	19
7	22	22	21	22	21	22	25	23	17	18	22	19
Elapsed time (day)	**25.00%**				**50.00%**				**100.00%**			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
4	27	26	28	27	27	30	25	27	25	27	24	25
5	25	26	28	26	26	29	25	27	23	23	22	23
6	25	25	27	26	25	28	25	26	23	20	22	22
7	25	23	24	24	25	26	25	25	23	20	22	22

Table 13. Overall survival proportions of olive flounder, *Paralichthys olivaceus* exposed to test concentration series of the > 32 PSU treated ballast water during the experiment period

Elapsed time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	100.0	96.7	100.0	98.9	100.0	100.0	100.0	100.0	100.0	100.0	93.3	97.8
2	96.7	90.0	100.0	95.6	100.0	96.7	90.0	95.6	100.0	96.7	86.7	94.4
3	96.7	90.0	100.0	95.6	100.0	96.7	86.7	94.4	100.0	96.7	80.0	92.2
4	96.7	86.7	96.7	93.3	100.0	93.3	83.3	92.2	96.7	96.7	76.7	90.0
5	96.7	80.0	93.3	90.0	100.0	93.3	83.3	92.2	96.7	93.3	76.7	88.9
6	96.7	73.3	93.3	87.8	100.0	90.0	83.3	91.1	96.7	93.3	76.7	88.9
7	96.7	73.3	90.0	86.7	100.0	86.7	83.3	90.0	96.7	86.7	66.7	83.3
Elapsed time (day)	**25.00%**				**50.00%**				**100.00%**			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	96.7	96.7	100.0	97.8	100.0	100.0	100.0	100.0	96.7	100.0	96.7	97.8
2	93.3	90.0	90.0	91.1	96.7	93.3	76.7	88.9	93.3	100.0	96.7	96.7
3	90.0	86.7	90.0	88.9	80.0	76.7	73.3	76.7	93.3	100.0	96.7	96.7
4	90.0	86.7	86.7	87.8	76.7	70.0	63.3	70.0	90.0	93.3	93.3	92.2
5	90.0	86.7	86.7	87.8	76.7	66.7	60.0	67.8	86.7	93.3	90.0	90.0
6	90.0	86.7	86.7	87.8	73.3	63.3	60.0	65.6	86.7	93.3	90.0	90.0
7	90.0	76.7	76.7	81.1	73.3	60.0	60.0	64.4	86.7	86.7	83.3	85.6

Table 14. Overall survival proportions of olive flounder, *Paralichthys olivaceus* exposed to test concentration series of the 3-32 PSU treated ballast water during the experiment period

Elapsed time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.3	96.7	90.0	93.3	96.7	90.0	96.7	94.4	90.0	86.7	93.3	90.0
2	93.3	96.7	83.3	91.1	96.7	80.0	93.3	90.0	83.3	76.7	93.3	84.4
3	86.7	80.0	70.0	78.9	73.3	76.7	90.0	80.0	80.0	70.0	80.0	76.7
4	76.7	80.0	70.0	75.6	70.0	76.7	90.0	78.9	76.7	70.0	80.0	75.6
5	76.7	73.3	70.0	73.3	70.0	73.3	90.0	77.8	73.3	70.0	76.7	73.3
6	73.3	73.3	70.0	72.2	70.0	73.3	86.7	76.7	56.7	60.0	73.3	63.3
7	73.3	73.3	70.0	72.2	70.0	73.3	83.3	75.6	56.7	60.0	73.3	63.3
Elapsed time (day)	**25.00%**				**50.00%**				**100.00%**			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.3	93.3	96.7	94.4	93.3	100.0	100.0	97.8	96.7	100.0	96.7	97.8
2	93.3	93.3	93.3	93.3	90.0	100.0	93.3	93.3	93.3	96.7	93.3	94.4
3	90.0	90.0	93.3	91.1	90.0	100.0	86.7	92.2	86.7	96.7	80.0	87.8
4	90.0	86.7	93.3	90.0	90.0	100.0	83.3	91.1	83.3	90.0	80.0	84.4
5	83.3	86.7	93.3	87.8	86.7	96.7	83.3	88.9	76.7	76.7	73.3	75.6
6	83.3	83.3	90.0	85.6	83.3	93.3	83.3	86.7	76.7	66.7	73.3	72.2
7	83.3	76.7	80.0	80.0	83.3	86.7	83.3	84.4	76.7	66.7	73.3	72.2

Table 15. NOEC, LOEC, LC25 and LC50 value calculated from survival data at the end of the experiment

Test substance	NOEC (%)	LOEC (%)	LC25 (%)	95 % confidence of limit (%)		LC50 (%)	95 % confidence of limit (%)	
				lower	upper		lower	upper
> 32 PSU treated ballast water	100.00	> 100.00	>100.00	-	-	> 100.00	-	-
3-32 PSU treated ballast water	100.00	> 100.00	>100.00	-	-	> 100.00	-	-

* -: no data; > 100.00: greater than 100.00 %

FIGURES

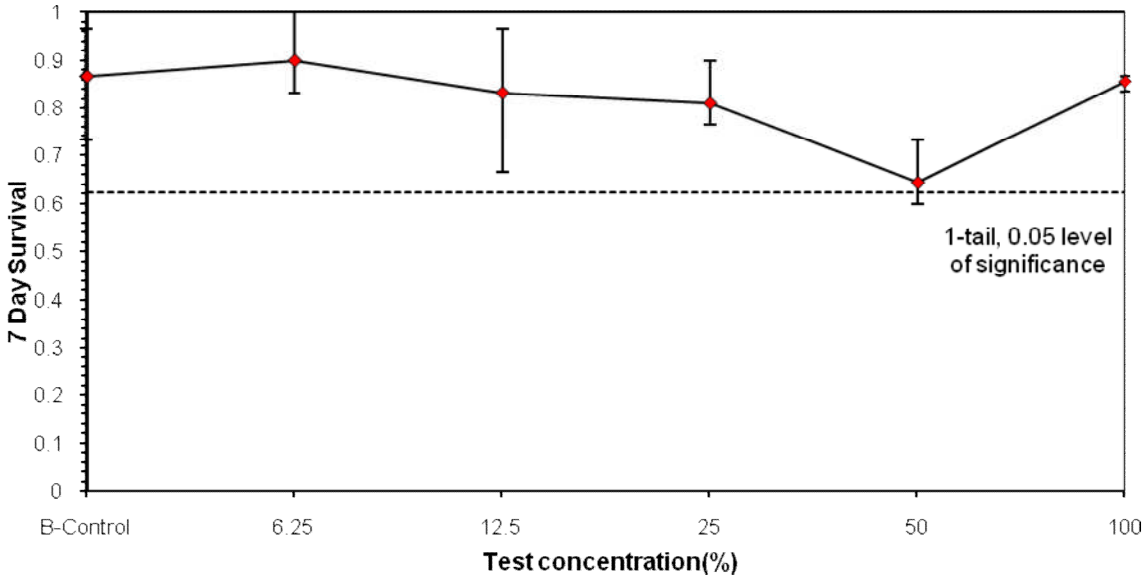


Fig. 1. Plot of the concentration-response data from the each test concentration of > 32 PSU treated ballast water. The dotted line was represented as 0.05 level of significant and vertical bar was represented maximum and minimum.

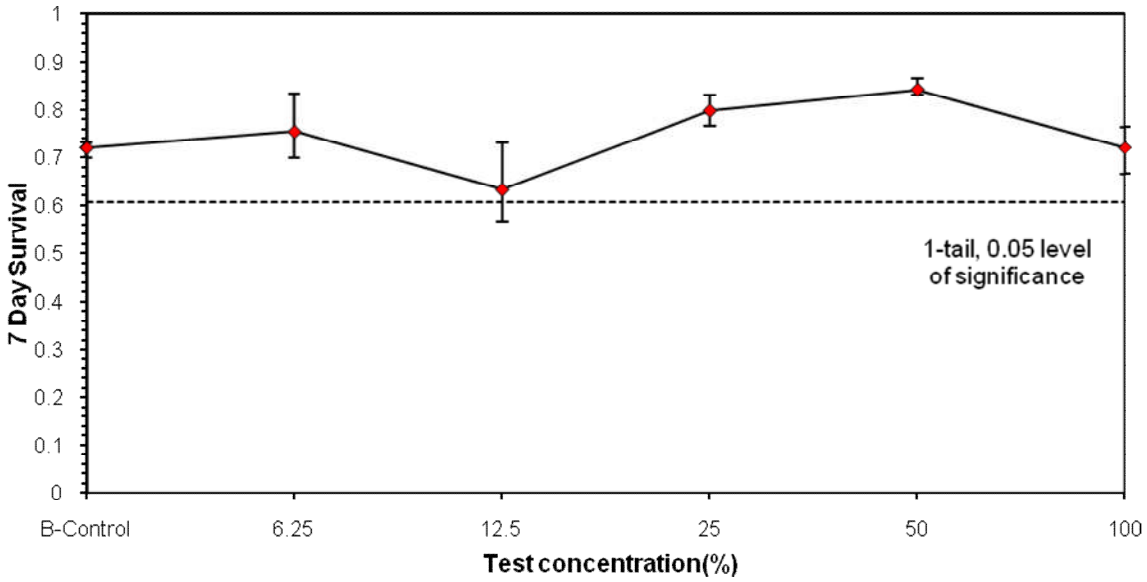


Fig. 2. Plot of the concentration-response data from the each test concentration of 3-32 PSU treated ballast water. The dotted line was represented as 0.05 level of significant and vertical bar was represented maximum and minimum.

ANNEX 1

Definitions

1. Semi-static test is a test without flow of solution, but with occasional batch wise renewal of the test solution after prolonged periods.

2. LC25 and LC50 in this study is the quarter and median lethal concentration, i.e. that concentration of the test substance in water which kills 25 and 50 percent of a test batch of fish within a particular period of exposure.

3. Lowest Observed Effect Concentration (LOEC) is the lowest tested concentration at which the substance is observed to have a statistically significant reducing effect on survival (at $p < 0.05$) when compared with the control, within a given exposure time. However, all test concentrations above the LOEC must have a harmful effect equal to or greater than those observed at the LOEC

4. No Observed Effect Concentration (NOEC) is the test concentration immediately below the LOEC.

ANNEX 2

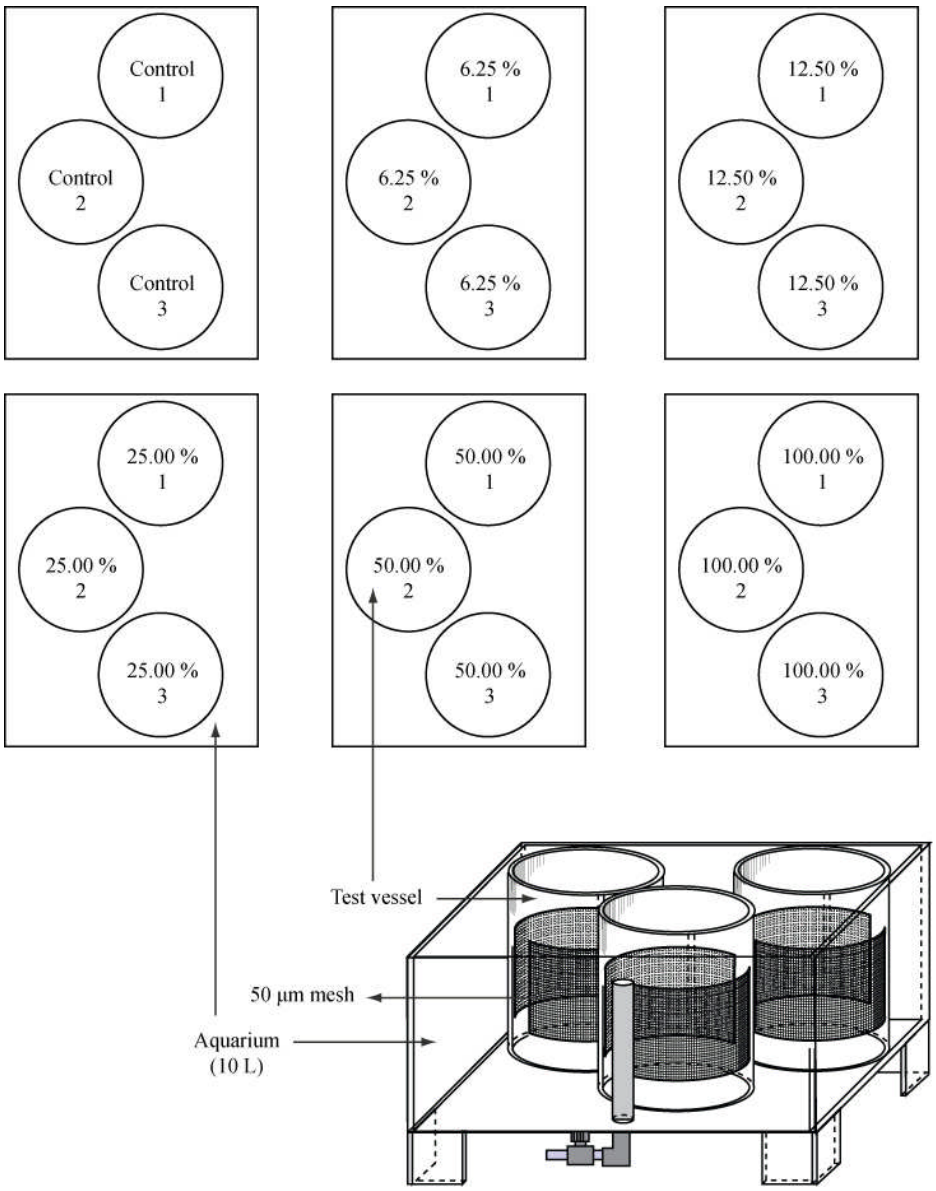
Experiment conditions and acceptability criteria for embryo and sac-fry stage of olive flounder, *Paralichthys olivaceus*, chronic mortality toxicity tests with the > 32 and 3-32 PSU seawater treated by the KTM-BWMS

Test parameter	Conditions
Test type	Semi-static
Temperature	20 ± 1 ˚C
Light quality	Cool white fluorescent lighting
Light intensity	2,000 lux
Photoperiod	16 h light : 8 h dark
Test camber size	1 L
Test solution volume	0.8 L
Renewal of test solutions	Every other day
Age of test organisms	Less than 8 h after fertilization
Number of individuals per test chamber	30
Replicate test chambers per concentration	3
Feeding regime	None
Cleaning	Exchange the test water
Aeration	To maintain DO (> 60 % of the air saturation value)
Dilution water	Filtered seawater (Control seawater) by 1μm CP Filter, Chisso Filter
Test concentration	5 including 100.00 % effluents and a control
Dilution factor	Effluents: 0.5
Test duration	7 days
Endpoints	Survival
Test acceptability criteria	- The water temperature change: less than ± 1.5 ˚C - Overall survival of fertilized eggs in the controls: more than 70 %

ANNEX 3

Application Site

Application site for the chronic toxicity test with the fertilized eggs of olive flounder, *Paralichthys olivaceus* is shown as follow.



ANNEX 4

Preparation of test substance and procedure of the experiment

Fig. 1. For aquatic toxicity test, collection of the control (untreated) and treated de-ballasting water from the KTM-BWMS (left down: sampling equipments).

Fig. 2. Transfer collected samples to the constant room (4 ± 1 ˚C).

Fig. 3. Preparation of test concentration diluted with non-treated seawater on treated seawater through flow-meter.

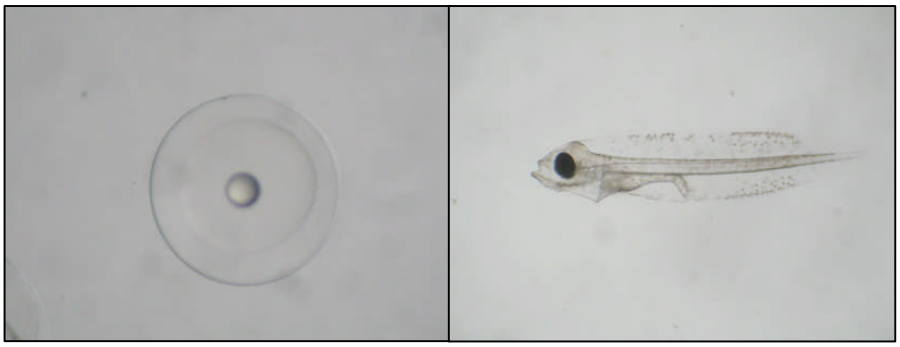


Fig. 4. Test organisms in this study: Fertilized eggs (left) and larva (right) of olive flounder, *Paralichthys olivaceus*.

Fig. 5. Test organisms in this study: Fertilized eggs and larvae of olive flounder, *Paralichthys olivaceus*.: (above: live, down: dead)

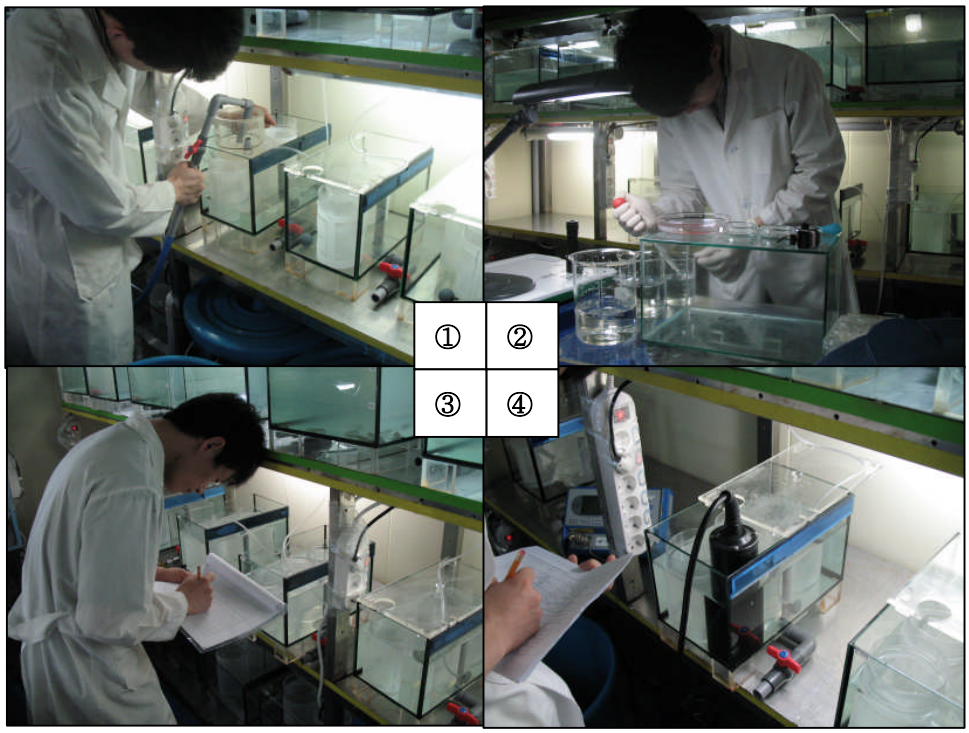


Fig. 6. Procedure of chronic fish toxicity test set up (① Test vessels set up; ② Sorting the fertilized eggs; ③ Observation of test organisms mortality; ④ Check the water quality of the test substance and recording)

ANNEX 5

▶ Total length and dry weight of larval olive flounder, *Paralichthys olivaceus*, at the end of the experiment for > 32 PSU seawater treated by the KTM-BWMS

▷ **Concentration :** Control						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	3.0	0.1	2.8	0.1	2.9	0.1
2	2.9	0.1	2.7	0.1	2.7	0.1
3	2.6	0.1	2.9	0.1	2.8	0.1
4	2.7	0.1	3.0	0.1	2.9	0.1
5	2.7	0.1	3.0	0.1	2.7	0.1
6	2.7	0.1	2.9	0.1	2.8	0.1
7	2.7	0.1	2.9	0.1	2.9	0.1
8	2.8	0.1	2.9	0.1	2.8	0.1
9	2.6	0.1	2.9	0.1	2.7	0.1
10	2.9	0.1	2.7	0.1	2.8	0.1
11	2.7	0.1	2.7	0.1	2.9	0.1
12	2.8	0.1	2.8	0.1	2.8	0.1
13	2.8	0.1	2.9	0.1	2.4	0.1
14	2.7	0.1	2.7	0.1	2.6	0.1
15	2.8	0.1	2.8	0.1	2.6	0.1
16	2.6	0.1	2.8	0.1	2.9	0.1
17	2.9	0.1	2.9	0.1	2.8	0.1
18	2.7	0.1	2.7	0.1	2.7	0.1
19	2.8	0.1	2.8	0.1	2.8	0.1
20	2.8	0.1	2.9	0.1	2.7	0.1
21	2.8	0.1	2.8	0.1	2.9	0.1
22	2.8	0.1	2.9	0.1	2.9	0.1
23	2.7	0.1			2.8	0.1
24	2.7	0.1			2.8	0.1
25	2.8	0.1			2.7	0.1
26	3.0	0.1			2.7	0.1
27	2.7	0.1			3.0	0.1
28	2.7	0.1				
29	2.8	0.1				
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 6.25 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.8	0.1	2.9	0.1	2.8	0.1
2	2.6	0.1	2.8	0.1	2.8	0.1
3	2.7	0.1	2.8	0.1	2.8	0.1
4	2.8	0.1	2.7	0.1	2.8	0.1
5	2.8	0.1	2.9	0.1	2.8	0.1
6	2.9	0.1	2.8	0.1	2.8	0.1
7	2.7	0.1	2.8	0.1	2.8	0.1
8	2.8	0.1	2.8	0.1	2.8	0.1
9	2.6	0.1	2.8	0.1	2.8	0.1
10	2.6	0.1	2.8	0.1	2.8	0.1
11	2.6	0.1	2.8	0.1	2.7	0.1
12	2.7	0.1	2.9	0.1	2.7	0.1
13	2.8	0.1	2.8	0.1	2.7	0.1
14	2.9	0.1	2.7	0.1	2.7	0.1
15	2.7	0.1	2.9	0.1	2.7	0.1
16	2.7	0.1	2.9	0.1	2.8	0.1
17	2.8	0.1	2.7	0.1	2.9	0.1
18	2.9	0.1	2.7	0.1	2.8	0.1
19	2.7	0.1	2.8	0.1	2.7	0.1
20	2.7	0.1	2.9	0.1	2.8	0.1
21	2.8	0.1	2.9	0.1	2.9	0.1
22	2.8	0.1	2.7	0.1	2.8	0.1
23	2.9	0.1	2.7	0.1	2.8	0.1
24	2.8	0.1	2.7	0.1	2.7	0.1
25	2.7	0.1	2.7	0.1	2.6	0.1
26	2.8	0.1	2.8	0.1		
27	2.8	0.1				
28	2.9	0.1				
29	2.8	0.1				
30	2.9	0.1				
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 12.50 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.8	0.1	2.7	0.1	2.8	0.1
2	2.7	0.1	2.7	0.1	2.8	0.1
3	2.8	0.1	2.7	0.1	2.7	0.1
4	2.9	0.1	2.7	0.1	2.7	0.1
5	2.9	0.1	2.8	0.1	2.7	0.1
6	2.8	0.1	2.8	0.1	2.8	0.1
7	2.7	0.1	2.8	0.1	2.7	0.1
8	2.8	0.1	2.8	0.1	2.8	0.1
9	2.8	0.1	2.7	0.1	2.8	0.1
10	2.7	0.1	2.8	0.1	2.7	0.1
11	2.7	0.1	2.9	0.1	2.7	0.1
12	2.7	0.1	2.6	0.1	2.8	0.1
13	2.8	0.1	2.7	0.1	2.9	0.1
14	2.7	0.1	2.8	0.1	2.7	0.1
15	2.8	0.1	2.7	0.1	2.7	0.1
16	2.6	0.1	2.9	0.1	2.7	0.1
17	2.8	0.1	2.8	0.1	2.7	0.1
18	2.7	0.1	2.7	0.1	2.8	0.1
19	2.8	0.1	2.8	0.1	2.8	0.1
20	2.9	0.1	2.7	0.1	2.8	0.1
21	2.7	0.1	2.8	0.1		
22	2.8	0.1	2.8	0.1		
23	2.7	0.1	2.9	0.1		
24	2.8	0.1	2.7	0.1		
25	2.8	0.1	2.8	0.1		
26	2.9	0.1	2.8	0.1		
27	2.7	0.1				
28	2.8	0.1				
29	2.8	0.1				
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 25.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.8	0.1	2.7	0.1	2.7	0.1
2	2.7	0.1	2.7	0.1	2.4	0.1
3	2.7	0.1	2.7	0.1	2.8	0.1
4	2.6	0.1	2.7	0.1	2.8	0.1
5	2.7	0.1	2.7	0.1	2.8	0.1
6	2.8	0.1	2.8	0.1	2.8	0.1
7	2.9	0.1	2.8	0.1	2.8	0.1
8	2.8	0.1	2.7	0.1	2.8	0.1
9	2.8	0.1	2.7	0.1	2.9	0.1
10	2.8	0.1	2.7	0.1	2.8	0.1
11	2.8	0.1	3.0	0.1	2.8	0.1
12	2.9	0.1	2.7	0.1	2.7	0.1
13	2.9	0.1	2.7	0.1	2.7	0.1
14	2.9	0.1	2.7	0.1	2.7	0.1
15	2.9	0.1	2.7	0.1	2.8	0.1
16	2.9	0.1	2.7	0.1	2.8	0.1
17	3.0	0.1	2.7	0.1	2.7	0.1
18	3.0	0.1	2.8	0.1	2.7	0.1
19	2.8	0.1	2.8	0.1	2.7	0.1
20	2.8	0.1	2.8	0.1	2.7	0.1
21	2.6	0.1	2.8	0.1	2.8	0.1
22	2.9	0.1	2.8	0.1	2.8	0.1
23	2.8	0.1	2.8	0.1	2.9	0.1
24	2.7	0.1				
25	2.7	0.1				
26	2.7	0.1				
27	2.8	0.1				
28						
29						
30						
Mean	2.8	0.1	2.7	0.1	2.8	0.1

▷ **Concentration :** 50.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.8	0.1	2.9	0.1	2.8	0.1
2	2.8	0.1	2.7	0.1	2.7	0.1
3	2.9	0.1	2.7	0.1	2.7	0.1
4	2.9	0.1	2.8	0.1	2.8	0.1
5	2.8	0.1	2.8	0.1	2.8	0.1
6	2.8	0.1	2.9	0.1	2.8	0.1
7	2.7	0.1	2.8	0.1	2.8	0.1
8	2.7	0.1	2.7	0.1	2.7	0.1
9	2.8	0.1	2.7	0.1	2.8	0.1
10	2.7	0.1	2.7	0.1	2.7	0.1
11	2.7	0.1	2.8	0.1	2.8	0.1
12	2.7	0.1	2.8	0.1	2.8	0.1
13	2.8	0.1	2.7	0.1	2.9	0.1
14	2.7	0.1	2.7	0.1	2.7	0.1
15	2.9	0.1	2.7	0.1	2.8	0.1
16	2.8	0.1	2.8	0.1	2.7	0.1
17	2.7	0.1	2.8	0.1	2.7	0.1
18	2.7	0.1	2.9	0.1	2.8	0.1
19	2.7	0.1				
20	2.7	0.1				
21	2.8	0.1				
22	2.8	0.1				
23						
24						
25						
26						
27						
28						
29						
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 100.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.7	0.1	2.9	0.1	2.8	0.1
2	2.8	0.1	2.7	0.1	2.7	0.1
3	2.7	0.1	2.8	0.1	2.7	0.1
4	2.7	0.1	2.7	0.1	2.7	0.1
5	2.7	0.1	2.8	0.1	2.7	0.1
6	2.7	0.1	2.8	0.1	2.8	0.1
7	2.7	0.1	2.7	0.1	2.8	0.1
8	2.8	0.1	2.7	0.1	2.9	0.1
9	2.7	0.1	2.8	0.1	2.7	0.1
10	2.8	0.1	2.9	0.1	2.8	0.1
11	2.9	0.1	2.8	0.1	2.8	0.1
12	2.7	0.1	2.7	0.1	2.7	0.1
13	2.8	0.1	2.7	0.1	2.7	0.1
14	2.7	0.1	2.7	0.1	2.7	0.1
15	2.8	0.1	2.8	0.1	2.8	0.1
16	2.7	0.1	2.7	0.1	2.7	0.1
17	2.7	0.1	2.8	0.1	2.8	0.1
18	2.8	0.1	2.7	0.1	2.7	0.1
19	2.8	0.1	2.8	0.1	2.8	0.1
20	2.8	0.1	2.8	0.1	2.9	0.1
21	2.9	0.1	2.9	0.1	2.8	0.1
22	2.8	0.1	2.8	0.1	2.7	0.1
23	2.7	0.1	2.7	0.1	2.7	0.1
24	2.7	0.1	2.7	0.1	2.7	0.1
25	2.7	0.1	2.7	0.1	2.8	0.1
26	2.8	0.1	2.8	0.1		
27						
28						
29						
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▶ Total length and dry weight of larval olive flounder, *Paralichthys olivaceus*, at the end of the experiment for 3-32 PSU seawater treated by the KTM-BWMS

▷ **Concentration :** Control						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.9	0.1	2.7	0.1	3.0	0.1
2	2.8	0.1	2.7	0.1	2.7	0.1
3	2.8	0.1	2.9	0.1	2.7	0.1
4	2.7	0.1	3.0	0.1	2.9	0.1
5	2.7	0.1	3.0	0.1	2.7	0.1
6	2.7	0.1	3.0	0.1	2.7	0.1
7	2.7	0.1	2.7	0.1	2.9	0.1
8	2.8	0.1	2.9	0.1	2.7	0.1
9	2.7	0.1	2.9	0.1	2.7	0.1
10	2.7	0.1	2.7	0.1	2.8	0.1
11	2.7	0.1	2.7	0.1	2.7	0.1
12	2.7	0.1	2.7	0.1	2.8	0.1
13	2.7	0.1	2.9	0.1	2.4	0.1
14	2.7	0.1	2.7	0.1	2.7	0.1
15	2.8	0.1	2.8	0.1	2.6	0.1
16	2.6	0.1	2.7	0.1	2.7	0.1
17	2.9	0.1	2.9	0.1	2.8	0.1
18	2.7	0.1	2.7	0.1	2.7	0.1
19	2.7	0.1	2.7	0.1	2.8	0.1
20	2.7	0.1	2.7	0.1	2.7	0.1
21	2.7	0.1	2.7	0.1	3.0	0.1
22	2.8	0.1	2.9	0.1		
23						
24						
25						
26						
27						
28						
29						
30						
Mean	2.7	0.1	2.8	0.1	2.7	0.1

▷ **Concentration :** 6.25 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.7	0.1	2.8	0.1	2.7	0.1
2	2.6	0.1	2.8	0.1	2.8	0.1
3	2.6	0.1	2.9	0.1	2.7	0.1
4	2.8	0.1	2.7	0.1	2.8	0.1
5	2.8	0.1	2.9	0.1	2.7	0.1
6	3.0	0.1	2.8	0.1	2.8	0.1
7	2.7	0.1	2.9	0.1	2.8	0.1
8	2.8	0.1	2.8	0.1	2.7	0.1
9	2.7	0.1	2.9	0.1	2.8	0.1
10	2.7	0.1	2.8	0.1	2.8	0.1
11	2.7	0.1	2.9	0.1	2.8	0.1
12	2.7	0.1	2.9	0.1	2.7	0.1
13	2.8	0.1	2.8	0.1	2.8	0.1
14	2.9	0.1	2.9	0.1	2.7	0.1
15	2.7	0.1	2.9	0.1	2.8	0.1
16	2.7	0.1	2.9	0.1	2.8	0.1
17	2.8	0.1	2.7	0.1	2.9	0.1
18	2.9	0.1	2.9	0.1	2.7	0.1
19	2.7	0.1	2.8	0.1	2.7	0.1
20	3.0	0.1	2.8	0.1	2.7	0.1
21	3.0	0.1	2.9	0.1	2.9	0.1
22			2.8	0.1	2.9	0.1
23					2.8	0.1
24					2.7	0.1
25					2.9	0.1
26						
27						
28						
29						
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 12.50 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.9	0.1	2.9	0.1	2.7	0.1
2	2.9	0.1	2.7	0.1	2.8	0.1
3	2.9	0.1	2.8	0.1	2.7	0.1
4	2.9	0.1	2.7	0.1	2.8	0.1
5	2.9	0.1	2.8	0.1	2.7	0.1
6	2.8	0.1	2.7	0.1	2.8	0.1
7	2.7	0.1	2.8	0.1	2.7	0.1
8	2.8	0.1	2.8	0.1	2.8	0.1
9	2.8	0.1	3.0	0.1	2.7	0.1
10	3.0	0.1	2.8	0.1	2.7	0.1
11	2.7	0.1	2.9	0.1	2.7	0.1
12	2.7	0.1	2.6	0.1	2.8	0.1
13	2.8	0.1	3.0	0.1	2.7	0.1
14	3.0	0.1	2.8	0.1	2.7	0.1
15	2.8	0.1	2.7	0.1	2.7	0.1
16	2.6	0.1	2.9	0.1	3.0	0.1
17	3.0	0.1	2.8	0.1	3.0	0.1
18			3.0	0.1	2.8	0.1
19					2.8	0.1
20					2.8	0.1
21					3.0	0.1
22					2.7	0.1
23						
24						
25						
26						
27						
28						
29						
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 25.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.7	0.1	2.9	0.1	2.9	0.1
2	2.7	0.1	2.9	0.1	2.9	0.1
3	2.8	0.1	2.7	0.1	2.8	0.1
4	2.6	0.1	2.7	0.1	2.8	0.1
5	2.9	0.1	2.7	0.1	2.8	0.1
6	2.8	0.1	2.8	0.1	2.8	0.1
7	2.7	0.1	2.8	0.1	2.9	0.1
8	2.8	0.1	2.7	0.1	2.8	0.1
9	2.7	0.1	2.9	0.1	2.9	0.1
10	2.8	0.1	2.7	0.1	2.8	0.1
11	2.6	0.1	3.0	0.1	2.9	0.1
12	2.7	0.1	2.9	0.1	2.7	0.1
13	2.9	0.1	2.7	0.1	2.7	0.1
14	3.0	0.1	2.7	0.1	2.7	0.1
15	2.9	0.1	2.9	0.1	2.8	0.1
16	2.9	0.1	2.7	0.1	2.9	0.1
17	3.0	0.1	2.7	0.1	2.7	0.1
18	3.0	0.1	2.8	0.1	2.7	0.1
19	2.9	0.1	2.7	0.1	2.7	0.1
20	2.9	0.1	2.8	0.1	2.7	0.1
21	2.6	0.1	2.8	0.1	2.9	0.1
22	2.9	0.1	2.8	0.1	2.8	0.1
23	2.8	0.1	2.7	0.1	2.9	0.1
24	2.8	0.1			3.0	0.1
25	2.8	0.1				
26						
27						
28						
29						
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 50.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.9	0.1	3.0	0.1	2.8	0.1
2	3.0	0.1	2.7	0.1	2.7	0.1
3	2.9	0.1	2.7	0.1	2.7	0.1
4	2.9	0.1	2.9	0.1	2.8	0.1
5	3.0	0.1	2.9	0.1	2.8	0.1
6	2.8	0.1	2.9	0.1	2.8	0.1
7	2.7	0.1	2.8	0.1	2.8	0.1
8	3.0	0.1	2.9	0.1	2.7	0.1
9	2.8	0.1	2.7	0.1	2.8	0.1
10	2.7	0.1	2.9	0.1	2.9	0.1
11	3.0	0.1	2.8	0.1	2.9	0.1
12	2.7	0.1	2.9	0.1	2.8	0.1
13	2.8	0.1	2.7	0.1	2.9	0.1
14	2.7	0.1	2.7	0.1	2.7	0.1
15	3.0	0.1	2.9	0.1	2.9	0.1
16	2.8	0.1	2.8	0.1	2.7	0.1
17	2.9	0.1	2.8	0.1	2.9	0.1
18	2.7	0.1	2.9	0.1	2.8	0.1
19	2.9	0.1	2.7	0.1	2.8	0.1
20	2.7	0.1	2.7	0.1	2.7	0.1
21	2.8	0.1	2.9	0.1	3.0	0.1
22	2.8	0.1	2.8	0.1	3.0	0.1
23	2.7	0.1	2.7	0.1	2.8	0.1
24	2.8	0.1	2.6	0.1	2.6	0.1
25	2.7	0.1	2.7	0.1	2.7	0.1
26			2.8	0.1		
27						
28						
29						
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

▷ **Concentration :** 100.00 %						
Replicate	**1**		**2**		**3**	
No.	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**	**Length (mm)**	**Dry weight (mg)**
1	2.8	0.1	3.0	0.1	2.6	0.1
2	2.8	0.1	2.7	0.1	2.8	0.1
3	2.7	0.1	2.8	0.1	2.7	0.1
4	2.7	0.1	2.8	0.1	2.9	0.1
5	2.7	0.1	2.8	0.1	2.9	0.1
6	2.8	0.1	2.8	0.1	2.8	0.1
7	2.7	0.1	2.8	0.1	2.9	0.1
8	2.8	0.1	2.7	0.1	2.9	0.1
9	2.7	0.1	2.8	0.1	2.7	0.1
10	2.8	0.1	2.9	0.1	2.9	0.1
11	2.9	0.1	2.8	0.1	2.8	0.1
12	2.7	0.1	2.7	0.1	2.7	0.1
13	2.8	0.1	2.8	0.1	2.9	0.1
14	2.7	0.1	2.7	0.1	2.7	0.1
15	2.8	0.1	2.8	0.1	2.8	0.1
16	2.8	0.1	2.8	0.1	2.9	0.1
17	2.7	0.1	2.8	0.1	2.8	0.1
18	3.0	0.1	2.7	0.1	2.7	0.1
19	2.8	0.1	2.7	0.1	2.9	0.1
20	3.0	0.1	2.9	0.1	2.9	0.1
21	2.9	0.1			2.8	0.1
22	2.8	0.1			2.9	0.1
23	2.8	0.1				
24						
25						
26						
27						
28						
29						
30						
Mean	2.8	0.1	2.8	0.1	2.8	0.1

4. COC (CHAIN OF CUSTODY) DATA

4.1 Sheet of raw data for toxicity test

4.1.1 Acute toxicity test

4.1.1.1 ANNEX 1

Sheet of raw data for "*Skeletonema costatum*, Growth Inhibition Test"

Marine Eco-Technology Institute Co., Ltd.
485-1 Yongdang, Namgu, Busan 608-830, Korea
Tel. +82-51-611-6200
Fax +82-51-611-0588

COC code : BW-DBNT1104-KT-P001

Sheet of Raw Data – Aquatic plant

[1] COC Client	KT Marine Co., Ltd.	Test date	2011.6.30 ~ 7.03
Lab. manager	Myung-Baek Shon	Observer	Myung-Baek Shon
Test organism	Skeletonema costatum	Test duration	72h
[2] COTS	0, 6.25, 12.5, 25, 50, 100	Test substance	KTM-SSDS-1 (>32 psu)

Test Conditions and Methods

Temperature	22 ℃	Salinity	33.6 psu	Light	3,000 Lux, LD cycle = 24 : 0D
Medium	f/2	Sterile	filtered by 0.2 um membrane filter		
Initial biomass	3,000 cells/ml	Vessel type and volume	15 ml test tube		
[3] MV / [4] VV	10 mL / 15 mL	Method of observation (SOP No.)	#TP-001 #TP-012		

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] MV: Medium volume; [4] VV: Vessel volume

▶ Result

Density of phytoplankton (unit: μg/L) **Date: 2011.6.30 (Initial)**

Replication	Concentration of test substance (%)						
	Control						
	Cell density (cells/mL)	Chl-*a*	6.25	12.5	25	50	100
1	298	1.40	1.70	1.20	1.25	1.82	1.36
2	350	1.34	1.15	1.30	1.30	1.26	1.17
3	371	1.34	1.05	1.22	1.14	1.23	1.43
4	380	1.36	1.45	1.25	1.18	1.17	1.23
Remarks	dilution factor : ×10, chl-a estimate date : 7/1						

Density of phytoplankton (unit: μg/L) **Date: 2011.7.1. (24 hours)**

Replication	Concentration of test substance (%)						
	Control						
	Cell density (cells/mL)	Chl-*a*	6.25	12.5	25	50	100
1	343	8.75	10.80	10.50	10.40	8.78	7.83
2	278	10.30	12.50	10.40	10.60	8.80	8.80
3	322	10.10	8.64	10.60	8.88	8.88	8.73
4	347	11.10	11.20	10.60	7.58	8.23	10.00
Remarks	dilution factor : ×100, chl-a estimation date : 7/2						

Density of phytoplankton (unit: μg/L) Date: 2011. 7. 2. (48 hours)

Replication	Concentration of test substance (%)						
	Control		6.25	12.5	25	50	100
	Cell density (cells/mL)	Chl-*a*					
1	581	143.00	140.00	134.00	127.00	109.00	23.20
2	713	147.00	163.00	139.00	137.00	108.00	24.00
3	558	150.00	104.00	135.00	115.00	109.00	24.20
4	616	136.00	120.00	132.00	98.00	107.00	23.50
Remarks	dilution factor: x500, chl-a estimate date: 7/3						

Density of phytoplankton (unit: μg/L) Date: 2011. 7. 3. (72 hours)

Replication	Concentration of test substance (%)						
	Control		6.25	12.5	25	50	100
	Cell density (cells/mL)	Chl-*a*					
1	228	233.00	229.00	245.00	223.00	180.00	13.50
2	258	248.00	241.00	251.00	230.00	179.00	12.60
3	186	255.00	243.00	252.00	239.00	187.00	14.00
4	212	270.00	263.00	260.00	243.00	186.00	13.40
Remarks	dilution factor: x2,000, chl-a estimate date: 7/4						

Density of phytoplankton (unit: μg/L) Date: 20 . . . (hours)

Replication	Concentration of test substance (%)						
	Control						
	Cell density (cells/mL)	Chl-*a*					
1							
2							
3							
4							
Remarks							

▶ pH determinations

Date : 2011. 6. 30 (Initial)

	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
Initial	7.25	7.29	7.30	7.34	7.15	7.19
Final	8.04	8.06	8.08	8.01	8.04	8.16
Remarks						

2011. 7. 4.

Approved by

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COC code : BW-DBWT1104-KT-P003

Sheet of Raw Data – Aquatic plant

[1] COC Client	KT Marine co.,Ltd.	Test date	2011. 7.13 ~7.16
Lab. manager	Myung-Baek Shon	Observer	Myung-Baek Shon
Test organism	Skeletonema costatum	Test duration	72h
[2] COTS	0,6.25,12.5,25,50,100	Test substance	KTM-SSDS-3 (3-32psu)
Test Conditions and Methods			
Temperature	22 ℃	Salinity	21.7 psu
Light	3,100 Lux, LD cycle = 24 L : 0 D		
Medium	f/2	Sterile	filtered by 0.2μm membrane filter
Initial biomass	3,100 cells/mL	Vessel type and volume	15mL test tube
[3] MV / [4] VV	10 mL / 15 mL	Method of observation (SOP No.)	#TP-001 #TP-012

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] MV: Medium volume; [4] VV: Vessel volume

▶ Result

Density of phytoplankton (unit: μg/L) Date: 2011. 7.13. (Initial)

Replication	Concentration of test substance (%)						
	Control		6.25	12.5	25	50	100
	Cell density (cells/mL)	Chl-*a*					
1	564	1.15	0.57	1.33	0.35	0.45	0.37
2	511	0.64	0.26	0.41	0.51	0.44	0.48
3	448	1.02	0.57	1.33	0.42	0.60	0.60
4	518	1.41	0.56	0.86	0.38	0.35	0.70
Remarks	dilution factor : ×10			Chl-a estimate date : 7/14			

Density of phytoplankton (unit: μg/L) Date: 2011. 7.14 (24 hours)

Replication	Concentration of test substance (%)						
	Control		6.25	12.5	25	50	100
	Cell density (cells/mL)	Chl-*a*					
1	616	26.80	22.20	25.10	18.50	16.30	5.48
2	601	28.40	24.80	24.80	18.60	20.10	4.87
3	486	26.10	24.70	27.70	25.10	20.60	4.81
4	540	18.20	18.10	22.40	22.50	18.40	4.85
Remarks	dilution factor : ×100			Chl-a estimate date : 7/15			

Density of phytoplankton (unit: μ g/L) Date: 2011. 7.15. (48 hours)

Replication	Concentration of test substance (%)						
	Control		6.25	12.5	25	50	100
	Cell density (cells/mL)	Chl-*a*					
1	402	196.00	173.00	175.00	94.70	38.20	13.50
2	535	192.00	168.00	169.00	83.70	39.60	12.60
3	463	147.00	165.00	168.00	78.70	31.40	11.20
4	410	164.00	125.00	143.00	66.40	30.90	9.99
Remarks	dilution factor : x1.00 chl-a estimate date: 7/16						

Density of phytoplankton (unit: μ g/L) Date: 2011.7.16. (72 hours)

Replication	Concentration of test substance (%)						
	Control		6.25	12.5	25	50	100
	Cell density (cells/mL)	Chl-*a*					
1	273	235.00	239.00	220.00	54.30	16.40	6.79
2	270	249.00	249.00	225.00	59.10	19.70	6.48
3	254	218.00	261.00	244.00	61.50	20.00	7.90
4	289	252.00	247.00	236.00	59.40	18.80	8.32
Remarks	dilution factor : x2500 chl-a estimate date : 7/17						

Density of phytoplankton (unit: μ g/L) Date: 20 . . . (hours)

Replication	Concentration of test substance (%)						
	Control						
	Cell density (cells/mL)	Chl-*a*					
1							
2							
3							
4							
Remarks							

▶ pH determinations

Date : 2011.7.13 (Initial)

	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
Initial	7.69	7.68	7.74	7.73	7.72	7.68
Final	8.66	8.76	8.76	8.54	7.94	7.72
Remarks						

2011.7.18.

Approved by [signature]

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COC Code. BW-DBWT1104-KT-P001

Test date (start - end)	110630-110703	**Test substance**	KTM-S5D5-1 (>32PSU)
End point	Growth	**Inoculation density**	3,000 cells/mL
Study director	Myung - Baek Shon	**Replication**	4
Test Observer	Myung - Baek Shon	**Test duration**	72 h
Test organism	*Skeletonema costatum*	**Observe duration**	24 h
Light	Continuous, Cool white, 3000 lux	**Temperature**	22 ± 1 ℃

▶Chlorophyll a values (μ g/mL)of the each test concentration over the test period

Concentration (%)	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	1.40	8.75	143.00	233.00
	2	1.34	10.30	147.00	248.00
	3	1.34	10.10	150.00	255.00
	4	1.36	11.10	136.00	270.00
6.25	1	1.70	10.80	140.00	229.00
	2	1.15	12.50	163.00	241.00
	3	1.05	8.64	104.00	243.00
	4	1.45	11.20	120.00	263.00
12.5	1	1.20	10.50	134.00	245.00
	2	1.30	10.40	139.00	251.00
	3	1.22	10.60	135.00	252.00
	4	1.25	10.60	132.00	260.00
25	1	1.25	10.40	127.00	223.00
	2	1.30	10.60	137.00	230.00
	3	1.14	8.98	115.00	239.00
	4	1.19	7.59	98.60	243.00
50	1	1.23	8.78	109.00	180.00
	2	1.26	8.90	108.00	179.00
	3	1.23	9.98	109.00	187.00
	4	1.17	9.23	107.00	196.00
100	1	1.36	7.93	23.20	13.50
	2	1.17	8.90	24.00	12.60
	3	1.43	9.73	24.20	14.00
	4	1.23	10.00	23.50	13.40

▶Cell density (cells/mL)of control at each time(h)

Concentration	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	2,790	34,300	293,500	456,000
	2	3,500	27,800	356,500	516,000
	3	3,710	32,200	379,000	372,000
	4	3,800	34,700	308,000	424,000

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▶ **Cell density and chlorophyll a of control at each time (h)** ▶ **Transformed log_{10}**

Replications	Chl-a (μg/ml	Cell density (cells/mL)	Replications	Chl-a (μg/mL)	Cell density (cells/mL)
0	1.40	2,790	**0**	0.15	3.45
0	1.34	3,500	**0**	0.13	3.54
0	1.34	3,710	**0**	0.13	3.57
0	1.36	3,800	**0**	0.13	3.58
24	8.75	34,300	**24**	0.94	4.54
24	10.30	27,800	**24**	1.01	4.44
24	10.10	32,200	**24**	1.00	4.51
24	11.10	34,700	**24**	1.05	4.54
48	143.00	293,500	**48**	2.16	5.47
48	147.00	356,500	**48**	2.17	5.55
48	150.00	379,000	**48**	2.18	5.58
48	136.00	308,000	**48**	2.13	5.49
72	233.00	456,000	**72**	2.37	5.66
72	248.00	516,000	**72**	2.39	5.71
72	255.00	372,000	**72**	2.41	5.57
72	270.00	424,000	**72**	2.43	5.63

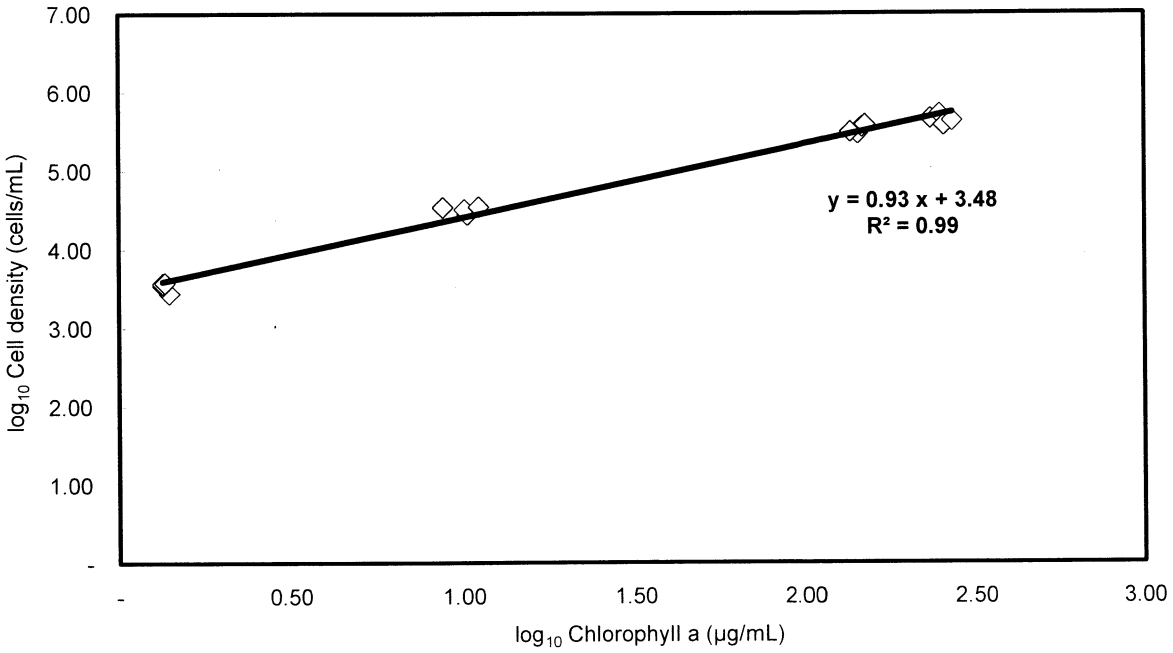

Figure. Cell density of *Skeletonema costatum* as a function of chlorophyll*a* in the control over the test period (n=16, P<0.001).

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▶ **Input data transformed from $\log_0$ Chl-a**

Concentration (%)	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	0.15	0.94	2.16	2.37
	2	0.13	1.01	2.17	2.39
	3	0.13	1.00	2.18	2.41
	4	0.13	1.05	2.13	2.43
6.25	1	0.23	1.03	2.15	2.36
	2	0.06	1.10	2.21	2.38
	3	0.02	0.94	2.02	2.39
	4	0.16	1.05	2.08	2.42
12.5	1	0.08	1.02	2.13	2.39
	2	0.11	1.02	2.14	2.40
	3	0.09	1.03	2.13	2.40
	4	0.10	1.03	2.12	2.41
25	1	0.10	1.02	2.10	2.35
	2	0.11	1.03	2.14	2.36
	3	0.06	0.95	2.06	2.38
	4	0.08	0.88	1.99	2.39
50	1	0.26	0.94	2.04	2.26
	2	0.10	0.95	2.03	2.25
	3	0.09	1.00	2.04	2.27
	4	0.07	0.97	2.03	2.29
100	1	0.13	0.90	1.37	1.13
	2	0.07	0.95	1.38	1.10
	3	0.16	0.99	1.38	1.15
	4	0.09	1.00	1.37	1.13

▶ **Output data through the equation from the linear regreession analysis ($\hat{y} = 0.93x + 3.48$)**

Concentration (%)	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	3.62	4.36	5.48	5.68
	2	3.60	4.42	5.50	5.71
	3	3.60	4.41	5.50	5.72
	4	3.60	4.45	5.46	5.74
6.25	1	3.69	4.44	5.48	5.67
	2	3.54	4.50	5.54	5.70
	3	3.50	4.35	5.36	5.70
	4	3.63	4.46	5.41	5.73
12.5	1	3.55	4.43	5.46	5.70
	2	3.59	4.43	5.47	5.71
	3	3.56	4.43	5.46	5.71
	4	3.57	4.43	5.45	5.73
25	1	3.57	4.43	5.44	5.66
	2	3.59	4.43	5.47	5.68
	3	3.53	4.37	5.40	5.69
	4	3.55	4.30	5.33	5.70
50	1	3.72	4.36	5.37	5.58
	2	3.57	4.36	5.37	5.58
	3	3.56	4.41	5.37	5.59
	4	3.54	4.38	5.37	5.61
100	1	3.60	4.32	4.75	4.53
	2	3.54	4.36	4.76	4.50
	3	3.62	4.40	4.77	4.55
	4	3.56	4.41	4.76	4.53

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▶ **Biomass (cells/mL) of *Skeletonema costatum* calculated by 10^x from output data**

Concentration	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	4,130	22,702	305,114	480,442
	2	3,965	26,420	313,044	509,143
	3	3,965	25,943	318,981	522,495
	4	4,020	28,324	291,200	551,021
6.25	1	4,947	27,611	299,157	472,767
	2	3,439	31,632	344,615	495,765
	3	3,160	22,437	226,903	499,590
	4	4,267	28,561	259,202	537,723
12.5	1	3,578	26,897	287,215	503,413
	2	3,854	26,659	297,169	514,869
	3	3,633	27,135	289,208	516,776
	4	3,716	27,135	283,226	532,017
25	1	3,716	26,659	273,236	461,236
	2	3,854	27,135	293,191	474,687
	3	3,411	23,257	249,143	491,938
	4	3,550	19,890	215,926	499,590
50	1	5,271	22,775	237,032	377,923
	2	3,744	23,064	235,009	375,970
	3	3,661	25,656	237,032	391,573
	4	3,495	23,858	232,984	409,070
100	1	4,020	20,717	56,222	33,979
	2	3,495	23,064	58,023	31,867
	3	4,212	25,058	58,472	35,148
	4	3,661	25,704	56,897	33,745

2011.7.6 Approved by

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COC Code. BW-DBWT1104-KT-P001

Test date (start - end)	110630-110703	**Test substar**	KTM-S5D5-1 (>32PSU)
End point	Growth	**Inoculation density**	3,000 cells/mL
Study director	Myung - Baek Shon	**Replication**	3
Test Observer	Myung - Baek Shon	**Test duration**	72 h
Test organism	*Skeletonema costatum*	**Observe duration**	24 h
Light	Continuous, Cool white, 3000 lux	**Temperature**	22 ± 1℃

■ **Raw Data**

▶ **Cell Density (cells/ml)**

Concentration	**Control**				**6.25%**			
Elapsed time (h)	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	3,965	3,965	4,020	3,983	3,439	3,160	4,267	3,622
24	26,420	25,943	28,324	26,896	31,632	22,437	28,561	27,543
48	313,044	318,981	291,200	307,742	344,615	226,903	259,202	276,907
72	509,143	522,495	551,021	527,553	495,765	499,590	537,723	511,026
96								

Concentration	**12.50%**				**25.00%**			
Elapsed time (h)	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	3,854	3,633	3,716	3,735	3,854	3,411	3,550	3,605
24	26,659	27,135	27,135	26,976	27,135	23,257	19,890	23,427
48	297,169	289,208	283,226	289,868	293,191	249,143	215,926	252,753
72	514,869	516,776	532,017	521,221	474,687	491,938	499,590	488,738
96								

Concentration	**50.00%**				**100.00%**			
Elapsed time (h)	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	3,744	3,661	3,495	3,633	4,020	4,212	3,661	3,964
24	23,064	25,656	23,858	24,193	20,717	25,058	25,704	23,826
48	235,009	237,032	232,984	235,008	56,222	58,472	56,897	57,197
72	375,970	391,573	409,070	392,205	33,979	35,148	33,745	34,291
96								

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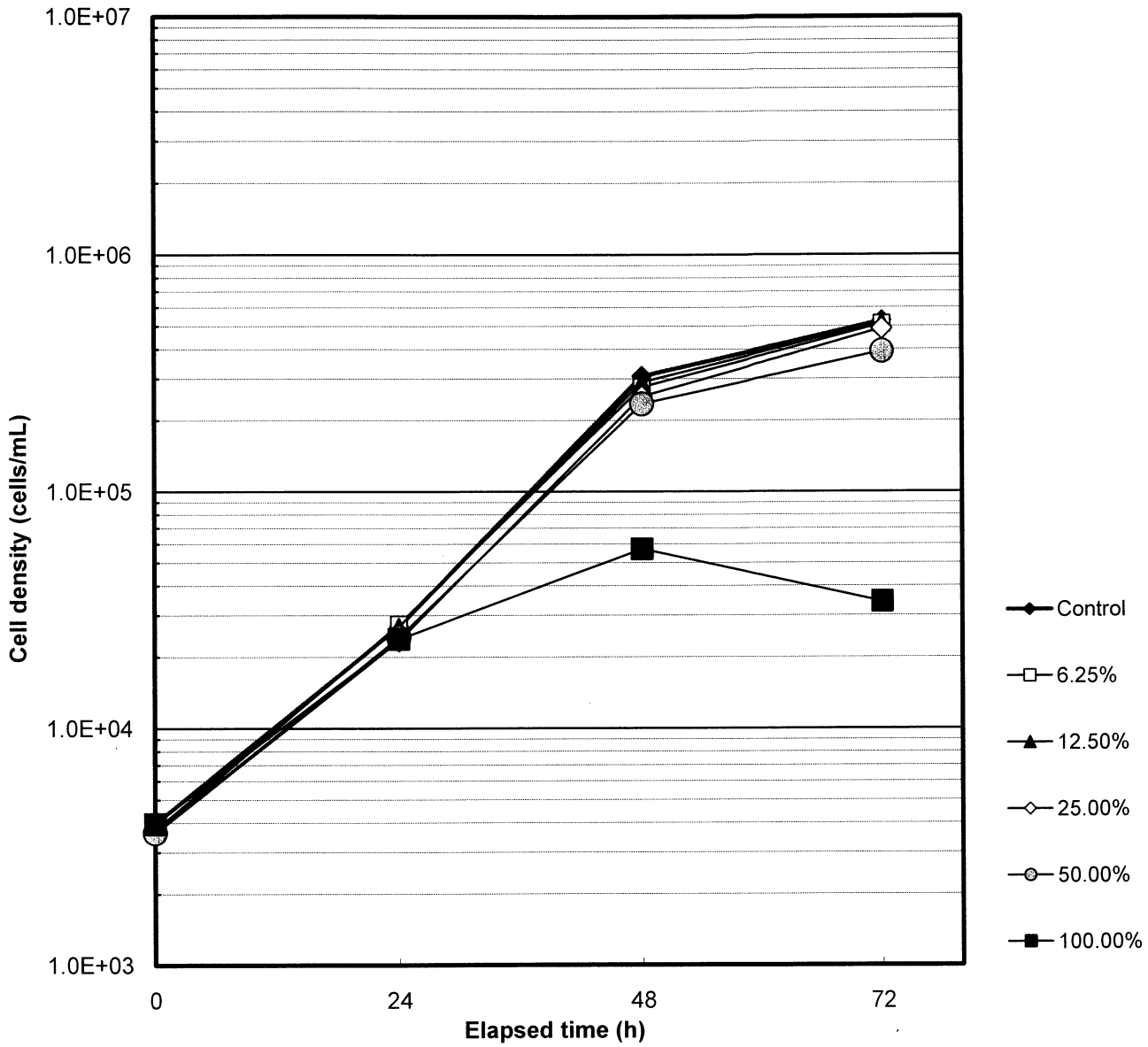

Fig. 1. Growth curves of *Skeletonema costatum* in the each test concentrations

▶ pH

Concentration	Concentration					
Elapsed time (h)	Control	6.25	12.5	25	50	100
0	7.25	7.29	7.30	7.34	7.15	7.19
72	8.94	8.96	8.98	9.01	8.94	8.16

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▶ **Average growth rate**

Concentration	Control				6.25%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	1.90	1.88	1.95	1.91	2.22	1.96	1.90	2.03
48	2.18	2.19	2.14	2.17	2.30	2.14	2.05	2.16
72	1.62	1.63	1.64	1.63	1.66	1.69	1.61	1.65
96								

Concentration	12.50%				25.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	1.93	2.01	1.99	1.98	1.95	1.92	1.72	1.86
48	2.17	2.19	2.17	2.18	2.17	2.15	2.05	2.12
72	1.63	1.65	1.65	1.65	1.60	1.66	1.65	1.64
96								

Concentration	50.00%				100.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	1.82	1.95	1.92	1.90	1.64	1.78	1.95	1.79
48	2.07	2.09	2.10	2.08	1.32	1.32	1.37	1.34
72	1.54	1.56	1.59	1.56	0.71	0.71	0.74	0.72
96								

▶ **Coefficient of variation (%)**

Elapsed time (h)	Concentration (%)					
	Control	6.25	12.5	25	50	100
24	2.0	8.3	2.0	6.6	3.6	8.6
48	1.3	5.9	0.5	2.8	0.7	2.4
72	0.7	2.3	0.8	1.7	1.6	2.5
mean	1.3	5.5	1.1	3.7	2.0	4.5

▶ **Inhibition of growth rate (*%Ir*)**

Elapsed time (h)	Concentration of substance				
	6.25%	12.50%	25.00%	50.00%	100.00%
24	-6.16	-3.58	2.33	0.73	6.21
48	0.39	-0.12	2.37	4.06	38.55
72	-1.46	-1.08	-0.51	4.18	55.81
96					

▶ **Yield (*%Iy*)**

Elapsed time (h)	Concentration of substance				
	6.25%	12.50%	25.00%	50.00%	100.00%
72	3.09	1.16	7.34	25.78	94.21

Concentration	Control				6.25%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
72					5.97	5.18	-1.89	3.09

Concetration	12.50%				25.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
72	2.40	1.99	-0.90	1.16	10.07	6.69	5.26	7.34

Concentration	50.00%				100.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
72	28.91	25.91	22.54	25.78	94.28	94.09	94.25	94.21

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▶ **Growth rate (% of control)**

Elapsed time (h)	Concentration of substance					
	0.00%	6.25%	12.50%	25.00%	50.00%	100.00%
24	100	106	104	98	99	94
48	100	100	100	98	96	61
72	100	101	101	101	96	44
96						

▶ **Average growth rate (% of control)**

Concentration	Control				6.25%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	100.00	100.00	100.00	100.00	116.22	102.66	99.58	106.16
48	100.00	100.00	100.00	100.00	106.00	98.33	94.49	99.61
72	100.00	100.00	100.00	100.00	101.74	103.63	98.99	101.46
96								

Concentration	12.50%				25.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	101.29	105.31	104.13	103.58	102.22	100.54	90.26	97.67
48	99.97	100.70	99.70	100.12	99.66	98.72	94.51	97.63
72	100.18	101.47	101.60	101.08	98.52	101.75	101.25	100.51
96								

Concentration	50.00%				100.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	95.23	101.98	100.61	99.27	85.89	93.40	102.08	93.79
48	95.24	95.95	96.62	95.94	60.70	60.52	63.12	61.45
72	94.34	95.63	97.48	95.82	43.69	43.43	45.46	44.19
96								

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▶ **Growth rate (0~72h)**

Replication	Concentration (In x%)					
	1.0	6.3	12.5	25.0	50.0	100.0
1	100.0	101.7	100.2	98.5	94.3	43.7
2	100.0	103.6	101.5	101.8	95.6	43.4
3	100.0	99.0	101.6	101.3	97.5	45.5
Mean	100.0	101.5	101.1	100.5	95.8	44.2

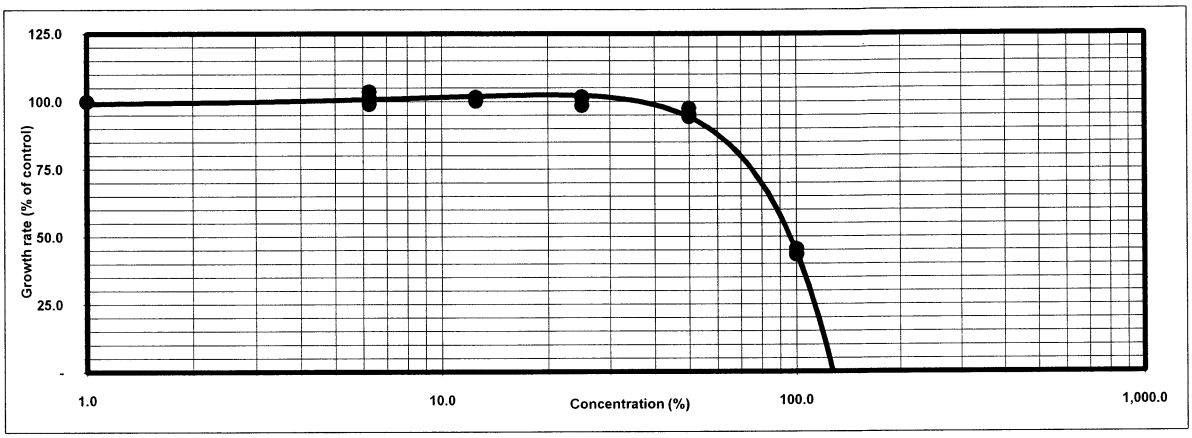

Fig. 2. Graph of concentration-response

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COC Code. BW-DBWT1104-KT-P003

Test date (start - end)	110713-110716	**Test substance**	KTM-S5D5-3 (3-32PSU)
End point	Growth	**Inoculation density**	3,000 cells/mL
Study director	Myung - Baek Shon	**Replication**	4
Test Observer	Myung - Baek Shon	**Test duration**	72 h
Test organism	*Skeletonema costatum*	**Observe duration**	24 h
Light	Continuous, Cool white, 3000 lux	**Temperature**	22 ± 1 ℃

▶Chlorophyll a values (μ g/mL)of the each test concentration over the test period

Concentration (%)	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	1.15	26.80	196.00	235.00
	2	0.64	29.40	192.00	249.00
	3	1.02	26.10	147.00	218.00
	4	1.41	18.20	164.00	252.00
6.25	1	0.57	22.20	173.00	239.00
	2	0.26	24.80	168.00	249.00
	3	0.57	24.70	165.00	261.00
	4	0.56	18.00	125.00	247.00
12.5	1	1.33	25.00	175.00	220.00
	2	0.41	24.90	169.00	225.00
	3	1.33	27.70	168.00	244.00
	4	0.96	22.40	143.00	236.00
25	1	0.35	19.50	94.70	54.30
	2	0.51	19.60	83.70	59.10
	3	0.42	25.00	78.70	61.50
	4	0.39	22.50	66.40	59.40
50	1	0.45	16.30	38.20	16.40
	2	0.44	20.10	39.60	19.70
	3	0.60	20.60	31.40	20.00
	4	0.35	19.40	30.90	18.80
100	1	0.37	5.48	13.50	6.79
	2	0.49	4.97	12.60	6.48
	3	0.60	4.81	11.20	7.90
	4	0.70	4.95	9.99	8.32

▶Cell density (cells/mL)of control at each time(h)

Concentration	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	5.640	61.600	402.000	682.500
	2	5.110	60.100	535.000	675.000
	3	4.490	48.600	463.000	635.000
	4	5.180	54.000	410.000	722.500

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▶ **Cell density and chlorophyll a of control at each time (h)** ▶ **Transformed log_{10}**

Replications	Chl-a (μg/ml	Cell density (cells/mL)	Replications	Chl-a (μg/mL)	Cell density (cells/mL)
0	1.15	5,640	**0**	0.06	3.75
0	0.64	5,110	**0**	- 0.19	3.71
0	1.02	4,490	**0**	0.01	3.65
0	1.41	5,180	**0**	0.15	3.71
24	26.80	61,600	**24**	1.43	4.79
24	29.40	60,100	**24**	1.47	4.78
24	26.10	48,600	**24**	1.42	4.69
24	18.20	54,000	**24**	1.26	4.73
48	196.00	402,000	**48**	2.29	5.60
48	192.00	535,000	**48**	2.28	5.73
48	147.00	463,000	**48**	2.17	5.67
48	164.00	410,000	**48**	2.21	5.61
72	235.00	682,500	**72**	2.37	5.83
72	249.00	675,000	**72**	2.40	5.83
72	218.00	635,000	**72**	2.34	5.80
72	252.00	722,500	**72**	2.40	5.86

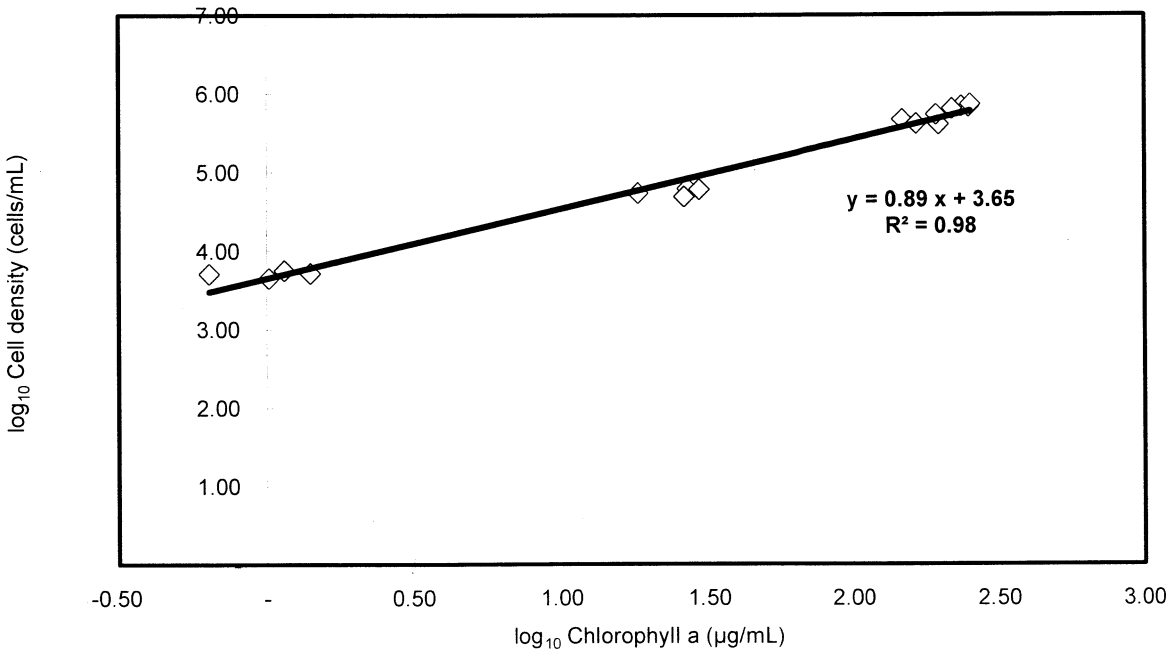

Figure. Cell density of *Skeletonema costatum* as a function of chlorophyll*a* in the control over the test period (n=16, P<0.001).

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▶ **Input data transformed from $\log_{10}$ Chl-a**

Concentration (%)	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	0.06	1.43	2.29	2.37
	2	-0.19	1.47	2.28	2.40
	3	0.01	1.42	2.17	2.34
	4	0.15	1.26	2.21	2.40
6.25	1	-0.24	1.35	2.24	2.38
	2	-0.59	1.39	2.23	2.40
	3	-0.24	1.39	2.22	2.42
	4	-0.25	1.26	2.10	2.39
12.5	1	0.12	1.40	2.24	2.34
	2	-0.39	1.40	2.23	2.35
	3	0.12	1.44	2.23	2.39
	4	-0.02	1.35	2.16	2.37
25	1	-0.46	1.29	1.98	1.73
	2	-0.29	1.29	1.92	1.77
	3	-0.38	1.40	1.90	1.79
	4	-0.41	1.35	1.82	1.77
50	1	-0.35	1.21	1.58	1.21
	2	-0.36	1.30	1.60	1.29
	3	-0.22	1.31	1.50	1.30
	4	-0.46	1.29	1.49	1.27
100	1	-0.43	0.74	1.13	0.83
	2	-0.31	0.70	1.10	0.81
	3	-0.22	0.68	1.05	0.90
	4	-0.15	0.69	1.00	0.92

▶ **Output data through the equation from the linear regreession analysis ($y = 0.89x + 3.65$)**

Concentration (%)	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	3.70	4.92	5.69	5.76
	2	3.48	4.96	5.68	5.78
	3	3.66	4.91	5.58	5.73
	4	3.78	4.77	5.62	5.79
6.25	1	3.43	4.85	5.64	5.77
	2	3.13	4.89	5.63	5.78
	3	3.43	4.89	5.62	5.80
	4	3.43	4.77	5.52	5.78
12.5	1	3.76	4.89	5.65	5.73
	2	3.31	4.89	5.63	5.74
	3	3.76	4.93	5.63	5.77
	4	3.63	4.85	5.57	5.76
25	1	3.24	4.80	5.41	5.19
	2	3.39	4.80	5.36	5.23
	3	3.31	4.89	5.34	5.24
	4	3.29	4.85	5.27	5.23
50	1	3.34	4.73	5.06	4.73
	2	3.33	4.81	5.07	4.80
	3	3.45	4.82	4.98	4.81
	4	3.24	4.80	4.98	4.78
100	1	3.27	4.31	4.66	4.39
	2	3.37	4.27	4.63	4.37
	3	3.45	4.26	4.58	4.45
	4	3.51	4.27	4.54	4.47

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▶ **Biomass (cells/mL) of *Skeletonema costatum* calculated by 10^x from output data**

Concentration	Replication	Elapsed time (h)			
		0	24	48	72
Control	1	5,058	83,376	489,901	575,772
	2	3,003	90,538	480,992	606,202
	3	4,546	81,435	379,239	538,550
	4	6,065	59,083	418,033	612,698
6.25	1	2,708	70,511	438,390	584,486
	2	1,347	77,815	427,096	606,202
	3	2,708	77,535	420,301	632,135
	4	2,666	58,505	328,284	601,867
12.5	1	5,757	78,373	442,898	542,945
	2	2,020	78,094	479,358	553,914
	3	5,757	85,863	427,096	595,356
	4	4,307	71,076	370,040	577,952
25	1	1,755	62,825	256,420	156,305
	2	2,453	63,111	229,734	168,544
	3	2,064	78,373	217,479	174,622
	4	1,932	71,358	186,952	169,305
50	1	2,195	53,561	114,297	53,853
	2	2,151	64,542	118,018	63,398
	3	2,835	65,969	95,999	64,256
	4	1,755	62,538	94,637	60,814
100	1	1,844	20,301	45,289	24,568
	2	2,367	18,610	42,592	23,567
	3	2,835	18,076	38,353	28,112
	4	3,252	18,544	34,643	29,438

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COC Code. BW-DBWT1104-KT-P003

Test date (start - end)	110713-110716	**Test substance**	KTM-S5D5-3 (3-32PSU)
End point	Growth	**Inoculation density**	3,000 cells/mL
Study director	Myung - Baek Shon	**Replication**	3
Test Observer	Myung - Baek Shon	**Test duration**	72 h
Test organism	*Skeletonema costatum*	**Observe duration**	24 h
Light	Continuous, Cool white, 3000 lux	**Temperature**	22 ± 1℃

■ **Raw Data**

▶ **Cell Density (cells/ml)**

Concentration	**Control**				**6.25%**			
Elapsed time (h)	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	5,058	3,003	4,546	4,202	2,708	2,708	2,666	2,694
24	83,376	90,538	81,435	85,116	70,511	77,535	58,505	68,850
48	489,901	480,992	379,239	450,044	438,390	420,301	328,284	395,659
72	575,772	606,202	538,550	573,508	584,486	632,135	601,867	606,163
96								

Concentration	**12.50%**				**25.00%**			
Elapsed time (h)	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	5,757	5,757	4,307	5,274	2,453	2,064	1,932	2,150
24	78,373	85,863	71,076	78,437	63,111	78,373	71,358	70,947
48	442,898	427,096	370,040	413,345	229,734	217,479	186,952	211,388
72	542,945	595,356	577,952	572,085	168,544	174,622	169,305	170,824
96								

Concentration	**50.00%**				**100.00%**			
Elapsed time (h)	**1**	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
0	2,195	2,151	2,835	2,394	2,367	2,835	3,252	2,818
24	53,561	64,542	65,969	61,357	18,610	18,076	18,544	18,410
48	114,297	118,018	95,999	109,438	42,592	38,353	34,643	38,529
72	53,853	63,398	64,256	60,502	23,567	28,112	29,438	27,039
96								

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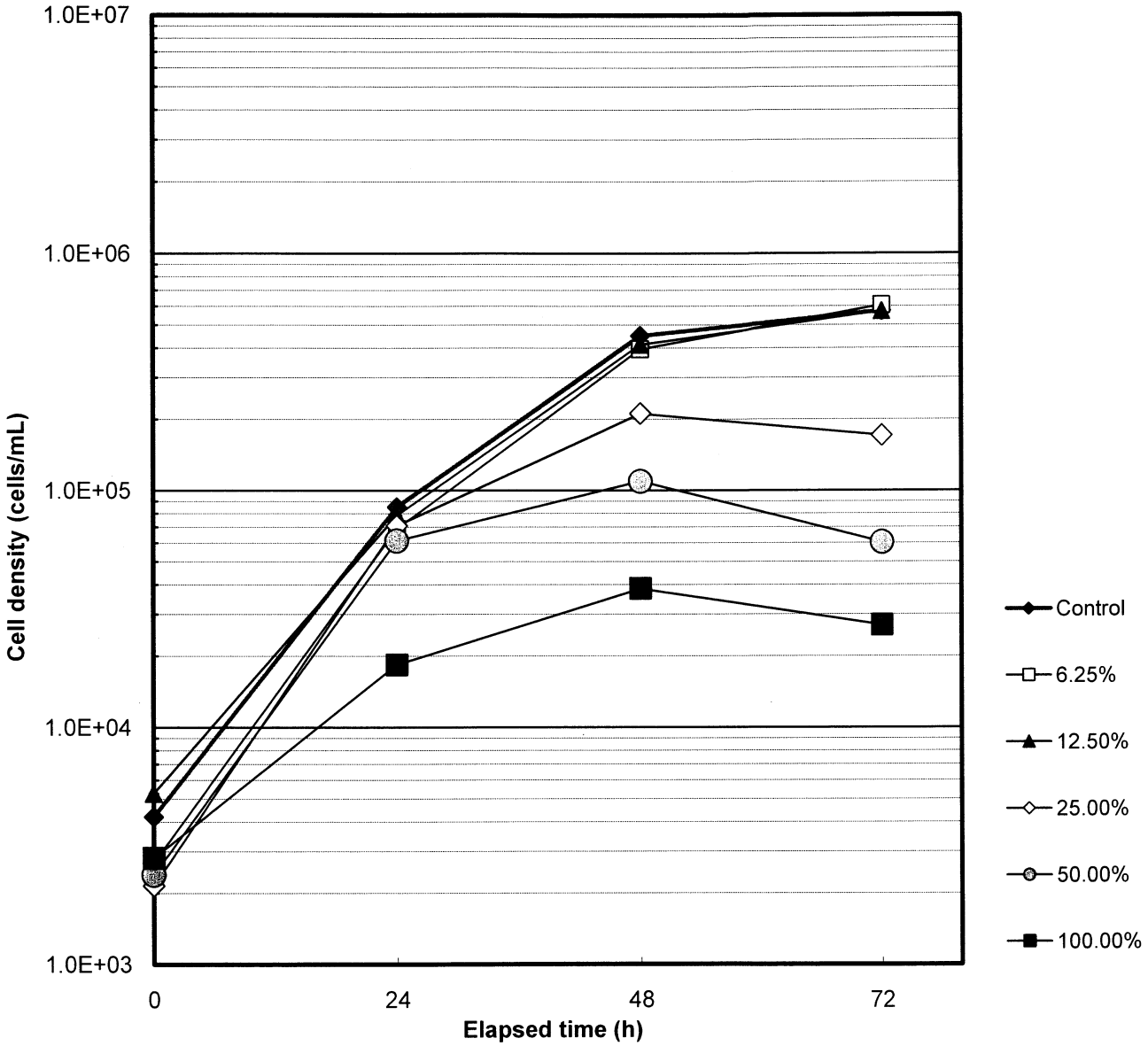

Fig. 1. Growth curves of *Skeletonema costatum* in the each test concentrations

▶ pH

Concentration	Concentration					
Elapsed time (h)	Control	6.25	12.5	25	50	100
0	7.69	7.68	7.74	7.73	7.72	7.68
72	8.66	8.76	8.76	8.54	7.94	7.72

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▶ Average growth rate

Concentration	Control				6.25%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	2.80	3.41	2.89	3.03	3.26	3.35	3.09	3.23
48	2.29	2.54	2.21	2.35	2.54	2.52	2.41	2.49
72	1.58	1.77	1.59	1.65	1.79	1.82	1.81	1.81
96								

Concentration	12.50%				25.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	2.61	2.70	2.80	2.71	3.25	3.64	3.61	3.50
48	2.17	2.15	2.23	2.18	2.27	2.33	2.29	2.29
72	1.52	1.55	1.63	1.56	1.41	1.48	1.49	1.46
96								

Concentration	50.00%				100.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	3.19	3.40	3.15	3.25	2.06	1.85	1.74	1.89
48	1.98	2.00	1.76	1.91	1.44	1.30	1.18	1.31
72	1.07	1.13	1.04	1.08	0.77	0.76	0.73	0.76
96								

▶ Coefficient of variation (%)

Elapsed time (h)	Concentration (%)					
	Control	6.25	12.5	25	50	100
24	10.8	4.2	3.6	6.2	4.2	8.6
48	7.3	3.0	1.7	1.3	6.9	10.0
72	6.5	0.7	3.9	3.0	4.2	2.4
mean	8.2	2.6	3.1	3.5	5.1	7.0

▶ Inhibition of growth rate (*%Ir*)

Elapsed time (h)	Concentration of substance				
	6.25%	12.50%	25.00%	50.00%	100.00%
24	-6.69	10.75	-15.39	-7.14	37.81
48	-6.19	6.90	2.16	18.43	44.15
72	-9.65	4.94	11.31	34.50	54.14
96					

▶ Yield (*%Iy*)

Elapsed time (h)	Concentration of substance				
	6.25%	12.50%	25.00%	50.00%	100.00%
72	-6.00	0.44	70.37	89.79	95.75

Concentration	Control				6.25%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
72					-2.19	-10.56	-5.25	-6.00

Concetration	12.50%				25.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
72	5.64	-3.56	-0.76	0.44	70.83	69.69	70.60	70.37

Concentration	50.00%				100.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
72	90.93	89.24	89.21	89.79	96.28	95.56	95.40	95.75

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▶ **Growth rate (% of control)**

Elapsed time (h)	Concentration of substance					
	0.00%	6.25%	12.50%	25.00%	50.00%	100.00%
24	100	107	89	115	107	62
48	100	106	93	98	82	56
72	100	110	95	89	65	46
96						

▶ **Average growth rate (% of control)**

Concentration	Control				6.25%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	100.00	100.00	100.00	100.00	107.52	110.65	101.88	106.69
48	100.00	100.00	100.00	100.00	108.43	107.53	102.60	106.19
72	100.00	100.00	100.00	100.00	108.81	110.40	109.73	109.65
96								

Concentration	12.50%				25.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	86.13	89.14	92.48	89.25	107.13	119.98	119.06	115.39
48	92.58	91.80	94.93	93.10	96.77	99.28	97.47	97.84
72	92.05	93.92	99.19	95.06	85.64	89.86	90.57	88.69
96								

Concentration	50.00%				100.00%			
Elapsed time (h)	1	2	3	Mean	1	2	3	Mean
24	105.39	112.20	103.82	107.14	68.02	61.11	57.43	62.19
48	84.26	85.37	75.08	81.57	61.60	55.53	50.43	55.85
72	64.80	68.50	63.19	65.50	46.53	46.45	44.61	45.86
96								

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▶ **Growth rate (0~72h)**

Replication	Concentration (ln x%)					
	1.0	6.3	12.5	25.0	50.0	100.0
1	100.0	108.8	92.1	85.6	64.8	46.5
2	100.0	110.4	93.9	89.9	68.5	46.4
3	100.0	109.7	99.2	90.6	63.2	44.6
Mean	100.0	109.6	95.1	88.7	65.5	45.9

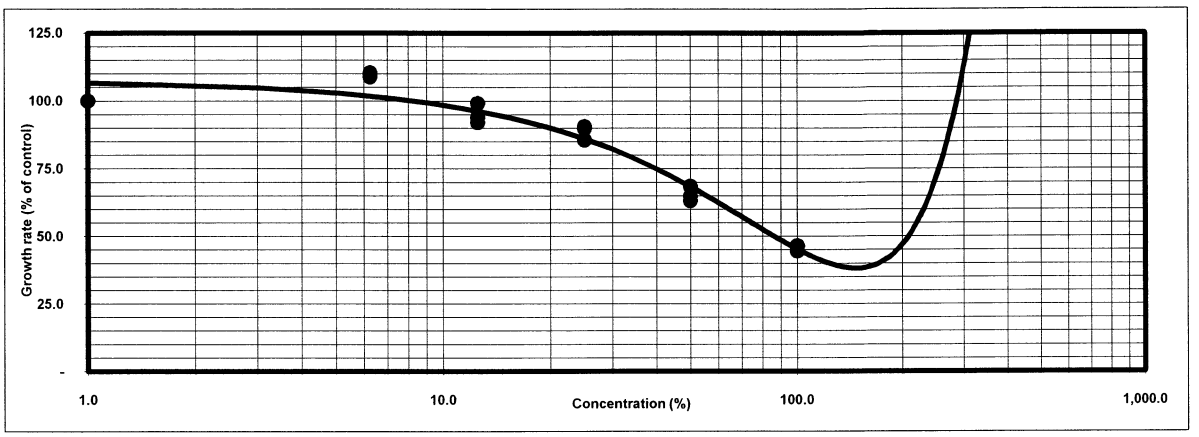

Fig. 2. Graph of concentration-response

20 11 . 7 . 18 Approved by

4.1.1.2 ANNEX 2

Sheet of raw data for

"*Brachionus plicatilis*, Acute Toxicity Test"

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COC code : BW-DBWT 1104-KT-I001

Sheet of Raw Data – Rotifer – Acute toxicity

[1] COC Client	KTM	Test date	2011. 6.30 ~ 7.1
Lab. manager	Myung - Baek Shon	Observer	So Eun Jo
Test organism	Brachionus plicatilis	Test duration	24h
[2] COTS	control, 6.25%, 12.5%, 25%, 50%, 100%	Test substance	KTM - SSDS-1 (> 32psu)

Test Conditions and Methods

Temperature	25±1 ℃	Salinity	27±1 psu	Light	0 Lux, LD cycle = 0 L : 24 D
Medium	Filtered Seawater (0.45 μm) or other ( )				
[3] No. Inds.	5 inds.	Vessel type and volume	48 well plate		
[4] MV / [5] VV	1 mL / 1.5 mL	Method of observation (SOP No.)	#TP-013		

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] No. Inds.: Number of individuals/replication; [4] MV: Medium volume; [5] VV: Vessel volume

▶ Results (Accumulated Number of Dead Organisms)

Date: 2011. 7. 1 .(1 days)

Repls.	Concentration of test substance (%)						
	Control	6.25	12.5	25	50	100	
1	0	0	0	0	0	0	
2	0	0	0	0	0	0	
3	0	0	0	0	0	0	
4	0	0	0	0	0	0	
5	0	0	0	0	0	0	
6	0	0	0	0	0	0	
7	0	0	0	0	0	0	
8	0	0	0	0	0	0	
9	0	0	0	0	0	0	
10	0	0	0	0	0	0	
11	0	0	0	0	0	0	
12	0	0	0	0	0	0	

▶ Routine chemical and physical determinations

Terms		Concentration of test substance (%)					
		Control	6.25	12.5	25	50	100
Temp. (℃)	Initial	25.4	25.1	24.7	24.3	24.3	24.8
	Final	25.2	25.3	25.6	25.5	25.5	25.5
DO (mg/L)	Initial	4.16	4.38	4.17	4.21	4.56	4.64
	Final	3.77	3.41	3.54	4.04	4.28	3.94
pH	Initial	7.47	7.74	7.73	7.85	7.91	7.71
	Final	7.58	7.73	7.76	7.80	7.83	7.83

2011. 7. 4

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COC code : BW-DBWT1104-KT-I003

Sheet of Raw Data – Rotifer – Acute toxicity

[1] COC Client	KTM	Test date	2011.7.13 ~ 7.14
Lab. manager	Myung-Back Shon	Observer	So Eun Jo
Test organism	Brachionus plicatilis	Test duration	24h
[2] COTS	Control, 6.25, 12.5, 25, 50, 100 %	Test substance	KTM-SSD5-3 (3-32psu)

Test Conditions and Methods

Temperature	25±1 ℃	Salinity	21±1 psu	Light	0 Lux, LD cycle = 0 L : 24 D
Medium	Filtered Seawater (0.45 μm) or other ( )				
[3] No. Inds.	5 inds.	Vessel type and volume	48 well plate		
[4] MV / [5] VV	1 mL / 1.5 mL	Method of observation (SOP No.)	#TP-013		

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] No. Inds.: Number of individuals/replication; [4] MV: Medium volume; [5] VV: Vessel volume

▶ Results (Accumulated Number of Dead Organisms)

Date: 2011. 7. 14.(1 days)

Repls.	Concentration of test substance (%)						
	Control	6.25	12.5	25	50	100	
1	0	0	0	0	0	0	
2	0	0	0	0	0	0	
3	0	0	0	0	0	0	
4	0	0	0	0	0	0	
5	0	0	0	0	0	0	
6	0	0	0	0	0	0	
7	0	0	0	0	0	0	
8	0	0	0	0	1	0	
9	0	0	0	0	0	0	
10	0	0	0	0	0	0	
11	0	0	0	0	0	1	
12	0	0	0	0	0	0	

▶ Routine chemical and physical determinations

Terms		Concentration of test substance (%)					
		Control	6.25	12.5	25	50	100
Temp. (℃)	Initial	24.3	24.5	25.6	25.0	25.1	25.3
	Final	25.3	25.4	24.6	25.6	25.5	25.3
DO (mg/L)	Initial	7.70	7.31	7.10	6.63	6.28	6.66
	Final	6.16	6.54	5.88	5.91	5.98	5.85
pH	Initial	7.61	7.57	7.54	7.47	7.43	7.48
	Final	7.76	7.85	7.82	7.84	7.85	7.84

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COC Code. BW-DBWT1104-KT-I001

Test date	110630-110701	Test substance	KTM-S5D5-1(> 32 PSU)
End point	Survival	Numbers of Initial individual	5 inds.
Study director	Jung-Im Park	Numbers of replications	12 repl.
Test Observer	So Eun Jo	Test duration	24h
Test organism	Rotifer, *Brachionus plicatilis*	Observe duration	24h
Light	Drkness	Temperature	25±1℃

■ **Raw Data**

Concentration of substance	Control				6.25%				12.50%			
Elapsed time (h)	0		24		0		24		0		24	
Replication	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead
1	5	0	5	0	5	0	5	0	5	0	5	0
2	5	0	5	0	5	0	5	0	5	0	5	0
3	5	0	5	0	5	0	5	0	5	0	5	0
4	5	0	5	0	5	0	5	0	5	0	5	0
5	5	0	5	0	5	0	5	0	5	0	5	0
6	5	0	5	0	5	0	5	0	5	0	5	0
7	5	0	5	0	5	0	5	0	5	0	5	0
8	5	0	5	0	5	0	5	0	5	0	5	0
9	5	0	5	0	5	0	5	0	5	0	5	0
10	5	0	5	0	5	0	5	0	5	0	5	0
11	5	0	5	0	5	0	5	0	5	0	5	0
12	5	0	5	0	5	0	5	0	5	0	5	0
Mean	5	0	5	0	5	0	5	0	5	0	5	0
Concentration of substance	25.00%				50.00%				100.00%			
Elapsed time (h)	0		24		0		24		0		24	
Replication	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead	Total	Dead
1	5	0	5	0	5	0	5	0	5	0	5	0
2	5	0	5	0	5	0	5	0	5	0	5	0
3	5	0	5	0	5	0	5	0	5	0	5	0
4	5	0	5	0	5	0	5	0	5	0	5	0
5	5	0	5	0	5	0	5	0	5	0	5	0
6	5	0	5	0	5	0	5	0	5	0	5	0
7	5	0	5	0	5	0	5	0	5	0	5	0
8	5	0	5	0	5	0	5	0	5	0	5	0
9	5	0	5	0	5	0	5	0	5	0	5	0
10	5	0	5	0	5	0	5	0	5	0	5	0
11	5	0	5	0	5	0	5	0	5	0	5	0
12	5	0	5	0	5	0	5	0	5	0	5	0
Mean	5	0	5	0	5	0	5	0	5	0	5	0

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▶ Survival rate (SR, %)

Concentration of substance	Control				6.25%				12.50%			
Elapsed time (h)	0		24		0		24		0		24	
Replication	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR
1	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
2	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
3	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
4	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
5	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
6	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
7	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
8	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
9	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
10	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
11	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
12	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Mean	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Concentration of substance	25.00%				50.00%				100.00%			
Elapsed time (h)	0		24		0		24		0		24	
Replication	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR
1	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
2	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
3	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
4	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
5	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
6	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
7	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
8	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
9	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
10	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
11	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
12	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Mean	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0

Concentration of substance	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
Elapsed time (h)	0	24	0	24	0	24	0	24	0	24	0	24
SR (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
STDEV (±)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

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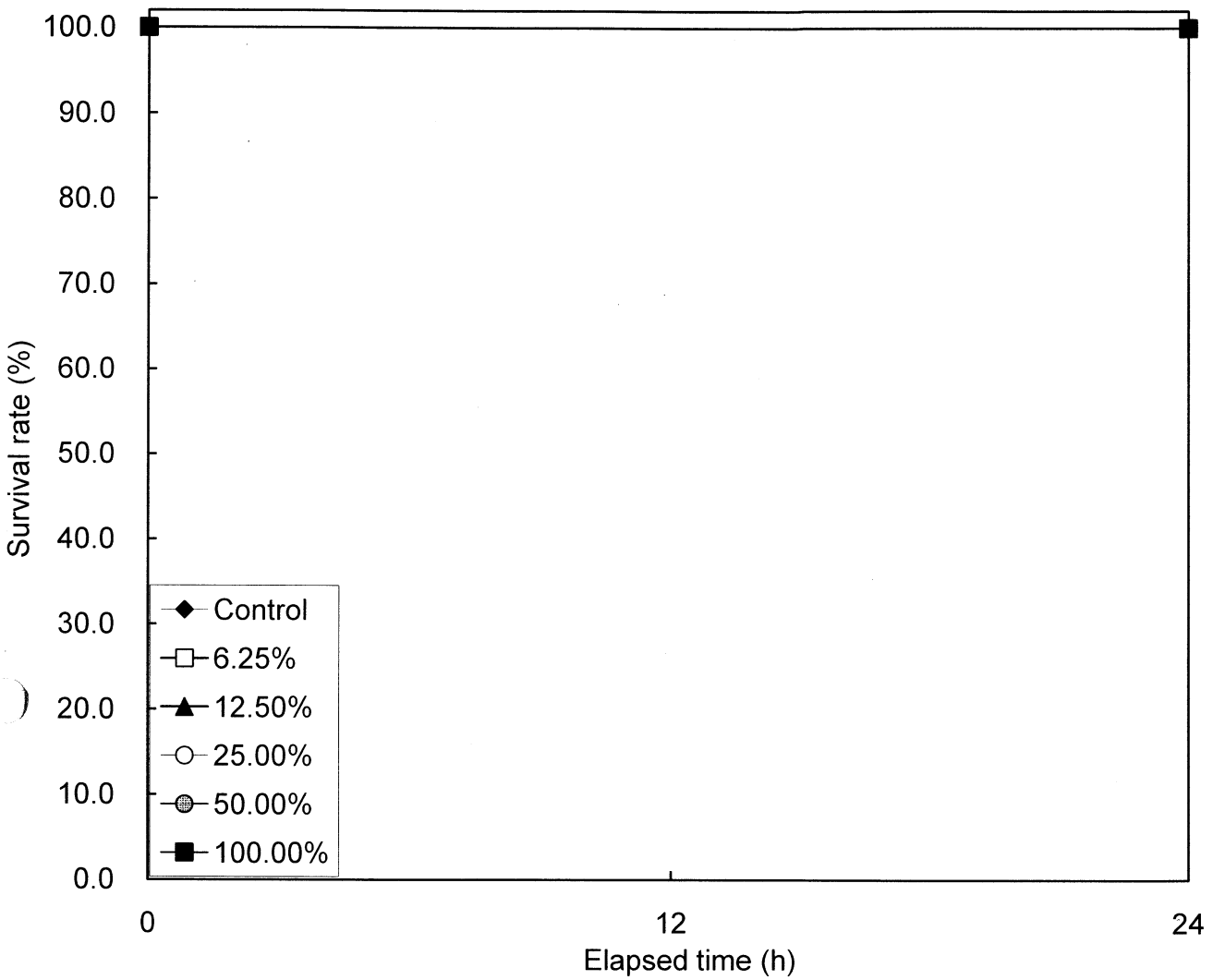

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COC Code. BW-DBWT1104-KT-I001

Test date	110630-110701	Test substance	KTM-S5D5-1(> 32 PSU)
Object of test	Survival	Numbers of Initial individual	5 inds.
Study director	Jung-Im Park	Numbers of groups	12 repl.
Test Observer	So Eun Jo	Test duration	24 h
Test organism	Rotifer, *Brachionus plicatilis*	Observe duration	24 h
Light	Darkness	Temperature	25 ± 1 ℃

▶ DO (mg/L)

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	4.16	4.38	4.17	4.21	4.56	4.64
Final	3.77	3.41	3.54	4.04	4.28	3.94

▶ pH

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	7.47	7.74	7.73	7.85	7.91	7.71
Final	7.58	7.73	7.76	7.80	7.83	7.83

▶ Temparature (℃)

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	25.40	25.10	24.70	24.30	24.30	24.80
Final	25.20	25.30	25.60	25.50	25.50	25.50

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COC Code. BW-DBWT1104-KT-I003

Test date	110713-110714	**Test substance**	KTM-S5D5-3(3-32 psu)
[illegible] point	Survival	**Numbers of Initial individual**	5 inds.
Study director	Jung-Im Park	**Numbers of replications**	12 repl.
Test Observer	So Eun Jo	**Test duration**	96h
Test organism	Rotifer, *Brachionus plicatilis*	**Observe duration**	24h
Light	Drkness	**Temperature**	25±1℃

■ **Raw Data**

Concentration of substance	**Control**				**6.25%**				**12.50%**			
Elapsed time (h)	**0**		**24**		**0**		**24**		**0**		**24**	
Replication	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**
1	5	0	5	0	5	0	5	0	5	0	5	0
2	5	0	5	0	5	0	5	0	5	0	5	0
3	5	0	5	0	5	0	5	0	5	0	5	0
4	5	0	5	0	5	0	5	0	5	0	5	0
5	5	0	5	0	5	0	5	0	5	0	5	0
6	5	0	5	0	5	0	5	0	5	0	5	0
7	5	0	5	0	5	0	5	0	5	0	5	0
8	5	0	5	0	5	0	5	0	5	0	5	0
9	5	0	5	0	5	0	5	0	5	0	5	0
10	5	0	5	0	5	0	5	0	5	0	5	0
11	5	0	5	0	5	0	5	0	5	0	5	0
12	5	0	5	0	5	0	5	0	5	0	5	0
Mean	5	0	5	0	5	0	5	0.00	5	0	5	0.00

Concentration of substance	**25.00%**				**50.00%**				**100.00%**			
Elapsed time (h)	**0**		**24**		**0**		**24**		**0**		**24**	
Replication	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**	**Total**	**Dead**
1	5	0	5	0	5	0	5	0	5	0	5	0
2	5	0	5	0	5	0	5	0	5	0	5	0
3	5	0	5	0	5	0	5	0	5	0	5	0
4	5	0	5	0	5	0	5	0	5	0	5	0
5	5	0	5	0	5	0	5	0	5	0	5	0
6	5	0	5	0	5	0	5	0	5	0	5	0
7	5	0	5	0	5	0	5	0	5	0	5	0
8	5	0	5	0	5	0	5	1	5	0	5	0
9	5	0	5	0	5	0	5	0	5	0	5	0
10	5	0	5	0	5	0	5	0	5	0	5	0
11	5	0	5	0	5	0	5	0	5	0	5	1
12	5	0	5	0	5	0	5	0	5	0	5	0
Mean	5	0	5	0.00	5	0	5	0.0833	5	0	5	0.0833

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▶ Survival rate (SR, %)

Concentration of substance	Control				6.25%				12.50%			
Elapsed time (h)	0		24		0		24		0		24	
Replication	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR
1	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
2	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
3	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
4	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
5	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
6	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
7	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
8	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
9	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
10	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
11	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
12	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Mean	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Concentration of substance	25.00%				50.00%				100.00%			
Elapsed time (h)	0		24		0		24		0		24	
Replication	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR	Total	SR
1	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
2	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
3	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
4	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
5	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
6	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
7	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
8	5	100.0	5	100.0	5	100.0	5	80.0	5	100.0	5	100.0
9	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
10	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
11	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	80.0
12	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0	5	100.0
Mean	5	100.0	5	100.0	5	100.0	5	98.3	5	100.0	5	98.3

Concentration of substance	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
Elapsed time (h)	0	24	0	24	0	24	0	24	0	24	0	24
SR (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.3	100.0	98.3
STDEV (±)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.8	0.0	5.8

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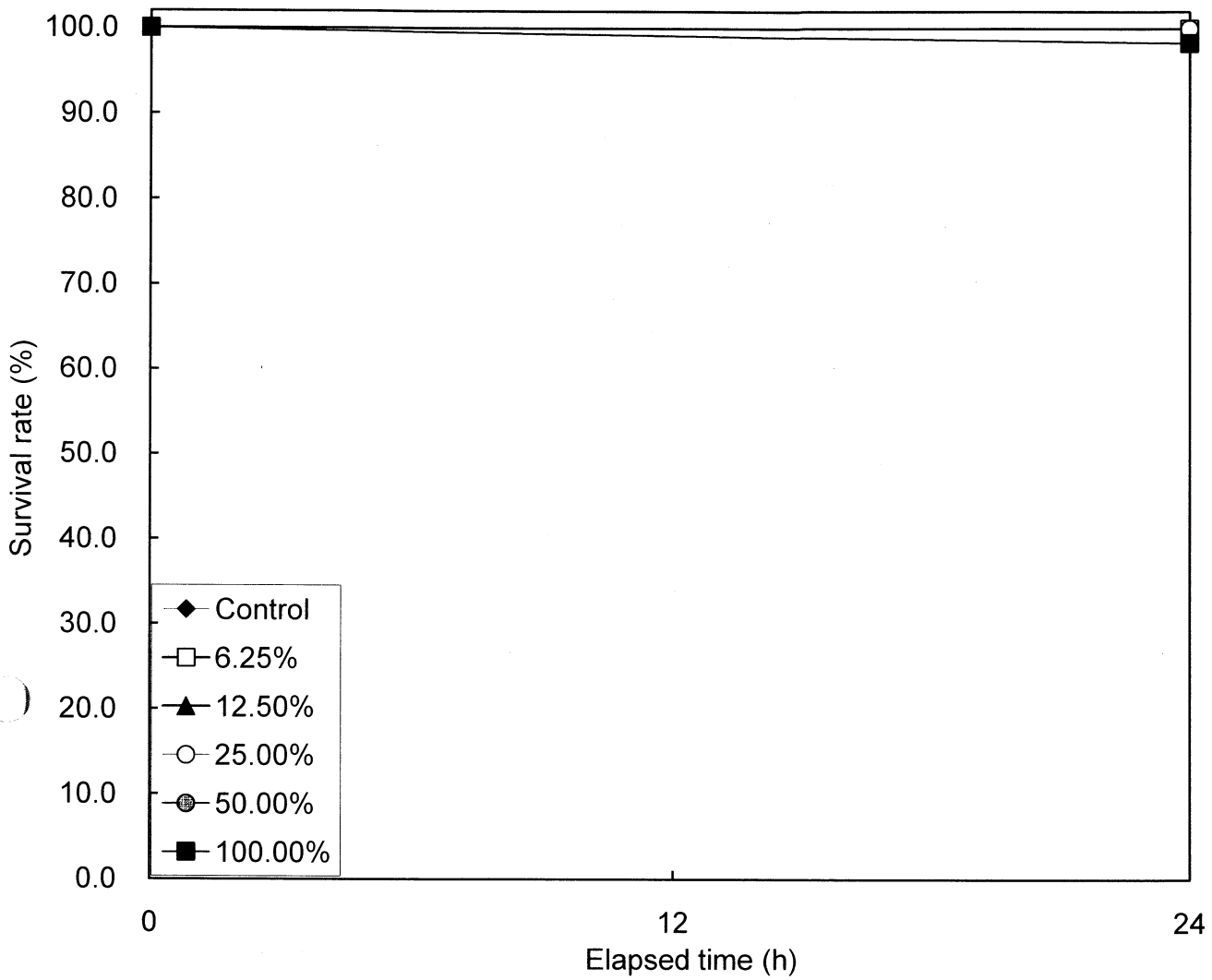

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COC Code. BW-DBWT1104-KT-I003

Test date	110713-110714	**Test substance**	KTM-S5D5-3(3-32 psu)
Object of test	Survival	**Numbers of Initial individual**	5 inds.
Study director	Jung-Im Park	**Numbers of groups**	12 repl.
Test Observer	So Eun Jo	**Test duration**	24 h
Test organism	Rotifer, *Brachionus plicatilis*	**Observe duration**	24 h
Light	Darkness	**Temperature**	25 ± 1 ℃

▶ **DO (mg/L)**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	7.70	7.31	7.10	6.63	6.28	6.66
Final	6.16	6.54	5.88	5.91	5.98	5.89

▶ **pH**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	7.61	7.57	7.54	7.47	7.43	7.44
Final	7.76	7.85	7.82	7.84	7.85	7.84

▶ **Temparature(℃)**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	24.30	24.50	25.60	25.00	25.10	25.30
Final	25.30	25.40	24.60	25.60	25.50	25.30

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4.1.1.3 ANNEX 3

Sheet of raw data for

"The olive flounder fish, *Paralichthys olivaceus*, Acute Toxicity Test"

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COC code : BW-DBWT1104-KT-F001

Sheet of Raw Data – Fish – Acute toxicity

[1] COC Client	KT Marine Co., Ltd.	Test date	110630 – 110704
Lab. manager	Myung Baek Shon	Observer	Tae Won Kim
Test organism	Juvenile of Paralichthys olivaceus	Test duration	96h
[2] COTS	0, 6.25, 12.5, 25, 50, 100%	Test substance	KTMSDS-1 (>32 psu)

Test Conditions and Methods

Temperature	20±1 ℃	Salinity	33 psu	Light	2000 Lux, LD cycle = 16 L : 8 D
Medium	Filtered Seawater (1 μm) or other ( )				
Initial biomass	20	Vessel type and volume	30L Glass Aquarium		
[3] MV / [4] VV	20 L / 30 L	Method of observation (SOP No.)	SOP#TP-006		

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] MV: Medium volume; [4] VV: Vessel volume

▶ Results (Accumulated Number of Dead Organisms)

Date : 2011.6.30 (Initial)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
Remarks						

Date : 2011. 11. 1. (24 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	0	0	1	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
Remarks						

Date : 2011. 11. 2. (48 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	1	0	1	1	0
2	0	0	1	0	0	0
3	0	0	0	0	0	0
Remarks						

Date : 2011. 11. 3. (72 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	2	0	2	1	0
2	0	0	2	0	1	0
3	0	0	0	0	0	0
Remarks						

Date : 20 11.7.4. (96 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	3	0	3	3	0
2	1	0	2	0	1	2
3	2	0	0	0	2	0
Remarks						

▶ **Routine chemical and physical determinations**

Terms		Concentration of test substance (%)																	
		Control			6.25			12.5			25			50			100		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Temp. (℃)	Initial	19.7	20.1	20.4	20.4	20.5	20.1	20.2	20.3	20.2	19.8	19.8	20.1	19.8	20.0	20.0	19.8	19.7	19.5
	24h	19.7	20.1	20.1	20.2	20.3	20.2	20.0	20.1	20.1	19.9	19.9	20.1	20.1	20.0	19.9	19.6	19.7	19.6
	48h	20.0	20.5	20.4	20.1	20.3	20.2	20.3	20.2	20.2	20.0	19.9	19.9	20.0	20.1	20.0	20.0	20.1	20.0
	72h	19.7	19.9	19.8	20.0	20.0	19.9	19.7	19.8	19.7	19.8	19.9	20.0	20.0	20.1	19.9	19.6	19.7	19.8
	96h	19.6	19.8	20.0	20.1	20.0	20.1	19.9	19.9	19.7	19.6	19.7	19.9	19.9	19.8	19.7	19.4	19.5	19.3
Salinity (psu)	Initial	33.3	33.3	33.3	33.3	33.1	33.6	33.3	32.4	33.1	33.4	33.0	32.5	32.4	33.3	32.5	32.7	33.0	33.1
	24h	33.0	33.0	33.5	33.3	33.3	33.1	33.5	33.3	33.2	32.9	33.3	33.2	32.5	33.4	33.4	32.8	33.2	33.0
	48h	33.2	33.3	33.1	33.2	33.2	33.2	33.4	33.5	33.4	33.5	33.4	33.5	33.5	33.5	33.5	33.0	33.3	33.0
	72h	33.2	33.3	33.2	33.2	33.2	33.2	33.1	33.2	33.2	33.2	33.2	33.2	33.1	33.2	33.1	33.2	33.2	33.1
	96h	33.5	33.6	33.5	33.3	33.5	33.6	33.5	33.2	33.4	33.1	33.4	33.4	33.5	33.6	33.0	33.5	33.0	33.4
DO (mg/L)	Initial	5.57	5.08	5.53	5.08	5.71	5.26	5.09	5.18	5.90	6.01	5.51	5.64	6.15	6.85	6.27	6.16	5.77	6.59
	24h	5.73	5.92	5.66	5.95	5.43	5.61	5.51	5.03	5.76	5.85	5.63	5.60	5.71	5.39	5.62	5.00	5.32	5.80
	48h	5.98	5.92	5.85	5.89	5.98	6.00	5.96	6.92	6.01	5.95	5.68	6.32	6.51	6.36	6.11	6.25	6.53	6.47
	72h	6.24	6.02	6.54	7.02	7.00	6.96	6.76	6.41	6.48	6.02	6.00	6.42	6.41	6.11	6.02	6.00	6.21	6.31
	96h	6.06	5.73	6.13	5.30	5.63	5.89	6.20	5.40	5.93	5.84	6.05	5.21	5.26	6.04	6.43	5.61	6.20	6.02
pH	Initial	7.88	7.87	7.85	7.85	7.82	7.83	7.82	7.82	7.82	7.78	7.71	7.77	7.71	7.73	7.73	7.75	7.75	7.74
	24h	7.69	7.83	7.82	7.80	7.80	[illegible]	[illegible]	[illegible]	7.78	[illegible]	7.81	[illegible]	7.71	7.78	7.78	7.72	7.73	7.74
	48h	7.79	7.77	7.78	7.77	7.78	7.75	7.80	7.79	7.79	7.75	7.75	7.74	7.78	7.78	7.79	7.76	7.77	7.75
	72h	7.80	7.78	7.77	7.81	7.82	7.80	7.81	7.80	7.81	7.80	7.79	7.77	7.80	[illegible]	7.80	7.77	7.75	7.74
	96h	7.87	7.88	7.96	7.95	7.89	7.79	7.93	7.83	7.89	7.84	7.88	7.85	7.77	7.88	7.83	7.74	7.83	7.82

2011. 7. 4

Approved by _____

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COC code : BW-DBWT1104-KT-F001

Sheet of Raw Data – Fish – Condition index

Date	110630 - 110704	Signature	Jin Hee Kim
Lab. manger	Myung Baek Shon		[signature]
Test Observer	Tae Won Kim		
Test organism	Juvenile of Paralichthys olivaceus	End point	Survival.
Test substance	KTMS5D5-1(S3 PSU)		

▶ Condition index of test organism

▷Concentration: Control-1 ▷ Concentration: Control-2

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.7	0.52	1	4.9	0.73
2	5.4	0.86	2	5.0	0.74
3	4.8	0.66	3	5.1	0.80
4	5.3	0.98	4	5.1	0.75
5	4.4	0.53	5	4.7	0.63
6	4.5	0.60	6	5.1	0.75
7	4.7	0.57	7	5.1	0.81
8	5.4	0.98	8	4.8	0.65
9	4.5	0.56	9	4.7	0.74
10	4.2	0.52	10	4.6	0.65
11	4.6	0.61	11	4.8	0.84
12	4.8	0.64	12	5.0	0.87
13	4.7	0.70	13	4.9	0.67
14	4.9	0.61	14	4.8	0.69
15	5.1	0.80	15	4.7	0.82
16	5.2	0.82	16	4.6	0.69
17	5.3	0.86	17	4.2	0.70
18	4.9	0.69	18	4.8	0.95
19	4.5	0.61	19	4.8	0.87
20	4.4	0.63	20	4.5	0.65
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.8	0.69	Mean	4.8	0.75

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▷ Concentration: Control -3 ▷ Concentration: 6.25% -1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	5.0	0.63	1	5.0	0.85
2	5.2	0.82	2	4.5	0.66
3	4.5	0.50	3	4.7	0.68
4	4.8	0.67	4	4.9	0.69
5	5.0	0.82	5	5.5	1.00
6	5.0	0.89	6	5.0	0.95
7	5.1	0.92	7	5.1	0.95
8	5.1	0.88	8	4.8	0.96
9	5.0	0.87	9	4.9	0.84
10	4.9	0.75	10	5.0	0.87
11	4.8	0.76	11	5.0	0.95
12	4.8	0.74	12	5.1	0.95
13	4.7	0.65	13	4.7	0.96
14	4.9	0.74	14	4.8	0.98
15	4.8	0.85	15	4.8	0.85
16	4.8	0.54	16	4.9	0.65
17	5.2	0.64	17	4.9	0.84
18	5.3	0.65	18	4.8	0.75
19	5.0	0.85	19	4.6	0.72
20	5.1	0.80	20	4.7	0.73
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	5.0	0.75	Mean	4.9	0.84

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▷ Concentration: 6.25% -2

No.	Length (cm)	Wet weight (g)
1	4.6	0.66
2	5.1	0.79
3	4.5	0.60
4	5.5	0.90
5	5.1	0.80
6	4.7	0.57
7	5.0	0.64
8	5.0	0.93
9	5.1	0.95
10	4.8	0.85
11	4.9	0.85
12	4.2	0.74
13	5.2	0.65
14	5.8	0.85
15	4.7	0.82
16	4.9	0.91
17	4.8	0.63
18	4.9	0.45
19	4.8	0.85
20	5.0	0.90
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.9	0.76

▷ Concentration: 6.25% -3

No.	Length (cm)	Wet weight (g)
1	5.0	0.87
2	4.8	0.66
3	4.8	0.67
4	5.0	0.71
5	4.5	0.62
6	4.9	0.72
7	4.8	0.62
8	4.8	0.68
9	4.7	0.95
10	4.9	0.85
11	4.5	0.54
12	4.8	0.89
13	4.8	0.68
14	4.9	0.69
15	5.0	0.95
16	5.0	0.85
17	5.1	0.82
18	5.0	0.83
19	4.9	0.95
20	4.8	0.84
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Mean	4.9	0.77

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▷Concentration: 12.5%-1

No.	Length (cm)	Wet weight (g)
1	5.4	0.82
2	5.4	0.92
3	4.8	0.68
4	4.7	0.74
5	4.9	0.59
6	5.1	0.65
7	4.9	0.76
8	5.0	0.73
9	4.9	0.82
10	4.8	0.75
11	4.7	0.68
12	4.9	0.95
13	5.1	0.84
14	5.2	0.95
15	5.0	0.84
16	5.3	0.75
17	4.9	0.64
18	4.7	0.68
19	4.8	0.69
20	4.6	0.70
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22		
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25		
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28		
29		
30		
Mean	5.0	0.76

▷ Concentration: 12.5%-2

No.	Length (cm)	Wet weight (g)
1	5.0	0.69
2	4.6	0.70
3	4.8	0.62
4	5.4	0.90
5	5.1	0.79
6	5.6	1.02
7	5.0	0.90
8	4.9	0.81
9	4.8	0.79
10	4.9	0.78
11	5.0	0.76
12	5.3	0.65
13	5.2	0.74
14	4.9	0.73
15	4.6	0.70
16	5.1	0.95
17	4.9	0.86
18	4.8	0.85
19	4.9	0.85
20	5.0	0.92
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Mean	5.0	0.80

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▷Concentration: 12.5% -3

No.	Length (cm)	Wet weight (g)
1	4.3	0.57
2	5.5	1.01
3	4.5	0.62
4	4.9	0.77
5	4.9	0.76
6	4.8	0.86
7	5.0	0.80
8	5.1	0.75
9	5.3	0.94
10	5.4	0.62
11	5.2	0.69
12	5.0	0.85
13	4.9	0.65
14	4.8	0.92
15	4.9	0.75
16	4.6	0.78
17	5.0	0.95
18	5.1	0.99
19	5.0	0.85
20	4.8	0.86
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29		
30		
Mean	4.9	0.78

▷ Concentration: 25% -1

No.	Length (cm)	Wet weight (g)
1	5.2	0.89
2	4.3	0.60
3	5.0	0.77
4	5.2	0.83
5	5.3	0.89
6	5.6	1.01
7	5.0	0.72
8	5.0	0.95
9	4.9	0.85
10	4.9	0.86
11	4.8	0.95
12	4.9	0.76
13	4.6	0.85
14	4.6	0.65
15	5.2	0.70
16	4.9	0.59
17	4.9	0.69
18	4.8	0.67
19	4.5	0.56
20	4.7	0.85
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Mean	4.9	0.77

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▷ Concentration: 25%-2

No.	Length (cm)	Wet weight (g)
1	5.1	0.79
2	5.2	0.83
3	4.9	0.70
4	5.5	1.01
5	4.7	0.63
6	4.7	0.65
7	5.2	0.88
8	5.6	1.01
9	5.2	0.81
10	5.1	0.74
11	5.0	0.95
12	4.9	0.85
13	4.8	0.75
14	4.8	0.65
15	4.9	0.84
16	4.9	0.75
17	5.0	0.65
18	5.3	0.65
19	4.8	0.95
20	4.9	0.85
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29		
30		
Mean	5.0	0.80

▷ Concentration: 25%-3

No.	Length (cm)	Wet weight (g)
1	4.3	0.57
2	4.5	0.60
3	4.5	0.58
4	5.3	0.88
5	4.7	0.63
6	4.6	0.60
7	5.0	0.75
8	4.6	0.85
9	5.0	0.84
10	5.2	0.68
11	5.0	0.69
12	4.9	0.65
13	4.8	0.75
14	4.7	0.85
15	4.6	0.68
16	4.8	0.75
17	4.7	0.88
18	4.6	0.95
19	5.0	0.85
20	5.0	0.86
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30		
Mean	4.8	0.74

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▷ Concentration: 50% -1

No.	Length (cm)	Wet weight (g)
1	5.6	1.03
2	5.4	0.99
3	5.5	1.00
4	5.2	0.83
5	5.0	0.85
6	5.0	0.65
7	4.9	0.85
8	4.8	0.84
9	4.9	0.75
10	5.0	0.76
11	5.1	0.85
12	5.2	0.84
13	4.8	0.82
14	4.9	0.76
15	4.5	0.75
16	4.9	0.86
17	5.0	0.75
18	5.2	0.86
19	5.2	0.99
20	5.1	0.89
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22		
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30		
Mean	5.1	0.85

▷ Concentration: 50% -2

No.	Length (cm)	Wet weight (g)
1	5.2	0.86
2	4.5	0.58
3	4.8	0.86
4	4.9	0.75
5	5.0	0.98
6	5.0	0.85
7	5.0	0.84
8	5.0	0.65
9	5.1	0.95
10	4.9	0.84
11	4.9	0.75
12	4.8	0.94
13	4.7	0.78
14	4.5	0.65
15	5.3	1.02
16	5.9	1.05
17	5.8	1.09
18	5.0	0.94
19	4.5	0.68
20	4.8	0.95
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30		
Mean	5.0	0.84

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▷ Concentration: 50%-3 ▷ Concentration: 100%-1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.1	0.50	1	5.1	0.97
2	4.5	0.59	2	5.4	1.02
3	4.6	0.61	3	5.6	0.98
4	4.6	0.77	4	5.1	1.10
5	5.0	0.75	5	5.0	0.90
6	4.9	0.65	6	5.5	0.77
7	5.0	0.95	7	4.9	1.02
8	4.6	0.65	8	5.5	0.85
9	4.9	0.85	9	5.5	1.03
10	4.8	0.65	10	5.4	1.02
11	4.8	0.65	11	5.2	0.95
12	4.8	0.69	12	4.9	0.85
13	4.7	0.75	13	4.8	0.69
14	4.6	0.85	14	4.8	0.75
15	4.9	0.65	15	4.6	0.85
16	5.0	0.95	16	4.9	0.75
17	5.0	0.65	17	4.8	0.59
18	5.4	0.99	18	5.0	0.68
19	5.1	0.85	19	4.9	0.85
20	5.0	0.95	20	4.8	0.85
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.8	0.75	Mean	5.1	0.87

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▷Concentration: 100% - 2

No.	Length (cm)	Wet weight (g)
1	4.4	0.58
2	4.2	0.50
3	4.3	0.60
4	4.4	0.68
5	4.5	0.65
6	4.6	0.64
7	4.9	0.72
8	4.9	0.85
9	4.8	0.65
10	5.0	0.68
11	4.0	0.95
12	4.9	0.84
13	4.8	0.82
14	5.0	0.86
15	4.9	0.87
16	4.6	0.68
17	5.0	0.85
18	4.9	0.75
19	4.8	0.78
20	4.9	0.65
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22		
23		
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26		
27		
28		
29		
30		
Mean	4.7	0.73

▷ Concentration: 100% - 3

No.	Length (cm)	Wet weight (g)
1	4.4	0.58
2	4.5	0.68
3	4.4	0.63
4	4.3	0.60
5	5.2	0.92
6	5.1	0.91
7	5.0	0.77
8	5.4	0.92
9	5.0	0.85
10	5.1	0.86
11	5.2	0.98
12	4.9	0.86
13	5.6	1.03
14	4.8	0.75
15	4.6	0.69
16	4.8	0.98
17	4.7	0.68
18	4.9	0.79
19	4.6	0.78
20	4.8	0.69
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27		
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29		
30		
Mean	4.9	0.80

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COC code : BW-DBWT1104-KT-F003

Sheet of Raw Data – Fish – Acute toxicity

[1] COC Client	KT Marine Co., Ltd.	Test date	110713 – 110717
Lab. manager	Myung Baek Shen	Observer	Tae Won Kim
Test organism	Juvenile of Paralichthys olivaceus	Test duration	96h
[2] COTS	0, 6.25, 12.5, 25, 50, 100%	Test substance	KTM-S5D5-3 (3-32 psu)

Test Conditions and Methods

Temperature	20±1 ℃	Salinity	22 psu	Light	2,000 Lux, LD cycle = 16 L : 8 D
Medium	Filtered Seawater (1 μm) or other ( )				
Initial biomass	20	Vessel type and volume	30L Glass aquarium		
[3] MV / [4] VV	20 L / 30 L	Method of observation (SOP No.)	SOP#TP-006		

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] MV: Medium volume; [4] VV: Vessel volume

▶ Results (Accumulated Number of Dead Organisms)

Date : 2011 . 7 . 13 (Initial)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
Remarks						

Date : 20 11 . 7 . 14 (24 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
Remarks						

Date : 20 11 . 7 . 15 (48 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
Remarks						

Date : 20 11 . 7 . 16 (72 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
Remarks						

Date : 2011 . 7. 17. (96 hours)

Replication	Concentration of test substance (%)					
	Control	6.25	12.5	25	50	100
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	P
Remarks						

▶ Routine chemical and physical determinations

Terms		Concentration of test substance (%)																	
		Control			6.25			12.5			25			50			100		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Temp. (℃)	Initial	19.6	20.6	20.9	20.2	20.5	20.1	20.3	20.5	20.5	20.2	20.0	20.3	20.5	20.4	20.2	20.4	20.2	20.0
	24h	19.7	20.0	20.2	20.2	20.3	20.4	20.4	20.3	20.2	20.0	20.0	20.2	20.3	20.3	20.1	19.9	19.7	19.6
	48h	20.0	20.0	20.2	20.3	20.3	20.4	20.0	20.3	20.3	20.1	20.1	20.3	20.3	20.2	20.1	19.8	19.8	19.7
	72h	19.9	20.0	20.0	20.2	20.1	20.2	20.0	20.0	20.0	19.4	19.2	19.3	19.3	19.2	19.4	19.1	20.0	20.1
	96h	19.4	20.0	20.0	20.2	20.1	20.2	19.7	20.0	20.0	19.6	19.9	19.8	19.6	20.0	20.0	19.1	19.8	19.8
Salinity (psu)	Initial	21.7	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.9	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8
	24h	21.9	21.9	21.9	21.9	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8
	48h	21.8	21.8	21.8	21.8	21.8	21.8	21.9	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.8	21.7	21.7
	72h	21.8	21.9	21.9	21.9	21.8	21.8	21.8	21.9	21.8	21.8	21.8	21.8	21.7	21.8	21.7	21.7	21.7	21.9
	96h	21.9	21.8	21.8	21.9	21.8	21.8	21.8	21.9	21.8	21.8	21.8	21.8	21.7	21.7	21.7	21.9	21.8	21.8
DO (mg/L)	Initial	8.58	7.78	7.18	6.95	6.50	6.21	5.54	6.05	6.03	5.75	5.89	5.42	5.61	5.66	5.18	5.42	5.20	5.31
	24h	6.28	6.06	5.94	6.46	5.71	6.15	5.49	5.90	5.56	5.76	5.86	6.00	5.90	5.28	5.28	5.21	5.30	5.30
	48h	5.17	4.81	5.96	4.63	5.09	5.73	5.48	5.66	5.16	5.08	5.57	5.58	5.01	5.72	5.44	5.37	5.49	5.30
	72h	6.21	6.42	6.43	6.21	6.71	6.93	6.74	6.27	6.41	6.42	6.81	6.42	6.71	7.02	7.11	7.04	7.03	7.11
	96h	7.09	6.02	6.12	6.41	6.72	6.71	6.42	6.41	6.71	6.79	6.81	6.42	6.41	6.02	6.04	6.88	6.78	6.44
pH	Initial	7.61	7.63	7.74	7.77	7.76	7.73	7.65	7.66	7.71	7.71	7.74	7.74	7.74	7.75	7.72	7.69	7.66	7.63
	24h	7.88	7.88	7.88	7.87	7.80	7.88	7.85	7.82	7.84	7.84	7.86	7.86	7.80	7.87	7.84	7.80	7.80	7.78
	48h	7.58	7.54	7.53	7.54	7.55	7.57	7.55	7.51	7.54	7.54	7.61	7.62	7.64	7.59	7.53	7.43	7.43	7.40
	72h	7.81	7.83	7.72	7.79	7.78	7.76	7.78	7.79	7.64	7.81	7.72	7.42	7.83	7.81	7.88	7.84	7.88	7.88
	96h	7.78	7.77	7.77	7.78	7.76	7.75	7.70	7.76	7.65	7.78	7.81	7.88	7.86	7.88	7.80	7.72	7.73	7.74

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Approved by

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COC code : BW-DBWT1104-KT-F003

Sheet of Raw Data – Fish – Condition index

Date	110713-110717	Signature	Jin Hee Kim
Lab. manger	Myung-Baek Shon		[signature]
Test Observer	Tae-Won Kim		
Test organism	Juvenile of Paralichthys olivaceus	End point	Survival
Test substance	KTM-S5D5-3 (3-32 psu)		

▶ Condition index of test organism

▷ Concentration: Control -1 ▷ Concentration: Control-2

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.6	0.93	1	4.5	0.97
2	4.2	0.62	2	4.2	0.89
3	3.8	0.41	3	4.6	1.20
4	4.2	0.85	4	4.5	1.13
5	4.6	1.00	5	4.4	1.10
6	4.4	0.71	6	4.6	1.18
7	3.8	0.45	7	4.5	0.99
8	4.6	1.01	8	4.4	1.09
9	4.5	0.69	9	4.2	1.07
10	4.8	0.80	10	4.5	1.06
11	4.5	0.88	11	4.1	0.96
12	4.4	0.89	12	4.2	0.72
13	4.4	0.90	13	4.3	1.02
14	4.3	0.54	14	4.5	1.03
15	4.8	1.00	15	4.3	0.98
16	4.7	0.89	16	4.8	0.76
17	4.5	0.82	17	4.7	0.75
18	4.5	0.74	18	4.3	0.98
19	4.7	0.99	19	4.4	0.99
20	4.5	0.74	20	4.5	1.12
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.4	0.79	Mean	4.3	1.00

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▷ Concentration: Control -3 ▷ Concentration: 6.25% -1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.6	0.85	1	4.9	1.08
2	4.7	0.91	2	4.5	0.90
3	5.0	1.05	3	4.3	0.82
4	4.7	0.86	4	4.3	0.72
5	4.5	0.88	5	4.6	0.92
6	4.2	0.76	6	4.3	0.88
7	4.7	0.97	7	4.5	0.94
8	4.8	0.95	8	4.4	0.79
9	4.5	0.80	9	4.6	0.88
10	4.6	0.92	10	4.8	0.99
11	4.5	0.65	11	4.9	1.02
12	4.5	0.83	12	4.8	1.00
13	4.6	0.91	13	4.4	0.78
14	4.3	0.80	14	4.9	1.00
15	4.5	0.88	15	4.5	0.92
16	4.5	0.82	16	4.3	0.90
17	4.6	0.92	17	4.5	0.88
18	4.7	0.91	18	4.8	0.92
19	4.7	0.94	19	4.9	1.04
20	4.8	0.95	20	4.8	1.10
21			21		
22			22		
23			23		
24			24		
25			25		
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27			27		
28			28		
29			29		
30			30		
Mean	4.6	0.88	Mean	4.6	0.92

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▷ Concentration: 6.25%-2

No.	Length (cm)	Wet weight (g)
1	4.0	0.63
2	4.3	0.76
3	4.5	0.91
4	4.1	0.66
5	4.0	0.53
6	3.8	0.51
7	4.1	0.82
8	4.2	0.80
9	3.8	0.45
10	3.9	0.53
11	4.5	0.82
12	4.3	0.92
13	4.4	0.94
14	3.7	0.48
15	4.1	0.69
16	4.2	0.84
17	4.4	0.90
18	4.1	0.80
19	4.2	0.82
20	4.5	0.92
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Mean	4.2	0.74

▷ Concentration: 6.25% -3

No.	Length (cm)	Wet weight (g)
1	4.0	0.71
2	4.0	0.47
3	4.6	0.82
4	4.0	0.51
5	4.1	0.62
6	4.3	0.70
7	4.2	0.80
8	4.2	0.74
9	4.3	0.84
10	4.0	0.55
11	4.4	0.76
12	3.8	0.52
13	3.9	0.54
14	3.9	0.60
15	3.9	0.60
16	4.0	0.62
17	4.0	0.59
18	4.4	0.68
19	4.3	0.73
20	4.0	0.65
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Mean	4.1	0.65

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▷Concentration: 12.5% -1

No.	Length (cm)	Wet weight (g)
1	3.0	0.98
2	4.7	0.74
3	3.0	0.92
4	4.6	0.90
5	4.4	0.83
6	4.8	0.90
7	4.8	0.92
8	4.4	0.79
9	4.3	0.83
10	4.8	0.92
11	4.7	0.90
12	4.4	0.81
13	4.7	1.00
14	4.7	0.95
15	4.4	0.79
16	4.4	0.81
17	4.7	0.98
18	5.0	0.90
19	4.9	1.00
20	4.7	0.99
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Mean	4.7	0.90

▷ Concentration: 12.5% - 2

No.	Length (cm)	Wet weight (g)
1	4.0	0.56
2	3.9	0.58
3	4.1	0.51
4	3.7	0.59
5	3.9	0.52
6	4.2	0.62
7	4.4	0.80
8	3.8	0.50
9	4.0	0.60
10	4.1	0.66
11	3.6	0.41
12	3.9	0.55
13	4.3	0.64
14	4.2	0.76
15	4.5	0.69
16	4.5	0.82
17	3.8	0.55
18	3.8	0.56
19	4.5	0.78
20	4.0	0.57
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Mean	4.1	0.61

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▷Concentration: 12.5% -3

No.	Length (cm)	Wet weight (g)
1	4.6	1.00
2	4.1	0.72
3	4.6	0.79
4	4.5	0.92
5	4.2	0.69
6	4.6	0.98
7	4.7	0.89
8	4.7	0.92
9	5.0	1.02
10	5.0	0.98
11	4.5	0.77
12	4.5	0.82
13	4.9	0.93
14	4.9	1.00
15	4.2	0.79
16	4.5	0.79
17	4.4	0.82
18	4.3	0.68
19	4.2	0.70
20	4.7	0.92
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Mean	4.6	0.86

▷ Concentration: 25% -1

No.	Length (cm)	Wet weight (g)
1	4.4	0.66
2	4.6	0.97
3	4.3	0.62
4	4.4	0.74
5	4.5	1.00
6	4.8	1.16
7	4.0	1.53
8	4.5	0.87
9	4.5	0.83
10	4.3	0.73
11	4.4	0.74
12	4.4	0.58
13	4.4	0.83
14	4.0	0.62
15	4.6	0.86
16	4.1	0.70
17	4.1	0.80
18	4.3	0.84
19	4.4	0.90
20	4.7	0.89
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Mean	4.4	0.79

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▷Concentration: 25%-2

No.	Length (cm)	Wet weight (g)
1	4.6	0.93
2	4.4	0.72
3	4.4	0.83
4	5.0	1.00
5	4.5	0.90
6	4.9	0.88
7	4.6	0.92
8	5.0	1.03
9	4.6	0.82
10	4.7	0.84
11	3.9	0.63
12	4.7	1.00
13	4.3	0.78
14	4.3	0.83
15	4.5	0.84
16	4.5	0.89
17	4.6	0.92
18	4.4	0.77
19	4.8	1.02
20	4.4	0.79
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Mean	4.6	0.87

▷ Concentration: 25%-3

No.	Length (cm)	Wet weight (g)
1	4.4	0.63
2	4.5	0.91
3	4.8	1.02
4	4.8	0.99
5	4.5	0.76
6	4.9	1.03
7	4.9	1.10
8	4.0	0.68
9	4.6	0.92
10	4.5	0.67
11	4.6	0.93
12	4.7	1.00
13	4.7	0.98
14	4.8	1.02
15	4.3	0.62
16	4.6	0.89
17	4.7	1.00
18	4.8	1.05
19	4.7	0.93
20	4.0	0.69
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Mean	4.6	0.89

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▷Concentration: 50 %-1

No.	Length (cm)	Wet weight (g)
1	4.6	0.83
2	4.5	0.92
3	5.1	1.10
4	4.8	0.88
5	4.9	1.00
6	4.9	1.04
7	4.3	0.78
8	4.7	0.84
9	4.3	0.85
10	5.0	0.92
11	4.5	0.90
12	4.6	0.70
13	4.1	0.65
14	5.1	0.84
15	5.0	0.91
16	4.7	0.84
17	4.6	0.94
18	4.9	1.00
19	4.5	0.94
20	5.0	1.05
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30		
Mean	4.7	0.90

▷ Concentration: 50 %-2

No.	Length (cm)	Wet weight (g)
1	4.9	0.94
2	5.0	1.00
3	4.1	0.69
4	4.6	0.92
5	3.9	0.65
6	4.2	0.67
7	3.8	0.60
8	4.6	0.92
9	4.4	0.83
10	4.7	1.00
11	5.1	1.10
12	4.3	0.67
13	4.9	1.02
14	4.8	0.83
15	4.5	0.81
16	3.9	0.50
17	4.3	0.64
18	4.7	0.84
19	4.4	0.72
20	4.7	0.90
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Mean	4.5	0.81

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▷Concentration: 50%-3 ▷ Concentration: 100%-1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	5.1	1.00	1	5.1	1.00
2	4.5	0.92	2	4.5	0.88
3	5.0	1.00	3	4.8	1.00
4	4.5	0.92	4	4.7	0.92
5	5.0	0.88	5	4.5	0.68
6	4.5	0.83	6	5.2	1.20
7	4.6	0.91	7	4.7	0.92
8	4.7	0.93	8	5.0	1.00
9	4.9	1.00	9	4.6	0.84
10	5.1	1.05	10	5.2	1.08
11	4.6	0.88	11	5.0	1.05
12	4.7	0.89	12	5.0	1.04
13	4.4	0.78	13	5.1	1.10
14	5.0	1.04	14	5.1	1.11
15	5.1	1.10	15	5.0	1.10
16	4.7	0.93	16	4.9	1.02
17	4.6	0.83	17	5.0	0.98
18	4.2	0.69	18	4.8	0.95
19	4.9	1.04	19	4.8	0.91
20	5.0	0.99	20	4.7	0.92
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.8	0.93	Mean	4.9	0.99

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▷Concentration: 100%-2

No.	Length (cm)	Wet weight (g)
1	4.8	0.83
2	5.0	1.00
3	4.1	0.83
4	4.7	0.93
5	5.0	1.05
6	4.7	0.84
7	5.4	1.15
8	5.1	0.89
9	4.7	0.88
10	5.0	1.00
11	4.9	0.86
12	5.1	1.15
13	4.7	1.02
14	4.6	0.86
15	5.0	1.00
16	5.0	1.05
17	4.7	0.97
18	5.0	1.08
19	4.8	0.97
20	4.7	0.88
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28		
29		
30		
Mean	4.9	0.96

▷ Concentration: 100%-3

No.	Length (cm)	Wet weight (g)
1	4.9	0.92
2	5.2	1.05
3	5.0	1.00
4	5.0	1.02
5	4.2	0.71
6	5.0	0.92
7	5.1	0.88
8	5.1	1.02
9	4.6	0.78
10	4.9	0.83
11	5.0	1.03
12	4.9	0.92
13	5.0	1.05
14	4.9	0.92
15	5.0	1.00
16	4.8	0.79
17	5.0	0.99
18	5.0	1.03
19	4.8	0.84
20	5.0	1.05
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25		
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30		
Mean	4.9	0.94

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COC Code. BW-DBWT1104-KT-F001

Test date	110630-110704	**Test substance**	KTM-S5D5-1(>32 psu)
End point	Survival	**Numbers of Initial individual**	20 inds. / Repl.
Study director	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	96 h
Test organism	Juvenile of *Paralichthys olivaceus*	**Observe duration**	24 h
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live

Elased time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	20	20	20	20	20	20	20	20	20	20	20	20
24	20	20	20	20	20	20	20	20	20	20	20	20
48	20	20	20	20	19	20	20	20	20	19	20	20
72	20	20	20	20	18	20	20	19	20	18	20	19
96	20	19	18	19	17	20	20	19	20	18	20	19

Elased time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	20	20	20	20	20	20	20	20	20	20	20	20
24	19	20	20	20	20	20	20	20	20	20	20	20
48	19	20	20	20	19	20	20	20	20	20	20	20
72	18	20	20	19	19	19	20	19	20	20	20	20
96	17	20	20	19	17	19	18	18	20	18	20	19

▶ Dead

Elased time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	1	0	0	0	0	1	0	0
72	0	0	0	0	2	0	0	1	0	2	0	1
96	0	1	2	1	3	0	0	1	0	2	0	1

Elased time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
24	1	0	0	0	0	0	0	0	0	0	0	0
48	1	0	0	0	1	0	0	0	0	0	0	0
72	2	0	0	1	1	1	0	1	0	0	0	0
96	3	0	0	1	3	1	2	2	0	2	0	1

▶ Survival rate (%)

Elased time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
24	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
48	100.0	100.0	100.0	100.0	95.0	100.0	100.0	98.3	100.0	95.0	100.0	98.3
72	100.0	100.0	100.0	100.0	90.0	100.0	100.0	96.7	100.0	90.0	100.0	96.7
96	100.0	95.0	90.0	95.0	85.0	100.0	100.0	95.0	100.0	90.0	100.0	96.7

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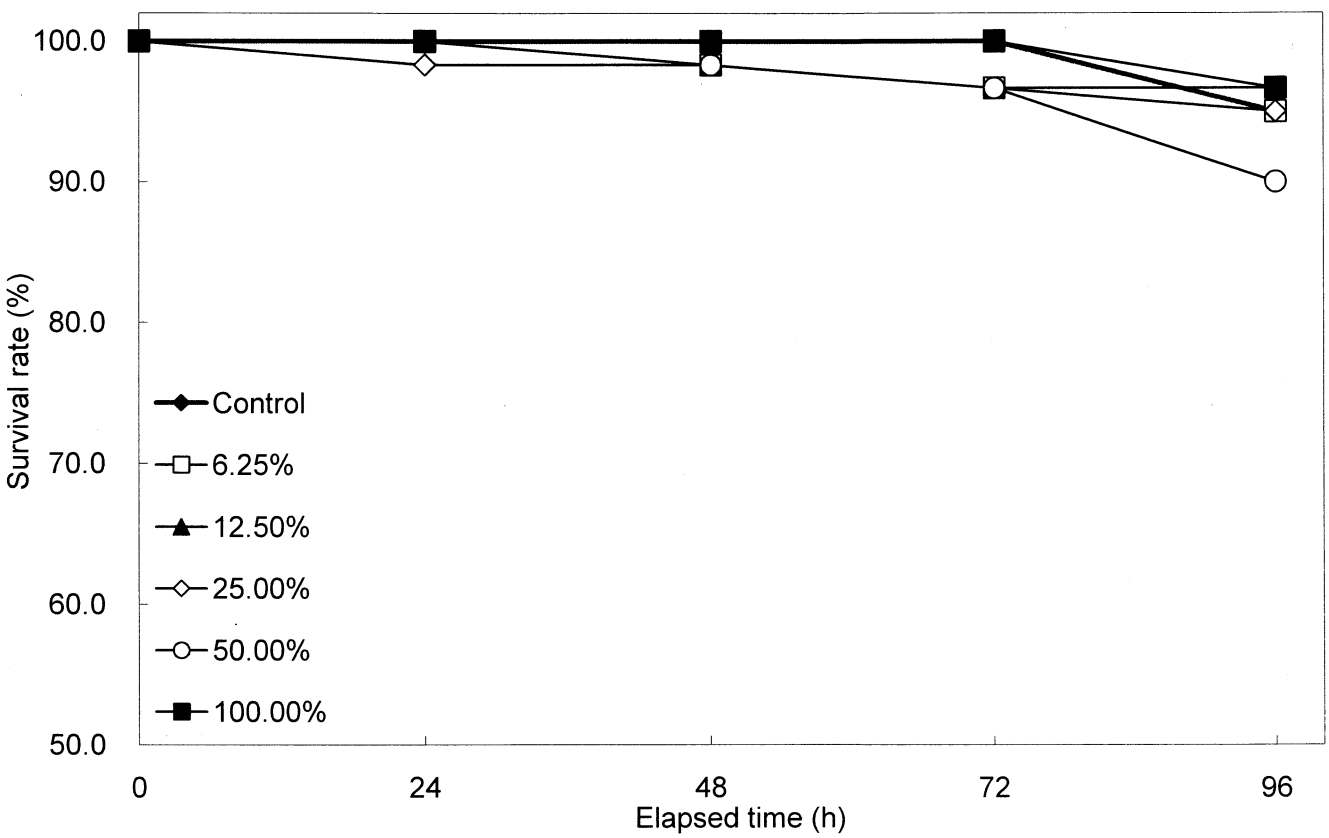
Elased time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
24	95.0	100.0	100.0	98.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
48	95.0	100.0	100.0	98.3	95.0	100.0	100.0	98.3	100.0	100.0	100.0	100.0
72	90.0	100.0	100.0	96.7	95.0	95.0	100.0	96.7	100.0	100.0	100.0	100.0
96	85.0	100.0	100.0	95.0	85.0	95.0	90.0	90.0	100.0	90.0	100.0	96.7



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COC Code. BW-DBWT1104-KT-F001

Test date	110630-110704	**Test substance**	KTM-S5D5-1(>32 psu)
Object of test	Test condition check	**Numbers of Initial individual**	20 inds. / Repl.
Study director	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	96 h
Test organism	Juvenile of *Paralichthys olivaceus*	**Observe duration**	24 h
Light	16L:8D, Cool white, 2000lux	**Temperature**	20 ± 1 ℃

▶ **Temperature (℃)**

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	mean	1	2	3	mean	1	2	3	mean
0	19.70	20.10	20.40	**20.07**	20.40	20.50	20.10	**20.33**	20.20	20.30	20.20	**20.23**
24	19.70	20.10	20.10	**19.97**	20.20	20.30	20.20	**20.23**	20.00	20.10	20.10	**20.07**
48	20.00	20.50	20.40	**20.30**	20.10	20.30	20.20	**20.20**	20.30	20.20	20.20	**20.23**
72	19.70	19.80	19.80	**19.77**	20.00	20.00	19.90	**19.97**	19.70	19.80	19.70	**19.73**
96	19.60	19.80	20.00	**19.80**	20.10	20.00	20.10	**20.07**	19.90	19.90	19.70	**19.83**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	mean	1	2	3	mean	1	2	3	mean
0	19.80	19.80	20.10	**19.90**	19.80	20.00	20.00	**19.93**	19.80	19.70	19.50	**19.67**
24	19.90	19.90	20.10	**19.97**	20.10	20.00	19.90	**20.00**	19.60	19.70	19.60	**19.63**
48	20.00	19.90	19.90	**19.93**	20.00	20.10	20.00	**20.03**	20.00	20.10	20.00	**20.03**
72	19.80	19.80	20.00	**19.87**	20.00	20.10	19.90	**20.00**	19.60	19.70	19.80	**19.70**
96	19.60	19.70	19.90	**19.73**	19.90	19.80	19.70	**19.80**	19.40	19.50	19.30	**19.40**

▶ **Salinity (psu)**

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	33.30	33.30	33.30	**33.30**	33.30	33.70	33.60	**33.53**	33.30	32.40	33.10	**32.93**
24	33.00	33.00	33.50	**33.17**	33.30	33.30	33.70	**33.43**	33.50	33.30	33.20	**33.33**
48	33.20	33.30	33.10	**33.20**	33.20	33.20	33.20	**33.20**	33.40	33.50	33.40	**33.43**
72	33.20	33.30	33.20	**33.23**	33.20	33.20	33.20	**33.20**	33.10	33.20	33.20	**33.17**
96	33.50	33.60	33.50	**33.53**	33.30	33.50	33.60	**33.47**	33.50	33.20	33.40	**33.37**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	33.40	33.00	32.50	**32.97**	33.40	33.30	32.50	**33.07**	32.70	33.00	33.10	**32.93**
24	32.90	33.30	33.20	**33.13**	32.50	33.40	33.40	**33.10**	32.80	33.20	33.00	**33.00**
48	33.50	33.40	33.50	**33.47**	33.50	33.50	33.50	**33.50**	33.00	33.30	33.00	**33.10**
72	33.20	33.20	33.20	**33.20**	33.10	33.20	33.10	**33.13**	33.20	33.20	33.10	**33.17**
96	33.10	33.40	33.40	**33.30**	33.50	33.60	33.00	**33.37**	33.50	33.00	33.40	**33.30**

▶ **DO (mg/L)**

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	5.57	5.08	5.53	**5.39**	5.08	5.71	5.26	**5.35**	5.09	5.18	5.90	**5.39**
24	5.73	5.92	5.66	**5.77**	5.95	5.43	5.67	**5.68**	5.51	5.03	5.76	**5.43**
48	5.98	5.92	5.85	**5.92**	5.89	5.98	6.00	**5.96**	5.96	6.92	6.01	**6.30**
72	6.24	6.02	6.54	**6.27**	7.02	7.00	6.96	**6.99**	6.76	6.41	6.48	**6.55**
96	6.06	5.73	6.13	**5.97**	5.50	5.62	5.89	**5.67**	6.20	5.40	5.93	**5.84**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	6.01	5.51	5.64	**5.72**	6.15	6.85	6.27	**6.42**	6.16	5.70	6.59	**6.15**
24	5.85	5.63	5.60	**5.69**	5.71	5.39	5.62	**5.57**	5.00	5.32	5.80	**5.37**
48	5.95	5.68	6.32	**5.98**	6.51	6.36	6.17	**6.35**	6.25	6.53	6.47	**6.42**
72	6.02	6.00	6.42	**6.15**	6.41	6.11	6.02	**6.18**	6.00	6.21	6.31	**6.17**
96	5.84	6.05	5.27	**5.72**	5.26	6.04	6.43	**5.91**	5.61	6.20	6.02	**5.94**

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▶ pH

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	7.88	7.87	7.85	**7.87**	7.85	7.82	7.83	**7.83**	7.82	7.82	7.82	**7.82**
24	7.69	7.83	7.82	**7.78**	7.80	7.80	7.77	**7.79**	7.67	7.77	7.78	**7.74**
48	7.79	7.77	7.78	**7.78**	7.77	7.78	7.75	**7.77**	7.80	7.79	7.79	**7.79**
72	7.80	7.78	7.77	**7.78**	7.81	7.82	7.82	**7.82**	7.81	7.80	7.81	**7.81**
96	7.87	7.88	7.96	**7.90**	7.95	7.89	7.74	**7.86**	7.83	7.83	7.87	**7.84**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	7.78	7.73	7.77	**7.76**	7.71	7.73	7.73	**7.72**	7.75	7.75	7.74	**7.75**
24	7.74	7.61	7.43	**7.59**	7.71	7.78	7.78	**7.76**	7.72	7.73	7.74	**7.73**
48	7.75	7.75	7.74	**7.75**	7.78	7.78	7.79	**7.78**	7.76	7.77	7.75	**7.76**
72	7.80	7.79	7.77	**7.79**	7.80	7.79	7.77	**7.79**	7.78	7.75	7.74	**7.76**
96	7.84	7.88	7.83	**7.85**	7.84	7.88	7.83	**7.85**	7.74	7.83	7.82	**7.80**

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■ COC code: BW-DBWT1104-KT-F001

Date	110630-110704	**Signature**	**Jin Hee Kim**
Lab. manger	Myung-Baek Shon		
Test Observer	Tae-Won Kim		
Test organism	Juvenile of *Paralichthys olivaceus*	**End point**	Survival
Test substance	KT-S5D5-1(>32 psu)		

▶ **Condition index of test organism**

▷ **Concentration:** Control-1 ▷ **Concentration:** Control-2

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.7	0.52	1	4.9	0.73
2	5.4	0.86	2	5.0	0.74
3	4.8	0.66	3	5.1	0.80
4	5.3	0.98	4	5.1	0.75
5	4.4	0.53	5	4.7	0.63
6	4.5	0.60	6	5.1	0.75
7	4.7	0.57	7	5.1	0.81
8	5.4	0.98	8	4.8	0.65
9	4.5	0.56	9	4.7	0.74
10	4.2	0.52	10	4.6	0.65
11	4.6	0.61	11	4.8	0.84
12	4.8	0.64	12	5.0	0.87
13	4.7	0.70	13	4.9	0.67
14	4.9	0.61	14	4.8	0.69
15	5.1	0.80	15	4.7	0.82
16	5.2	0.82	16	4.6	0.69
17	5.3	0.86	17	4.2	0.70
18	4.9	0.69	18	4.8	0.95
19	4.5	0.61	19	4.8	0.87
20	4.4	0.63	20	4.5	0.65
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.8	0.69	Mean	4.8	0.75

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▷ **Concentration:** Control-3 ▷ **Concentration:** 6.25%-1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	5.0	0.63	1	5.0	0.85
2	5.2	0.82	2	4.5	0.66
3	4.5	0.50	3	4.7	0.68
4	4.8	0.67	4	4.9	0.69
5	5.0	0.82	5	5.5	1.00
6	5.0	0.89	6	5.0	0.95
7	5.1	0.92	7	5.1	0.95
8	5.1	0.88	8	4.8	0.96
9	5.0	0.87	9	4.9	0.84
10	4.9	0.75	10	5.0	0.87
11	4.8	0.76	11	5.0	0.95
12	4.8	0.74	12	5.1	0.95
13	4.7	0.65	13	4.7	0.96
14	4.9	0.74	14	4.8	0.98
15	4.8	0.85	15	4.8	0.85
16	4.8	0.54	16	4.9	0.65
17	5.2	0.64	17	4.9	0.84
18	5.3	0.65	18	4.8	0.75
19	5.0	0.85	19	4.6	0.72
20	5.1	0.80	20	4.7	0.73
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	5.0	0.75	Mean	4.9	0.84

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▷ **Concentration:** 6.25%-2

No.	Length (cm)	Wet weight (g)
1	4.6	0.66
2	5.1	0.79
3	4.5	0.60
4	5.5	0.90
5	5.1	0.80
6	4.7	0.57
7	5.0	0.64
8	5.0	0.73
9	5.1	0.95
10	4.8	0.85
11	4.9	0.85
12	4.2	0.74
13	5.2	0.65
14	5.8	0.85
15	4.7	0.82
16	4.9	0.91
17	4.8	0.63
18	4.9	0.45
19	4.8	0.85
20	5.0	0.90
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.9	0.76

▷ **Concentration:** 6.25%-3

No.	Length (cm)	Wet weight (g)
1	5.0	0.87
2	4.8	0.66
3	4.8	0.67
4	5.0	0.71
5	4.5	0.62
6	4.9	0.72
7	4.8	0.62
8	4.8	0.68
9	4.7	0.95
10	4.9	0.85
11	4.5	0.54
12	4.8	0.87
13	4.8	0.68
14	4.9	0.69
15	5.0	0.95
16	5.0	0.85
17	5.1	0.82
18	5.0	0.83
19	4.9	0.95
20	4.8	0.84
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.9	0.77

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▷ **Concentration:** 12.5%-1

No.	Length (cm)	Wet weight (g)
1	5.4	0.82
2	5.4	0.92
3	4.8	0.68
4	4.7	0.74
5	4.9	0.59
6	5.1	0.65
7	4.9	0.76
8	5.0	0.73
9	4.9	0.82
10	4.8	0.75
11	4.7	0.68
12	4.9	0.95
13	5.1	0.84
14	5.2	0.95
15	5.0	0.84
16	5.3	0.75
17	4.9	0.64
18	4.7	0.68
19	4.8	0.69
20	4.6	0.70
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
mean	5.0	0.76

▷ **Concentration:** 12.5%-2

No.	Length (cm)	Wet weight (g)
1	5.0	0.69
2	4.6	0.70
3	4.8	0.62
4	5.4	0.90
5	5.1	0.79
6	5.6	1.02
7	5.0	0.90
8	4.9	0.81
9	4.8	0.79
10	4.9	0.78
11	5.0	0.76
12	5.3	0.65
13	5.2	0.74
14	4.9	0.73
15	4.6	0.70
16	5.1	0.95
17	4.9	0.86
18	4.8	0.85
19	4.9	0.85
20	5.0	0.92
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	5.0	0.80

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▷ **Concentration:** 12.5%-3 ▷ **Concentration:** 25%-1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.3	0.57	1	5.2	0.89
2	5.5	1.01	2	4.3	0.60
3	4.5	0.62	3	5.0	0.77
4	4.9	0.77	4	5.2	0.83
5	4.9	0.76	5	5.3	0.89
6	4.8	0.86	6	5.6	1.01
7	5.0	0.80	7	5.0	0.72
8	5.1	0.75	8	5.0	0.95
9	5.3	0.74	9	4.9	0.85
10	5.4	0.62	10	4.9	0.86
11	5.2	0.69	11	4.8	0.75
12	5.0	0.85	12	4.9	0.76
13	4.9	0.65	13	4.6	0.85
14	4.8	0.72	14	4.6	0.65
15	4.7	0.75	15	5.2	0.70
16	4.6	0.78	16	4.9	0.59
17	5.0	0.95	17	4.9	0.69
18	5.1	0.99	18	4.8	0.67
19	5.0	0.85	19	4.5	0.56
20	4.8	0.86	20	4.7	0.85
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.9	0.78	Mean	4.9	0.77

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▷ **Concentration:** 25%-2

No.	Length (cm)	Wet weight (g)
1	5.1	0.79
2	5.2	0.83
3	4.9	0.70
4	5.5	1.01
5	4.7	0.63
6	4.7	0.65
7	5.2	0.88
8	5.6	1.01
9	5.2	0.81
10	5.1	0.74
11	5.0	0.95
12	4.9	0.85
13	4.8	0.75
14	4.8	0.65
15	4.9	0.84
16	4.9	0.75
17	5.0	0.65
18	5.3	0.65
19	4.8	0.95
20	4.9	0.85
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	5.0	0.80

▷ **Concentration:** 25%-3

No.	Length (cm)	Wet weight (g)
1	4.3	0.57
2	4.5	0.60
3	4.5	0.58
4	5.3	0.88
5	4.7	0.63
6	4.6	0.60
7	5.0	0.75
8	4.6	0.85
9	5.0	0.84
10	5.2	0.68
11	5.0	0.69
12	4.9	0.65
13	4.8	0.75
14	4.7	0.85
15	4.6	0.68
16	4.8	0.75
17	4.9	0.88
18	4.6	0.95
19	5.0	0.85
20	5.0	0.86
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.8	0.74

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▷ **Concentration:** 50%-1

No.	Length (cm)	Wet weight (g)
1	5.6	1.03
2	5.4	0.99
3	5.5	1.00
4	5.2	0.83
5	5.0	0.85
6	5.0	0.65
7	4.9	0.85
8	4.8	0.84
9	4.9	0.75
10	5.0	0.76
11	5.1	0.85
12	5.2	0.84
13	4.8	0.82
14	4.9	0.76
15	4.5	0.75
16	4.7	0.86
17	5.0	0.75
18	5.2	0.86
19	5.2	0.99
20	5.1	0.89
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	5.1	0.85

▷ **Concentration:** 50%-2

No.	Length (cm)	Wet weight (g)
1	5.2	0.86
2	4.5	0.58
3	4.8	0.86
4	4.9	0.75
5	5.0	0.98
6	5.0	0.85
7	5.0	0.84
8	5.0	0.65
9	5.1	0.95
10	4.9	0.84
11	4.9	0.75
12	4.8	0.84
13	4.7	0.78
14	4.5	0.65
15	5.3	1.02
16	5.9	1.05
17	5.8	1.09
18	5.0	0.74
19	4.5	0.68
20	4.8	0.95
21		
22		
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24		
25		
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27		
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30		
Mean	5.0	0.84

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▷ **Concentration:** 50%-3

No.	Length (cm)	Wet weight (g)
1	4.1	0.50
2	4.5	0.59
3	4.6	0.61
4	4.6	0.77
5	5.0	0.75
6	4.9	0.65
7	5.0	0.95
8	4.6	0.65
9	4.9	0.85
10	4.8	0.65
11	4.8	0.65
12	4.8	0.69
13	4.7	0.75
14	4.6	0.85
15	4.9	0.65
16	5.0	0.95
17	5.0	0.65
18	5.4	0.99
19	5.1	0.85
20	5.0	0.95
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.8	0.75

▷ **Concentration:** 100%-1

No.	Length (cm)	Wet weight (g)
1	5.1	0.97
2	5.4	1.02
3	5.6	0.98
4	5.1	1.10
5	5.0	0.90
6	5.5	0.77
7	4.9	1.02
8	5.5	0.85
9	5.5	1.03
10	5.4	1.02
11	5.2	0.95
12	4.9	0.85
13	4.8	0.69
14	4..8	0.75
15	4.6	0.85
16	4.9	0.75
17	4.8	0.59
18	5.0	0.68
19	4.9	0.85
20	4.8	0.85
21		
22		
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25		
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28		
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30		
Mean	5.1	0.87

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▷ **Concentration:** 100%-2

No.	Length (cm)	Wet weight (g)
1	4.4	0.58
2	4.2	0.50
3	4.3	0.60
4	4.4	0.68
5	4.5	0.65
6	4.6	0.64
7	4.9	0.72
8	4.9	0.85
9	4.8	0.65
10	5.0	0.68
11	4.0	0.95
12	4.9	0.84
13	4.8	0.82
14	5.0	0.86
15	4.9	0.87
16	4.6	0.68
17	5.0	0.85
18	4.9	0.75
19	4.8	0.78
20	4.9	0.65
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.7	0.73

▷ **Concentration:** 100%-3

No.	Length (cm)	Wet weight (g)
1	4.4	0.58
2	4.5	0.68
3	4.4	0.63
4	4.3	0.60
5	5.2	0.92
6	5.1	0.91
7	5.0	0.77
8	5.4	0.92
9	5.0	0.85
10	5.1	0.86
11	5.2	0.98
12	4.9	0.86
13	5.6	1.03
14	4.8	0.75
15	4.6	0.69
16	4.8	0.98
17	4.7	0.68
18	4.9	0.79
19	4.6	0.78
20	4.8	0.69
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.9	0.80

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COC Code. BW-DBWT1104-KT-F003

Test date	110713-110717	**Test substance**	KTM-S5D5-3(3-32 psu)
End point	Survival	**Numbers of Initial individual**	20 inds. / Repl.
Study director	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	96 h
Test organism	Juvenile of *Paralichthys olivaceus*	**Observe duration**	24 h
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live

Elased time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	20	20	20	20	20	20	20	20	20	20	20	20
24	20	20	20	20	20	20	20	20	20	20	20	20
48	20	20	20	20	20	20	20	20	20	20	20	20
72	20	20	20	20	20	20	20	20	20	20	20	20
96	20	20	20	20	20	20	20	20	20	20	20	20

Elased time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	20	20	20	20	20	20	20	20	20	20	20	20
24	20	20	20	20	20	20	20	20	20	20	20	20
48	20	20	20	20	20	20	20	20	20	20	20	20
72	20	20	20	20	20	20	20	20	20	20	20	20
96	20	20	20	20	20	20	20	20	20	20	20	20

▶ Dead

Elased time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0

Elased time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0

▶ Survival rate (%)

Elased time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
24	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
48	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
72	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
96	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

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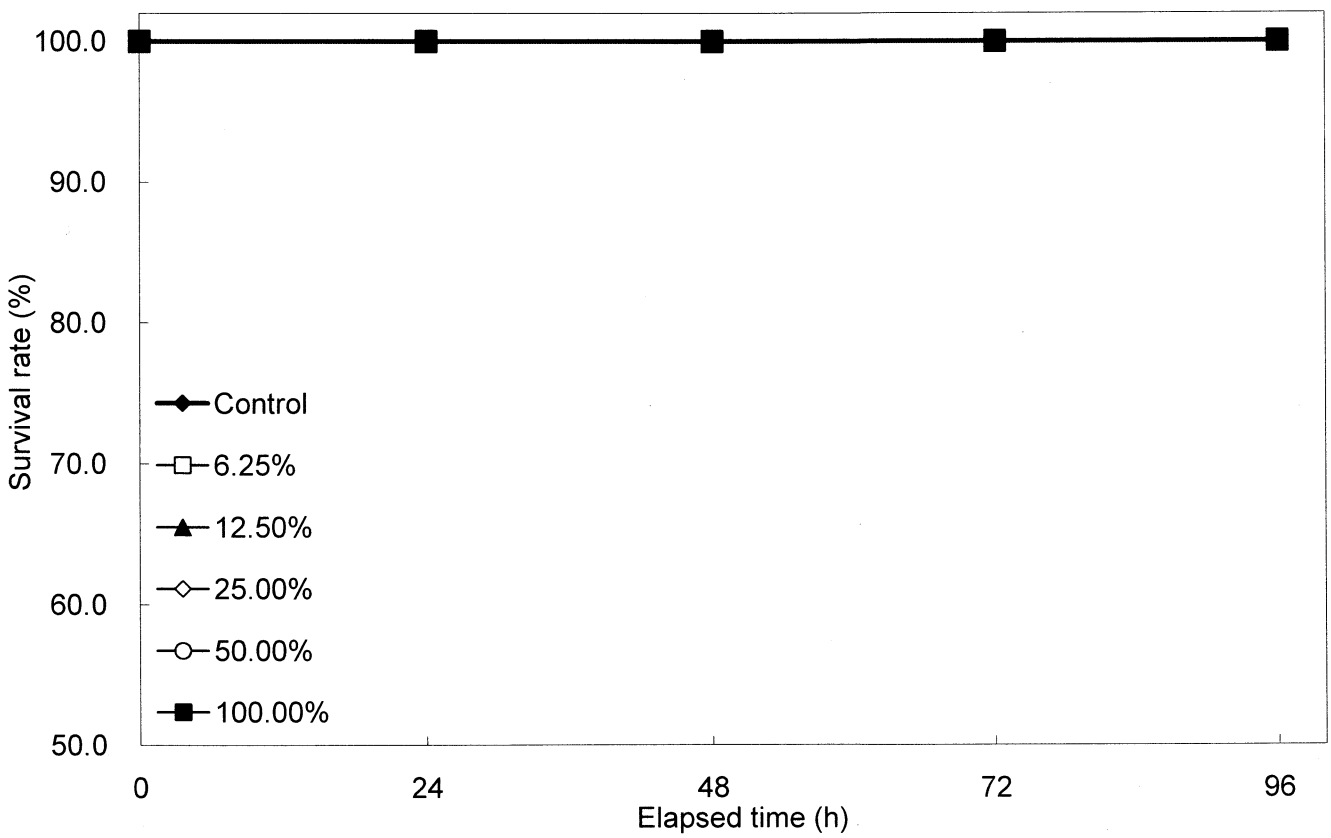
Elased time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
24	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
48	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
72	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
96	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0



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COC Code. BW-DBWT1104-KT-F003

Test date	110713-110717	**Test substance**	KTM-S5D5-3(3-32 psu)
Object of test	Test condition check	**Numbers of Initial individual**	20 inds. / Repl.
Study director	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	96 h
Test organism	Juvenile of *Paralichthys olivaceus*	**Observe duration**	24 h
Light	16L:8D, Cool white, 2000lux	**Temperature**	20 ± 1 ℃

▶ **Temperature (℃)**

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	mean	1	2	3	mean	1	2	3	mean
0	19.60	20.60	20.90	**20.37**	20.20	20.50	20.10	**20.27**	20.30	20.50	20.50	**20.43**
24	19.90	20.00	20.20	**20.03**	20.20	20.30	20.40	**20.50**	20.40	20.30	20.20	**20.30**
48	20.00	20.00	20.20	**20.07**	20.30	20.30	20.40	**20.33**	20.00	20.30	20.30	**20.20**
72	19.90	20.00	20.00	**19.97**	20.20	20.10	20.20	**20.17**	20.00	20.00	20.00	**20.00**
96	19.40	20.00	20.00	**19.80**	20.20	20.10	20.20	**20.17**	19.70	20.00	20.00	**19.90**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	mean	1	2	3	mean	1	2	3	mean
0	20.20	20.00	20.30	**20.17**	20.50	20.40	20.20	**20.37**	20.40	20.20	20.00	**20.20**
24	20.00	20.00	20.20	**20.07**	20.30	20.30	20.10	**20.23**	19.90	19.70	19.60	**19.73**
48	20.10	20.10	20.30	**20.17**	20.30	20.20	20.10	**20.20**	19.80	19.80	19.70	**19.77**
72	19.40	19.20	19.30	**19.30**	19.30	19.20	19.40	**19.30**	19.10	20.00	20.10	**19.73**
96	19.60	19.90	19.80	**19.77**	19.60	20.00	20.00	**19.87**	19.10	19.80	19.80	**19.57**

▶ **Salinity (psu)**

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	21.70	21.80	21.80	**21.77**	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**
24	21.90	21.90	21.90	**21.90**	21.90	21.80	21.80	**21.83**	21.80	21.80	21.80	**21.80**
48	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**	21.90	21.80	21.80	**21.83**
72	21.80	21.90	21.90	**21.87**	21.90	21.80	21.80	**21.83**	21.80	21.90	21.80	**21.83**
96	21.90	21.80	21.80	**21.83**	21.90	21.80	21.80	**21.83**	21.80	21.90	21.80	**21.83**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	21.90	21.80	21.80	**21.83**	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**
24	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**
48	21.80	21.80	21.80	**21.80**	21.80	21.80	21.80	**21.80**	21.80	21.70	21.70	**21.73**
72	21.80	21.80	21.80	**21.80**	21.70	21.80	21.70	**21.73**	21.90	21.70	21.90	**21.83**
96	21.80	21.80	21.80	**21.80**	21.70	21.70	21.70	**21.70**	21.90	21.80	21.80	**21.83**

▶ **DO (mg/L)**

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	8.58	7.78	7.18	**7.85**	6.95	6.50	6.21	**6.55**	5.59	6.05	6.03	**5.89**
24	6.28	6.06	5.94	**6.09**	6.46	5.71	6.15	**6.11**	5.49	5.90	5.56	**5.65**
48	5.17	5.81	5.96	**5.65**	5.63	5.09	5.73	**5.48**	5.48	5.66	5.16	**5.43**
72	6.21	6.42	6.43	**6.35**	6.21	6.71	6.93	**6.62**	6.34	6.27	6.41	**6.34**
96	7.09	6.02	6.12	**6.41**	6.41	6.92	6.71	**6.68**	6.42	6.41	6.71	**6.51**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	5.75	5.89	5.42	**5.69**	5.61	5.66	5.18	**5.48**	5.42	5.20	5.31	**5.31**
24	5.76	5.86	6.00	**5.87**	5.90	5.28	5.28	**5.49**	5.21	5.30	5.30	**5.27**
48	5.08	5.57	5.58	**5.41**	6.01	5.72	5.44	**5.72**	5.37	5.49	5.30	**5.39**
72	6.42	6.81	6.92	**6.72**	5.71	7.02	7.11	**6.61**	7.03	7.02	7.11	**7.05**
96	6.79	6.81	6.92	**6.84**	6.41	6.02	6.04	**6.16**	6.88	6.97	6.44	**6.76**

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▶ pH

Elapsed time (h)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	7.67	7.63	7.74	**7.68**	7.77	7.76	7.73	**7.75**	7.65	7.66	7.71	**7.67**
24	7.88	7.88	7.88	**7.88**	7.87	7.88	7.88	**7.88**	7.85	7.82	7.84	**7.84**
48	7.58	7.54	7.53	**7.55**	7.54	7.55	7.57	**7.55**	7.55	7.51	7.54	**7.53**
72	7.81	7.83	7.82	**7.82**	7.79	7.78	7.76	**7.78**	7.78	7.79	7.64	**7.74**
96	7.78	7.77	7.79	**7.78**	7.78	7.76	7.75	**7.76**	7.70	7.76	7.65	**7.70**

Elapsed time (h)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	7.71	7.74	7.74	**7.73**	7.74	7.75	7.72	**7.74**	7.69	7.66	7.63	**7.66**
24	7.84	7.85	7.86	**7.85**	7.80	7.87	7.84	**7.84**	7.80	7.80	7.78	**7.79**
48	7.54	7.61	7.62	**7.59**	7.64	7.59	7.53	**7.59**	7.43	7.43	7.40	**7.42**
72	7.81	7.92	7.42	**7.72**	7.83	7.87	7.88	**7.86**	7.84	7.88	7.88	**7.87**
96	7.78	7.87	7.88	**7.84**	7.86	7.88	7.80	**7.85**	7.72	7.73	7.74	**7.73**

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■ COC code: BW-DBWT1104-KT-F003

Date	110713-110717	**Signature**	**Jin Hee Kim**
Lab. manger	Myung-Baek Shon		
Test Observer	Tae-Won Kim		
Test organism	Juvenile of *Paralichthys olivaceus*	**End point**	Survival
Test substance	KTM-S5D5-3(3-32 psu)		

▶ **Condition index of test organism**

▷ **Concentration:** Control-1 ▷ **Concentration:** Control-2

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.6	0.93	1	4.5	0.97
2	4.2	0.62	2	4.2	0.89
3	3.8	0.41	3	4.6	1.20
4	4.2	0.85	4	4.5	1.13
5	4.6	1.00	5	4.4	1.10
6	4.4	0.71	6	4.6	1.18
7	3.8	0.45	7	4.5	0.99
8	4.6	1.01	8	4.4	1.09
9	4.5	0.69	9	4.2	1.07
10	4.8	0.80	10	4.5	1.06
11	4.5	0.88	11	4.1	0.96
12	4.4	0.89	12	4.2	0.72
13	4.4	0.90	13	4.3	1.02
14	4.3	0.54	14	4.5	1.03
15	4.8	1.00	15	4.3	0.98
16	4.7	0.89	16	3.8	0.76
17	4.5	0.82	17	3.7	0.75
18	4.5	0.74	18	4.3	0.98
19	4.7	0.99	19	4.4	0.99
20	4.5	0.74	20	4.5	1.12
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.4	0.79	Mean	4.3	1.00

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▷ **Concentration:** Control-3 ▷ **Concentration:** 6.25%-1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.6	0.85	1	4.9	1.08
2	4.7	0.91	2	4.5	0.90
3	5.0	1.05	3	4.3	0.82
4	4.7	0.86	4	4.3	0.72
5	4.5	0.88	5	4.6	0.92
6	4.2	0.76	6	4.3	0.88
7	4.7	0.97	7	4.5	0.94
8	4.8	0.95	8	4.4	0.79
9	4.5	0.80	9	4.6	0.88
10	4.6	0.92	10	4.8	0.99
11	4.5	0.65	11	4.9	1.02
12	4.5	0.83	12	4.8	1.00
13	4.6	0.91	13	4.4	0.78
14	4.3	0.80	14	4.9	1.00
15	4.5	0.88	15	4.5	0.92
16	4.5	0.82	16	4.3	0.90
17	4.6	0.92	17	4.5	0.88
18	4.7	0.91	18	4.8	0.92
19	4.7	0.94	19	4.9	1.04
20	4.8	0.95	20	4.8	1.10
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	4.6	0.88	Mean	4.6	0.92

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▷ **Concentration:** 6.25%-2

No.	Length (cm)	Wet weight (g)
1	4.0	0.63
2	4.3	0.76
3	4.5	0.91
4	4.1	0.66
5	4.0	0.53
6	3.8	0.51
7	4.1	0.82
8	4.2	0.80
9	3.8	0.45
10	3.9	0.53
11	4.5	0.82
12	4.3	0.92
13	4.4	0.94
14	3.7	0.48
15	4.1	0.69
16	4.2	0.84
17	4.4	0.90
18	4.1	0.80
19	4.2	0.82
20	4.5	0.92
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.2	0.74

▷ **Concentration:** 6.25%-3

No.	Length (cm)	Wet weight (g)
1	4.0	0.71
2	4.0	0.47
3	4.6	0.82
4	4.0	0.51
5	4.1	0.62
6	4.3	0.70
7	4.2	0.80
8	4.2	0.74
9	4.3	0.84
10	4.0	0.55
11	4.4	0.76
12	3.8	0.52
13	3.9	0.54
14	3.9	0.60
15	3.9	0.60
16	4.0	0.62
17	4.0	0.59
18	4.4	0.68
19	4.3	0.73
20	4.0	0.65
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.1	0.65

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▷ **Concentration:** 12.5%-1 ▷ **Concentration:** 12.5%-2

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	5.0	0.98	**1**	4.0	0.56
2	4.7	0.74	**2**	3.9	0.58
3	5.0	0.92	**3**	4.1	0.51
4	4.6	0.90	**4**	3.7	0.59
5	4.4	0.83	**5**	3.9	0.52
6	4.8	0.90	**6**	4.2	0.62
7	4.8	0.92	**7**	4.4	0.80
8	4.4	0.79	**8**	3.8	0.50
9	4.3	0.83	**9**	4.0	0.60
10	4.8	0.92	**10**	4.1	0.66
11	4.7	0.90	**11**	3.6	0.41
12	4.4	0.81	**12**	3.9	0.55
13	4.7	1.00	**13**	4.3	0.64
14	4.7	0.95	**14**	4.2	0.76
15	4.4	0.79	**15**	4.5	0.69
16	4.4	0.87	**16**	4.5	0.82
17	4.7	0.98	**17**	3.8	0.55
18	5.0	0.90	**18**	3.8	0.56
19	4.9	1.00	**19**	4.5	0.78
20	4.7	0.99	**20**	4.0	0.57
21			**21**		
22			**22**		
23			**23**		
24			**24**		
25			**25**		
26			**26**		
27			**27**		
28			**28**		
29			**29**		
30			**30**		
mean	4.7	0.90	**Mean**	4.1	0.61

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▷ **Concentration:** 12.5%-3 ▷ **Concentration:** 25%-1

No.	Length (cm)	Wet weight (g)	No.	Length (cm)	Wet weight (g)
1	4.6	1.00	**1**	4.4	0.66
2	4.1	0.72	**2**	4.6	0.97
3	4.6	0.79	**3**	4.3	0.62
4	4.5	0.92	**4**	4.4	0.74
5	4.2	0.69	**5**	4.5	1.00
6	4.6	0.98	**6**	4.8	1.16
7	4.7	0.89	**7**	4.0	0.53
8	4.7	0.92	**8**	4.5	0.87
9	5.0	1.02	**9**	4.5	0.83
10	5.0	0.98	**10**	4.3	0.73
11	4.5	0.77	**11**	4.4	0.74
12	4.5	0.82	**12**	4.4	0.58
13	4.9	0.93	**13**	4.4	0.83
14	4.9	1.00	**14**	4.0	0.62
15	4.2	0.79	**15**	4.6	0.86
16	4.5	0.79	**16**	4.1	0.70
17	4.4	0.82	**17**	4.1	0.80
18	4.3	0.68	**18**	4.3	0.84
19	4.2	0.70	**19**	4.4	0.90
20	4.7	0.92	**20**	4.7	0.89
21			**21**		
22			**22**		
23			**23**		
24			**24**		
25			**25**		
26			**26**		
27			**27**		
28			**28**		
29			**29**		
30			**30**		
Mean	4.6	0.86	**Mean**	4.4	0.79

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▷ **Concentration:** 25%-2

No.	Length (cm)	Wet weight (g)
1	4.6	0.93
2	4.4	0.72
3	4.4	0.83
4	5.0	1.00
5	4.5	0.90
6	4.9	0.88
7	4.6	0.92
8	5.0	1.03
9	4.6	0.82
10	4.7	0.84
11	3.9	0.63
12	4.7	1.00
13	4.3	0.78
14	4.3	0.83
15	4.5	0.84
16	4.5	0.89
17	4.6	0.92
18	4.4	0.77
19	4.8	1.02
20	4.4	0.79
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.6	0.87

▷ **Concentration:** 25%-3

No.	Length (cm)	Wet weight (g)
1	4.4	0.63
2	4.5	0.91
3	4.8	1.02
4	4.8	0.99
5	4.5	0.76
6	4.9	1.03
7	4.9	1.10
8	4.0	0.68
9	4.6	0.92
10	4.5	0.67
11	4.6	0.93
12	4.7	1.00
13	4.7	0.98
14	4.8	1.02
15	4.3	0.62
16	4.6	0.89
17	4.7	1.00
18	4.8	1.05
19	4.7	0.93
20	4.0	0.69
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.6	0.89

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▷ **Concentration:** 50%-1

No.	Length (cm)	Wet weight (g)
1	4.6	0.83
2	4.5	0.92
3	5.1	1.10
4	4.8	0.88
5	4.9	1.00
6	4.9	1.04
7	4.3	0.78
8	4.7	0.84
9	4.3	0.85
10	5.0	0.92
11	4.5	0.90
12	4.6	0.70
13	4.1	0.65
14	5.1	0.84
15	5.0	0.91
16	4.7	0.84
17	4.6	0.94
18	4.9	1.00
19	4.5	0.94
20	5.0	1.05
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.7	0.90

▷ **Concentration:** 50%-2

No.	Length (cm)	Wet weight (g)
1	4.9	0.94
2	5.0	1.00
3	4.1	0.69
4	4.6	0.92
5	3.9	0.65
6	4.2	0.67
7	3.8	0.60
8	4.6	0.92
9	4.4	0.83
10	4.7	1.00
11	5.1	1.10
12	4.3	0.67
13	4.9	1.02
14	4.8	0.83
15	4.5	0.81
16	3.9	0.50
17	4.3	0.64
18	4.7	0.84
19	4.4	0.72
20	4.7	0.90
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.5	0.81

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▷ **Concentration:** 50%-3

No.	Length (cm)	Wet weight (g)
1	5.1	1.00
2	4.5	0.92
3	5.0	1.00
4	4.5	0.92
5	5.0	0.88
6	4.5	0.83
7	4.6	0.91
8	4.7	0.93
9	4.9	1.00
10	5.1	1.05
11	4.6	0.88
12	4.7	0.89
13	4.4	0.78
14	5.0	1.04
15	5.1	1.10
16	4.7	0.93
17	4.6	0.83
18	4.2	0.69
19	4.9	1.04
20	5.0	0.99
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.8	0.93

▷ **Concentration:** 100%-1

No.	Length (cm)	Wet weight (g)
1	5.1	1.00
2	4.5	0.88
3	4.8	1.00
4	4.7	0.92
5	4.5	0.68
6	5.2	1.20
7	4.7	0.92
8	5.0	1.00
9	4.6	0.84
10	5.2	1.08
11	5.0	1.05
12	5.0	1.04
13	5.1	1.10
14	5.1	1.11
15	5.0	1.10
16	4.9	1.02
17	5.0	0.98
18	4.8	0.95
19	4.8	0.91
20	4.7	0.92
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.9	0.99

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▷ **Concentration:** 100%-2

No.	Length (cm)	Wet weight (g)
1	4.8	0.83
2	5.0	1.00
3	4.1	0.83
4	4.7	0.93
5	5.0	1.05
6	4.7	0.84
7	5.4	1.15
8	5.1	0.89
9	4.7	0.88
10	5.0	1.00
11	4.9	0.86
12	5.1	1.15
13	4.7	1.02
14	4.6	0.86
15	5.0	1.00
16	5.0	1.05
17	4.7	0.97
18	5.0	1.08
19	4.8	0.97
20	4.7	0.88
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.9	0.96

▷ **Concentration:** 100%-3

No.	Length (cm)	Wet weight (g)
1	4.9	0.92
2	5.2	1.05
3	5.0	1.00
4	5.0	1.02
5	4.2	0.71
6	5.0	0.92
7	5.1	0.88
8	5.1	1.02
9	4.6	0.78
10	4.9	0.83
11	5.0	1.03
12	4.9	0.92
13	5.0	1.05
14	4.9	0.92
15	5.0	1.00
16	4.8	0.79
17	5.0	0.99
18	5.0	1.03
19	4.8	0.84
20	5.0	1.05
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	4.9	0.94

4.1.2 Chronic toxicity test

4.1.2.1 ANNEX 1

Sheet of raw data for

"*Brachionus plicatilis*, Chronic Toxicity Test"

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COC code : BW-DBWT1104-KT-I002

Sheet of Raw Data – Rotifer – Chronic toxicity

[1] COC Client	KTM	Test date	2011. 6. 30 ~ 7. 4
Lab. manager	Myung-Baek Shon	Observer	So Eun Jo
Test organism	Brachionus plicatilis	Test duration	96h
[2] COTS	control, 6.25%, 12.5%, 25%, 50%, 100%	Test substance	KTM-SED5-1 (>32 psu)

Test Conditions and Methods

Temperature	25±1 ℃	Salinity	33±1 psu	Light	0 Lux, LD cycle = 0 L : 24 D
Medium	Filtered Seawater (0.45 μm) or other ( )				
[3] No. Inds.	5 inds.	Vessel type and volume	48 well plate		
[4] MV / [5] VV	1 mL / 1.5 mL	Method of observation (SOP No.)	#TP-014		

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] No. Inds.: Number of individuals/replication; [4] MV: Medium volume; [5] VV: Vessel volume

▶ Results (Accumulated Number of Live and Dead Organisms)

Date: 20 11. 7. 1 .(1 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	5	0	5	0	5	0	5	0	5	0	5	0		
2	4	1	5	0	5	0	5	0	5	0	5	0		
3	5	0	5	0	5	0	5	0	5	0	5	0		
4	5	0	5	0	5	0	5	0	5	0	5	0		
5	5	0	5	0	5	0	5	0	5	0	5	0		
6	~~5~~	~~0~~	5	0	5	0	5	0	~~5~~	~~0~~	5	0		
7	~~5~~	~~0~~	~~5~~	~~0~~	5	0	5	0	5	0	5	0		
8	5	0	~~5~~	~~0~~	5	0	~~5~~	~~0~~	~~5~~	~~0~~	~~5~~	~~0~~		
9	5	0	5	0	5	0	5	0	5	0	5	0		
10	5	0	5	0	5	0	~~5~~	~~0~~	5	0	5	0		
11	5	0	5	0	5	0	5	0	5	0	5	0		
12	5	0	5	0	~~5~~	~~0~~	5	0	5	0	~~5~~	~~0~~		
13	5	0	5	0	~~5~~	~~0~~	5	0	5	0	5	0		
14	~~5~~	~~0~~	~~5~~	~~0~~	~~5~~	~~1~~	~~5~~	~~0~~	5	0	5	0		
15	5	0	5	0	5	0	5	0	~~5~~	~~0~~	~~5~~	~~0~~		

*N: Total number of individuals. D: Number of dead individuals

Date: 20 11. 7 . 2 .(2 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	14	0	9	0	16	0	10	0	10	0	10	0		
2	10	1	12	0	11	0	10	0	10	0	8	0		
3	9	0	10	0	8	0	9	0	7	0	8	0		
4	10	0	11	0	10	0	9	0	7	0	9	0		
5	10	0	9	0	9	0	10	0	8	0	8	0		
6	~~9~~	~~0~~	7	0	8	0	10	0	~~6~~	~~0~~	8	0		
7	~~11~~	~~0~~	~~8~~	~~0~~	10	0	8	0	8	0	9	0		
8	12	0	~~9~~	~~0~~	9	0	~~8~~	~~0~~	~~5~~	~~0~~	~~9~~	~~0~~		
9	14	0	9	0	8	0	10	0	15	0	10	0		
10	13	0	8	0	7	0	~~10~~	~~0~~	8	0	8	0		
11	9	0	11	0	7	0	7	0	7	0	6	0		
12	10	0	9	0	~~6~~	~~0~~	10	0	7	0	~~9~~	~~0~~		
13	12	0	10	0	~~8~~	~~0~~	8	0	11	0	6	0		
14	~~10~~	~~0~~	~~9~~	~~0~~	~~8~~	~~1~~	~~7~~	~~0~~	11	0	9	0		
15	8	0	7	0	9	0	9	0	~~8~~	~~0~~	~~6~~	~~1~~		

Date: 20 11 . 7 . 3 .(3 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	23	0	17	0	22	0	23	0	18	0	14	0		
2	26	1	25	0	24	0	20	0	21	0	19	0		
3	17	0	21	0	21	0	19	0	13	0	14	0		
4	17	0	19	0	16	0	16	0	19	0	21	0		
5	15	0	13	0	16	0	22	0	12	0	16	0		
6	~~18~~	~~0~~	15	0	15	0	19	0	~~17~~	~~0~~	15	0		
7	~~19~~	~~0~~	~~16~~	~~0~~	14	0	17	0	9	0	18	0		
8	26	0	~~18~~	~~0~~	17	0	~~13~~	~~0~~	~~20~~	~~0~~	~~4~~	~~2~~		
9	27	0	14	0	12	0	19	0	19	0	18	0		
10	31	0	20	0	14	0	~~22~~	~~0~~	16	0	11	0		
11	21	0	18	0	13	0	13	0	17	0	16	0		
12	21	0	17	0	~~10~~	~~0~~	19	0	21	0	~~12~~	~~0~~		
13	21	0	18	0	~~4~~	~~0~~	18	0	20	0	18	0		
14	~~18~~	~~0~~	~~18~~	~~0~~	~~12~~	~~1~~	~~17~~	~~0~~	17	0	16	0		
15	20	0	13	0	12	0	15	0	~~15~~	~~0~~	~~12~~	~~1~~		

Date: 2011 . 7 . 4 .(4 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	51	0	39	0	47	0	44	0	49	0	51	0		
2	44	1	51	0	48	0	52	0	47	0	50	0		
3	44	0	40	0	48	0	48	0	43	0	54	0		
4	49	0	38	0	32	0	53	0	54	0	48	0		
5	45	0	36	0	41	0	52	0	39	0	38	0		
6	~~33~~	~~0~~	36	0	39	0	43	0	~~20~~	~~0~~	46	0		
7	~~42~~	~~0~~	~~31~~	~~0~~	29	0	39	0	47	0	50	0		
8	50	0	~~23~~	~~0~~	30	0	~~23~~	~~0~~	~~44~~	~~0~~	~~40~~	~~1~~		
9	52	0	49	0	34	0	27	0	42	0	48	0		
10	50	0	44	0	39	0	~~1~~	~~0~~	50	0	36	0		
11	47	0	44	0	31	0	25	0	53	0	40	0		
12	43	0	42	0	~~4~~	~~0~~	29	0	45	0	~~29~~	~~0~~		
13	44	0	39	0	~~20~~	~~0~~	35	0	54	0	34	0		
14	~~38~~	~~0~~	~~31~~	~~0~~	~~18~~	~~1~~	~~19~~	~~1~~	49	0	37	0		
15	46	0	36	0	28	0	37	0	~~29~~	~~0~~	~~25~~	~~1~~		

▶ Routine chemical and physical determinations

Date: 2011 . 6 .30 .(initial)

Terms		Concentration of test substance (%)					
		Control	6.25	12.5	25	50	100
Temp. (℃)	Initial	25.7	25.6	25.6	25.7	25.8	25.8
	Final	25.5	25.5	25.4	25.5	25.5	25.5
DO (mg/L)	Initial	4.48	4.50	4.58	4.43	4.52	4.53
	Final	4.04	3.95	4.05	4.07	4.28	4.03
pH	Initial	8.00	8.02	8.03	8.00	8.00	8.01
	Final	7.66	7.65	7.68	7.64	7.69	7.66

2011 . 7 . 5

Approved by _____

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COC code : BW-DBWT1104-KT-I004

Sheet of Raw Data – Rotifer – Chronic toxicity

[1] COC Client	KTM	Test date	2011.7.13 – 7.17
Lab. manager	Myung-Baek Shon	Observer	So Eun Jo
Test organism	Brachionus plicatilis	Test duration	96h
[2] COTS	control, 6.25, 12.5, 25, 50, 100%	Test substance	KTM-SHD5-3 (3-32psu)

Test Conditions and Methods

Temperature	25±1 ℃	Salinity	21±1 psu	Light	0 Lux, LD cycle = 0 L 24 D
Medium	Filtered Seawater (0.45 μm) or other ( )				
[3] No. Inds.	5 inds.	Vessel type and volume	4ft well plate		
[4] MV / [5] VV	1 mL / 1.5 mL	Method of observation (SOP No.)	#TP-014		

[1] COC: Chain Of Custody; [2] COTS: Concentration of test Substance; [3] No. Inds.: Number of individuals/replication; [4] MV: Medium volume; [5] VV: Vessel volume

▶ Results (Accumulated Number of Live and Dead Organisms)

Date: 2011.7.13.(1 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	5	0	~~5~~	~~0~~	5	0	~~5~~	~~0~~	5	0	5	0		
2	5	0	5	0	~~5~~	~~0~~	~~5~~	~~0~~	5	0	5	0		
3	5	0	5	0	5	0	5	0	~~5~~	~~0~~	5	0		
4	~~5~~	~~0~~	~~5~~	~~0~~	5	0	5	0	~~5~~	~~0~~	~~5~~	~~0~~		
5	5	0	5	0	~~5~~	~~0~~	5	0	5	0	~~5~~	~~0~~		
6	5	0	5	0	5	0	5	0	6	0	5	0		
7	5	0	5	0	5	0	5	0	5	0	5	0		
8	5	0	5	0	5	0	5	0	5	0	~~5~~	~~0~~		
9	5	0	5	0	~~5~~	~~0~~	5	0	~~5~~	~~0~~	5	0		
10	~~5~~	~~0~~	5	0	5	0	~~5~~	~~0~~	5	0	5	0		
11	5	0	5	0	5	0	5	0	5	0	5	0		
12	5	0	5	0	5	0	5	0	5	0	5	0		
13	5	0	5	0	5	0	5	0	5	0	5	0		
14	5	0	5	0	5	0	5	0	5	0	5	0		
15	~~5~~	~~0~~	~~5~~	~~0~~	5	0	5	0	5	0	5	0		

*N: Total number of individuals. D: Number of dead individuals

Date: 2011 . 7 . 14 .(2 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	11	1	~~16~~	~~0~~	14	0	~~14~~	~~0~~	13	1	21	0		
2	19	0	12	1	~~15~~	~~0~~	~~13~~	~~0~~	14	0	14	0		
3	17	0	16	0	13	0	17	0	~~16~~	~~0~~	15	0		
4	~~19~~	~~0~~	~~18~~	~~1~~	12	0	12	0	~~15~~	~~0~~	~~13~~	~~0~~		
5	18	0	17	0	~~13~~	~~0~~	13	0	13	0	~~14~~	~~0~~		
6	22	0	16	0	14	0	14	0	12	0	14	0		
7	21	0	18	0	15	0	17	1	13	0	15	0		
8	16	0	15	0	16	0	19	0	11	0	~~5~~	~~1~~		
9	19	1	12	0	~~14~~	~~1~~	17	0	~~12~~	~~0~~	10	0		
10	~~16~~	~~0~~	15	0	13	0	~~20~~	~~0~~	8	0	14	1		
11	14	0	12	0	16	0	13	0	9	0	15	1		
12	12	0	15	0	14	0	12	0	12	0	16	1		
13	16	0	14	0	15	0	12	1	11	0	17	0		
14	18	0	12	0	13	0	13	1	13	0	12	0		
15	~~18~~	~~0~~	~~11~~	~~1~~	13	0	12	1	11	0	13	0		

Date: 20 11 . 7 .15 .(3 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	32	1	~~39~~	~~0~~	27	0	~~22~~	~~0~~	35	1	34	0		
2	36	0	28	1	~~30~~	~~0~~	~~31~~	~~0~~	42	0	28	0		
3	45	0	42	0	27	0	28	0	~~30~~	~~0~~	34	0		
4	~~46~~	~~0~~	~~39~~	~~1~~	29	0	31	0	~~28~~	~~0~~	~~31~~	~~0~~		
5	47	0	33	0	~~22~~	~~0~~	31	0	34	0	~~30~~	~~0~~		
6	44	0	44	0	35	0	28	0	31	0	26	0		
7	44	0	44	0	34	0	28	1	32	0	32	1		
8	39	0	45	0	31	0	32	0	31	0	~~13~~	~~0~~		
9	36	1	28	0	~~18~~	~~1~~	37	0	~~32~~	~~0~~	23	0		
10	~~35~~	~~0~~	42	0	37	0	~~30~~	~~0~~	32	0	30	1		
11	36	0	38	0	48	0	32	0	27	0	33	1		
12	42	0	37	0	32	0	34	0	26	0	35	1		
13	44	0	47	0	35	0	37	1	29	0	38	0		
14	48	0	40	0	30	0	27	1	22	0	25	0		
15	~~44~~	~~0~~	~~28~~	~~1~~	35	0	33	1	28	0	39	0		

Date: 2011 . 7 . 17 .(4 days)

Repls.	Concentration of test substance (%)													
	Control		6.25		12.5		25		50		100			
	N	D	N	D	N	D	N	D	N	D	N	D	N	D
1	50	1	~~39~~	~~0~~	54	0	~~48~~	~~0~~	49	1	55	0		
2	52	0	46	1	~~48~~	~~0~~	~~45~~	~~0~~	60	0	51	0		
3	58	0	57	0	52	0	57	0	~~44~~	~~0~~	52	0		
4	~~48~~	~~0~~	~~43~~	~~1~~	61	0	56	0	~~48~~	~~0~~	~~45~~	~~0~~		
5	50	0	47	0	~~52~~	~~0~~	58	0	52	0	~~48~~	~~0~~		
6	59	0	50	0	52	0	57	0	47	0	51	0		
7	62	0	57	0	57	0	55	1	61	0	61	0		
8	63	0	51	0	51	0	60	0	54	0	~~27~~	~~1~~		
9	56	1	46	0	~~49~~	~~1~~	60	0	~~47~~	~~0~~	49	0		
10	~~45~~	~~0~~	53	0	50	0	~~40~~	~~0~~	53	0	48	1		
11	50	0	46	0	50	0	56	0	50	0	53	1		
12	52	0	46	0	50	0	52	0	62	0	49	1		
13	56	0	57	0	58	0	55	1	~~46~~	~~0~~	56	0		
14	59	0	55	0	57	0	53	1	48	0	62	0		
15	~~43~~	~~0~~	~~36~~	~~1~~	56	0	54	1	54	0	60	0		

▶ Routine chemical and physical determinations

Date: 20 11 . 7 . 13 .(initial)

Terms		Concentration of test substance (%)					
		Control	6.25	12.5	25	50	100
Temp. (℃)	Initial	25.2	25.0	25.2	25.1	25.6	25.7
	Final	24.6	24.6	24.5	25.0	24.5	24.2
DO (mg/L)	Initial	4.37	4.68	4.46	4.41	4.01	4.28
	Final	5.68	4.67	4.03	4.71	4.98	4.40
pH	Initial	7.72	7.67	7.69	7.68	7.67	7.65
	Final	7.76	7.75	7.78	7.76	7.74	7.72

2011. 7 . 18.

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COC Code. BW-DBWT1104-KT-I002

Test date	110630-110704	**Test substance**	KTM-S5D5-1(> 32 PSU)
End point	Population growth	**Numbers of Initial individual**	5 inds.
Study director	Jung-Im Park	**Numbers of replications**	12 repl.
Test Observer	So Eun Jo	**Test duration**	96h
Test organism	Rotifer, *Brachionus plicatilis*	**Observe duration**	24h
Light	Darkness	**Temperature**	25±1℃

■ **Raw Data**

Concentration of substance	**Control**					**6.25%**					**12.50%**				
Elapsed time (h) / **Replication**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**
1	5	5	14	23	51	5	5	9	17	39	5	5	15	22	47
2	5	4	10	26	44	5	5	12	25	51	5	5	11	24	48
3	5	5	9	17	44	5	5	10	21	40	5	5	8	21	48
4	5	5	10	17	49	5	5	11	19	38	5	5	10	16	32
5	5	5	10	15	45	5	5	9	13	36	5	5	9	16	41
6	5	5	12	26	50	5	5	7	15	36	5	5	8	15	39
7	5	5	14	27	52	5	5	9	14	49	5	5	10	14	29
8	5	5	13	31	50	5	5	8	20	44	5	5	9	17	30
9	5	5	9	21	47	5	5	11	18	44	5	5	8	12	34
10	5	5	10	21	43	5	5	9	17	42	5	5	7	14	39
11	5	5	12	21	44	5	5	10	18	39	5	5	7	13	31
12	5	5	8	20	46	5	5	7	13	36	5	5	9	12	28
Mean	**5**	**5**	**11**	**22**	**47**	**5**	**5**	**9**	**18**	**41**	**5**	**5**	**9**	**16**	**37**
STDEV (±)	0	0	2	5	3	0	0	2	4	5	0	0	2	4	8

Concentration of substance	**25.00%**					**50.00%**					**100.00%**				
Elapsed time (h) / **Replication**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**
1	5	5	10	23	44	5	5	10	18	49	5	5	10	14	57
2	5	5	10	20	52	5	5	10	21	47	5	5	8	19	50
3	5	5	9	19	48	5	5	7	13	43	5	5	8	14	54
4	5	5	9	16	53	5	5	7	19	54	5	5	9	21	48
5	5	5	10	22	52	5	5	8	12	39	5	5	8	16	38
6	5	5	10	19	43	5	5	8	9	41	5	5	8	15	46
7	5	5	8	17	39	5	5	15	19	42	5	5	9	18	50
8	5	5	10	19	27	5	5	8	16	50	5	5	10	18	48
9	5	5	7	13	25	5	5	7	17	53	5	5	8	11	36
10	5	5	10	19	29	5	5	7	21	45	5	5	6	16	40
11	5	5	8	18	35	5	5	11	20	54	5	5	6	18	34
12	5	5	9	15	37	5	5	11	17	49	5	5	9	16	37
Mean	**5**	**5**	**9**	**18**	**40**	**5**	**5**	**9**	**17**	**47**	**5**	**5**	**8**	**16**	**45**
STDEV (±)	0	0	1	3	10	0	0	2	4	5	0	0	1	3	8

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▶ **Population growth rate(r)**

Concentration of substance	Control					6.25%					12.50%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Replication	Total					Total					Total				
1	0.00	0.00	0.51	0.51	0.58	0.00	0.00	0.29	0.41	0.51	0.00	0.00	0.55	0.49	0.56
2	0.00	-0.22	0.35	0.55	0.54	0.00	0.00	0.44	0.54	0.58	0.00	0.00	0.39	0.52	0.57
3	0.00	0.00	0.29	0.41	0.54	0.00	0.00	0.35	0.48	0.52	0.00	0.00	0.24	0.48	0.57
4	0.00	0.00	0.35	0.41	0.57	0.00	0.00	0.39	0.45	0.51	0.00	0.00	0.35	0.39	0.46
5	0.00	0.00	0.35	0.37	0.55	0.00	0.00	0.29	0.32	0.49	0.00	0.00	0.29	0.39	0.53
6	0.00	0.00	0.44	0.55	0.58	0.00	0.00	0.17	0.37	0.49	0.00	0.00	0.24	0.37	0.51
7	0.00	0.00	0.51	0.56	0.59	0.00	0.00	0.29	0.34	0.57	0.00	0.00	0.35	0.34	0.44
8	0.00	0.00	0.48	0.61	0.58	0.00	0.00	0.24	0.46	0.54	0.00	0.00	0.29	0.41	0.45
9	0.00	0.00	0.29	0.48	0.56	0.00	0.00	0.39	0.43	0.54	0.00	0.00	0.24	0.29	0.48
10	0.00	0.00	0.35	0.48	0.54	0.00	0.00	0.29	0.41	0.53	0.00	0.00	0.17	0.34	0.51
11	0.00	0.00	0.44	0.48	0.54	0.00	0.00	0.35	0.43	0.51	0.00	0.00	0.17	0.32	0.46
12	0.00	0.00	0.24	0.46	0.55	0.00	0.00	0.17	0.32	0.49	0.00	0.00	0.29	0.29	0.43
Mean	0.00	-0.02	0.38	0.49	0.56	0.00	0.00	0.31	0.41	0.53	0.00	0.00	0.30	0.39	0.50

Concentration of substance	25.00%					50.00%					100.00%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Replication	Total					Total					Total				
1	0.00	0.00	0.35	0.51	0.54	0.00	0.00	0.35	0.43	0.57	0.00	0.00	0.35	0.34	0.61
2	0.00	0.00	0.35	0.46	0.59	0.00	0.00	0.35	0.48	0.56	0.00	0.00	0.24	0.45	0.58
3	0.00	0.00	0.29	0.45	0.57	0.00	0.00	0.17	0.32	0.54	0.00	0.00	0.24	0.34	0.59
4	0.00	0.00	0.29	0.39	0.59	0.00	0.00	0.17	0.45	0.59	0.00	0.00	0.29	0.48	0.57
5	0.00	0.00	0.35	0.49	0.59	0.00	0.00	0.24	0.29	0.51	0.00	0.00	0.24	0.39	0.51
6	0.00	0.00	0.35	0.45	0.54	0.00	0.00	0.24	0.20	0.53	0.00	0.00	0.24	0.37	0.55
7	0.00	0.00	0.24	0.41	0.51	0.00	0.00	0.55	0.45	0.53	0.00	0.00	0.29	0.43	0.58
8	0.00	0.00	0.35	0.45	0.42	0.00	0.00	0.24	0.39	0.58	0.00	0.00	0.35	0.43	0.57
9	0.00	0.00	0.17	0.32	0.40	0.00	0.00	0.17	0.41	0.59	0.00	0.00	0.24	0.26	0.49
10	0.00	0.00	0.35	0.45	0.44	0.00	0.00	0.17	0.48	0.55	0.00	0.00	0.09	0.39	0.52
11	0.00	0.00	0.24	0.43	0.49	0.00	0.00	0.39	0.46	0.59	0.00	0.00	0.09	0.43	0.48
12	0.00	0.00	0.29	0.37	0.50	0.00	0.00	0.39	0.41	0.57	0.00	0.00	0.29	0.39	0.50
Mean	0.00	0.00	0.30	0.43	0.51	0.00	0.00	0.28	0.40	0.56	0.00	0.00	0.24	0.39	0.55

Concentration of	Control					6.25%					12.50%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Mean	**0.00**	**-0.02**	**0.38**	**0.49**	**0.56**	**0.00**	**0.00**	**0.31**	**0.41**	**0.53**	**0.00**	**0.00**	**0.30**	**0.39**	**0.50**
STDEV (±)	0.00	0.06	0.09	0.07	0.02	0.00	0.00	0.09	0.07	0.03	0.00	0.00	0.11	0.08	0.05
Concentration of	25.00%					50.00%					100.00%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Mean	**0.00**	**0.00**	**0.30**	**0.43**	**0.51**	**0.00**	**0.00**	**0.28**	**0.40**	**0.56**	**0.00**	**0.00**	**0.24**	**0.39**	**0.55**
STDEV (±)	0.00	0.00	0.06	0.05	0.07	0.00	0.00	0.12	0.09	0.03	0.00	0.00	0.08	0.06	0.04

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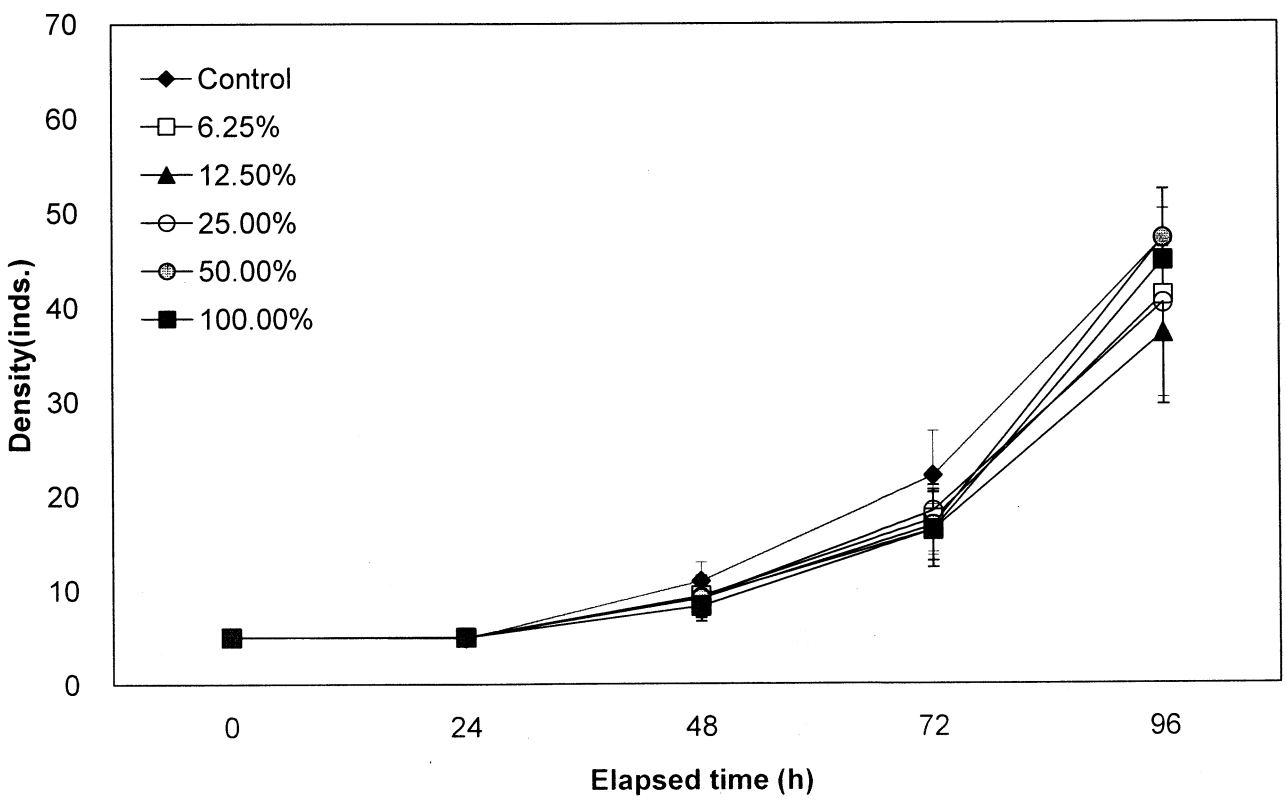
▶ **Growth inhibition(I, %)**

Elapsed time (h)	6.25%	12.50%	25.00%	50.00%	100.00%
24	-1.69	-1.69	-1.69	-1.69	-1.69
48	14.50	15.27	16.03	16.79	24.43
72	20.75	26.04	16.98	23.77	26.04
96	12.57	21.06	14.34	-0.18	4.78

▶ **Coefficient of variation (%)**

Elapsed time (h)	Control	6.25%	12.50%	25.00%	50.00%	100.00%
24	5.87	0.00	0.00	0.00	0.00	0.00
48	18.51	6.74	23.56	11.23	26.74	15.61
72	21.37	20.17	24.40	15.31	22.35	16.37
96	6.74	12.16	20.36	24.73	10.99	16.93



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COC Code. BW-DBWT1104-KT-I002

Test date	110630-110704	**Test substance**	KTM-S5D5-1(> 32 PSU)
Object of test	Population growth	**Numbers of Initial individual**	5 inds.
Study director	Jung-Im Park	**Numbers of groups**	12 repl.
Test Observer	So Eun Jo	**Test duration**	96 h
Test organism	Rotifer, *Brachionus plicatilis*	**Observe duration**	24 h
Light	Darkness	**Temperature**	25 ± 1 ℃

▶ **DO (mg/L)**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	4.48	4.50	4.58	4.43	4.52	4.53
Final	4.04	3.95	4.05	4.07	4.28	4.03

▶ **pH**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	8.00	8.02	8.03	8.00	8.00	8.01
Final	7.66	7.65	7.68	7.64	7.69	7.66

▶ **Temparature (℃)**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	25.70	25.60	25.60	25.70	25.80	25.80
Final	25.50	25.50	25.40	25.50	25.50	25.50

20 11 . 7 . 5 **Approved by** _____

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COC Code. BW-DBWT1104-KT-I004

Test date	110713-110717	**Test substance**	KTM-S5D5-3(3-32 psu)
End point	Population growth	**Numbers of Initial individual**	5 inds.
Study director	Jung-Im Park	**Numbers of replications**	12 repl.
Test Observer	So Eun Jo	**Test duration**	96h
Test organism	Rotifer, *Brachionus plicatilis*	**Observe duration**	24h
Light	Darkness	**Temperature**	25±1℃

■ **Raw Data**

Concentration of substance	**Control**					**6.25%**					**12.50%**				
Elapsed time (h) / **Replication**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**
1	5	5	11	32	50	5	5	12	28	46	5	5	14	27	54
2	5	5	19	36	52	5	5	16	42	57	5	5	13	27	52
3	5	5	17	45	58	5	5	17	33	47	5	5	12	29	61
4	5	5	18	47	50	5	5	16	44	50	5	5	14	35	52
5	5	5	22	44	59	5	5	19	44	57	5	5	15	34	57
6	5	5	21	44	62	5	5	15	45	51	5	5	16	37	51
7	5	5	16	39	63	5	5	12	28	46	5	5	13	37	50
8	5	5	19	36	56	5	5	15	42	53	5	5	16	48	50
9	5	5	14	36	50	5	5	12	38	46	5	5	14	32	50
10	5	5	12	42	52	5	5	15	39	46	5	5	15	35	58
11	5	5	16	44	56	5	5	14	44	57	5	5	13	30	57
12	5	5	18	48	59	5	5	12	40	55	5	5	13	35	56
Mean	**5**	**5**	**17**	**41**	**56**	**5**	**5**	**15**	**39**	**51**	**5**	**5**	**14**	**34**	**54**
STDEV (±)	0	0	3	5	5	0	0	2	6	5	0	0	1	6	4

Concentration of substance	**25.00%**					**50.00%**					**100.00%**				
Elapsed time (h) / **Replication**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**	**0**	**24**	**48**	**72**	**96**
1	5	5	17	28	57	5	5	13	35	49	5	5	21	34	55
2	5	5	12	31	56	5	5	14	42	60	5	5	14	28	51
3	5	5	13	37	58	5	5	13	34	52	5	5	15	34	52
4	5	5	14	28	57	5	6	12	31	47	5	5	14	26	51
5	5	5	17	28	55	5	5	13	32	61	5	5	15	32	61
6	5	5	19	32	60	5	5	11	31	54	5	5	10	23	49
7	5	5	17	37	60	5	5	8	32	53	5	5	14	30	48
8	5	5	13	32	56	5	5	9	27	50	5	5	15	33	53
9	5	5	12	34	52	5	5	12	26	62	5	6	16	35	49
10	5	5	12	37	55	5	5	11	29	46	5	5	17	38	56
11	5	5	13	27	53	5	5	13	22	48	5	5	12	25	62
12	5	5	12	33	54	5	5	11	28	54	5	5	13	39	60
Mean	**5**	**5**	**14**	**32**	**56**	**5**	**5**	**12**	**31**	**53**	**5**	**5**	**15**	**31**	**54**
STDEV (±)	0	0	3	4	3	0	0	2	5	5	0	0	3	5	5

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▶ **Population growth rate(r)**

Concentration of substance	Control					6.25%					12.50%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Replication	Total					Total					Total				
1	0.00	0.00	0.39	0.62	0.58	0.00	0.00	0.44	0.57	0.55	0.00	0.00	0.51	0.56	0.59
2	0.00	0.00	0.67	0.66	0.59	0.00	0.00	0.58	0.71	0.61	0.00	0.00	0.48	0.56	0.59
3	0.00	0.00	0.61	0.73	0.61	0.00	0.00	0.61	0.63	0.56	0.00	0.00	0.44	0.59	0.63
4	0.00	0.00	0.64	0.75	0.58	0.00	0.00	0.58	0.72	0.58	0.00	0.00	0.51	0.65	0.59
5	0.00	0.00	0.74	0.72	0.62	0.00	0.00	0.67	0.72	0.61	0.00	0.00	0.55	0.64	0.61
6	0.00	0.00	0.72	0.72	0.63	0.00	0.00	0.55	0.73	0.58	0.00	0.00	0.58	0.67	0.58
7	0.00	0.00	0.58	0.68	0.63	0.00	0.00	0.44	0.57	0.55	0.00	0.00	0.48	0.67	0.58
8	0.00	0.00	0.67	0.66	0.60	0.00	0.00	0.55	0.71	0.59	0.00	0.00	0.58	0.75	0.58
9	0.00	0.00	0.51	0.66	0.58	0.00	0.00	0.44	0.68	0.55	0.00	0.00	0.51	0.62	0.58
10	0.00	0.00	0.44	0.71	0.59	0.00	0.00	0.55	0.68	0.55	0.00	0.00	0.55	0.65	0.61
11	0.00	0.00	0.58	0.72	0.60	0.00	0.00	0.51	0.72	0.61	0.00	0.00	0.48	0.60	0.61
12	0.00	0.00	0.64	0.75	0.62	0.00	0.00	0.44	0.69	0.60	0.00	0.00	0.48	0.65	0.60
Mean	0.00	0.00	0.60	0.70	0.60	0.00	0.00	0.53	0.68	0.58	0.00	0.00	0.51	0.63	0.59
Concentration of substance	25.00%					50.00%					100.00%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Replication	Total					Total					Total				
1	0.00	0.00	0.61	0.57	0.61	0.00	0.00	0.48	0.65	0.57	0.00	0.00	0.72	0.64	0.60
2	0.00	0.00	0.44	0.61	0.60	0.00	0.00	0.51	0.71	0.62	0.00	0.00	0.51	0.57	0.58
3	0.00	0.00	0.48	0.67	0.61	0.00	0.00	0.48	0.64	0.59	0.00	0.00	0.55	0.64	0.59
4	0.00	0.00	0.51	0.57	0.61	0.00	0.18	0.44	0.61	0.56	0.00	0.00	0.51	0.55	0.58
5	0.00	0.00	0.61	0.57	0.60	0.00	0.00	0.48	0.62	0.63	0.00	0.00	0.55	0.62	0.63
6	0.00	0.00	0.67	0.62	0.62	0.00	0.00	0.39	0.61	0.59	0.00	0.00	0.35	0.51	0.57
7	0.00	0.00	0.61	0.67	0.62	0.00	0.00	0.24	0.62	0.59	0.00	0.00	0.51	0.60	0.57
8	0.00	0.00	0.48	0.62	0.60	0.00	0.00	0.29	0.56	0.58	0.00	0.00	0.55	0.63	0.59
9	0.00	0.00	0.44	0.64	0.59	0.00	0.00	0.44	0.55	0.63	0.00	0.18	0.58	0.65	0.57
10	0.00	0.00	0.44	0.67	0.60	0.00	0.00	0.39	0.59	0.55	0.00	0.00	0.61	0.68	0.60
11	0.00	0.00	0.48	0.56	0.59	0.00	0.00	0.48	0.49	0.57	0.00	0.00	0.44	0.54	0.63
12	0.00	0.00	0.44	0.63	0.59	0.00	0.00	0.39	0.57	0.59	0.00	0.00	0.48	0.68	0.62
Mean	0.00	0.00	0.52	0.62	0.60	0.00	0.02	0.42	0.60	0.59	0.00	0.02	0.53	0.61	0.59

Concentration of	Control					6.25%					12.50%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Mean	**0.00**	**0.00**	**0.60**	**0.70**	**0.60**	**0.00**	**0.00**	**0.53**	**0.68**	**0.58**	**0.00**	**0.00**	**0.51**	**0.63**	**0.59**
STDEV (±)	0.00	0.00	0.11	0.04	0.02	0.00	0.00	0.08	0.06	0.02	0.00	0.00	0.05	0.05	0.02
Concentration of	25.00%					50.00%					100.00%				
Elapsed time (h)	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Mean	**0.00**	**0.00**	**0.52**	**0.62**	**0.60**	**0.00**	**0.02**	**0.42**	**0.60**	**0.59**	**0.00**	**0.02**	**0.53**	**0.61**	**0.59**
STDEV (±)	0.00	0.00	0.08	0.04	0.01	0.00	0.05	0.08	0.05	0.03	0.00	0.05	0.09	0.06	0.02

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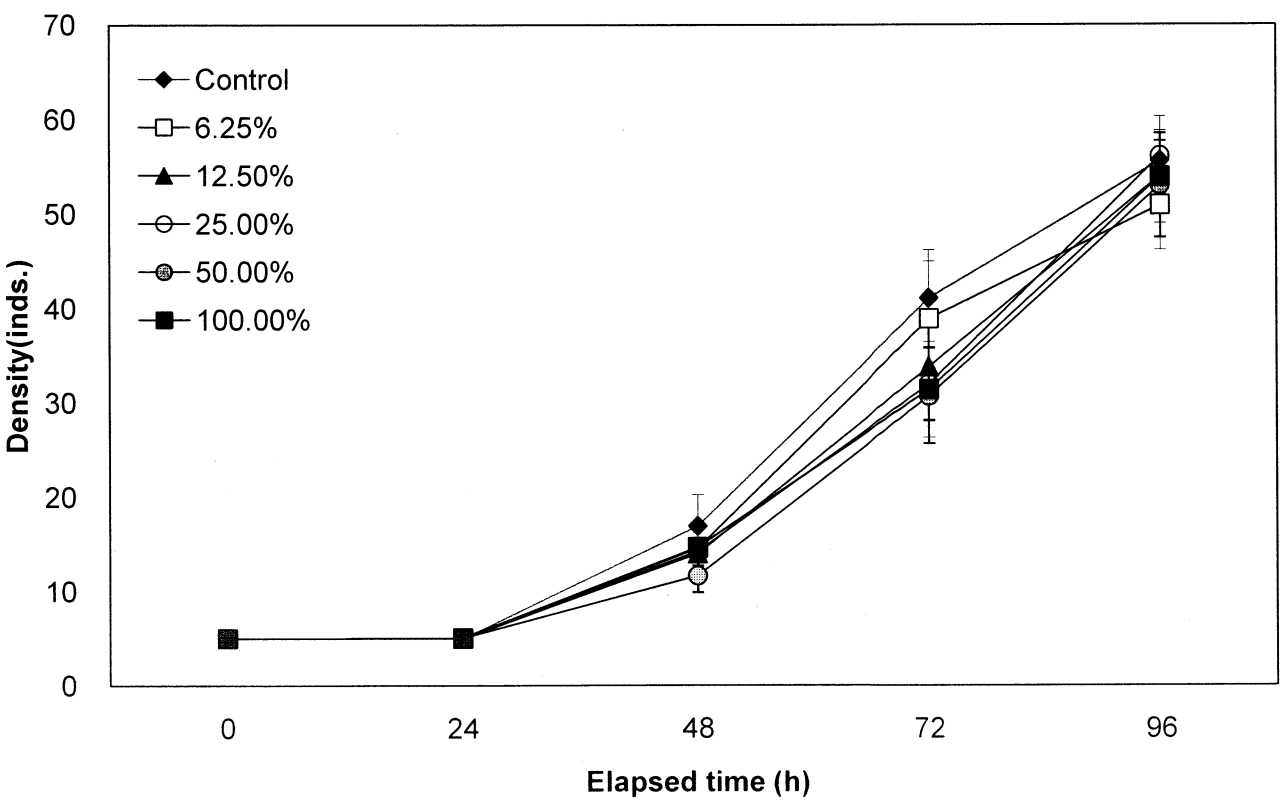
▶ **Growth inhibition(I, %)**

Elapsed time (h)	6.25%	12.50%	25.00%	50.00%	100.00%
24	0.00	0.00	0.00	-1.67	-1.67
48	13.79	17.24	15.76	31.03	13.30
72	5.27	17.65	22.11	25.15	23.53
96	8.40	2.85	-0.90	4.65	3.00

▶ **Coefficient of variation (%)**

Elapsed time (h)	Control	6.25%	12.50%	25.00%	50.00%	100.00%
24	0.00	0.00	0.00	0.00	5.68	5.68
48	19.76	8.49	9.14	17.73	15.22	18.46
72	12.47	15.67	16.90	11.69	16.47	16.20
96	8.49	9.27	6.88	4.46	10.37	9.07



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COC Code. BW-DBWT1104-KT-I004

Test date	110713-110717	**Test substance**	KTM-S5D5-3(3-32 psu)
Object of test	Population growth	**Numbers of Initial individual**	5 inds.
Study director	Jung-Im Park	**Numbers of groups**	12 repl.
Test Observer	So Eun Jo	**Test duration**	96 h
Test organism	Rotifer, *Brachionus plicatilis*	**Observe duration**	24 h
Light	Darkness	**Temperature**	25 ± 1 ℃

▶ **DO (mg/L)**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	4.37	4.68	4.86	4.41	4.01	4.78
Final	5.18	4.67	4.73	4.71	4.98	4.40

▶ **pH**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	7.72	7.67	7.69	7.68	7.67	7.65
Final	7.76	7.75	7.78	7.76	7.74	7.72

▶ **Temparature (℃)**

Concentration	Control	6.25%	12.50%	25.00%	50.00%	100.00%
Initial	25.20	25.00	25.20	25.10	25.60	25.70
Final	24.60	24.60	24.50	25.00	24.50	24.20

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4.1.2.2 ANNEX 2

Sheet of raw data for

"The olive flounder fish, *Paralichthys olivaceus*, Chronic Toxicity Test"

Marine Eco-Technology Institute Co., Ltd.
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COC code : BW-DBWT1104-KT-F1002

Date : 20 11 . 6 . 20.

Sheet of Raw Data – Fish – Chronic toxicity

[1] COC Client	KT Marine Co., Ltd.	End point	Survival
Lab. manager	Myung Baek Shon	Observer	Tae Won Kim
Test organism	Eggs of Paralichthys olivaceus	Test duration	7 days
[2] COTS	0. 6.25, 12.5, 25, 50, 100%	Test substance	KTM-S5D5-1.2 (>32 psu)

Test Conditions and Methods

Temperature	20±1 ℃	Salinity	33 psu	Light	2,000 Lux, LD cycle = 16 L : 8 D
Medium	Filtered Seawater (1 μm) or other ( )				
Initial biomass	20	Vessel type and volume	1l Circle Chamber with mesh (50μm)		
[3] MV / [4] VV	0.8 L / 1 L	Method of observation (SOP No.)	SOP#TP-001		

▶ Results (Accumulated Number of Dead Organisms)

Conc.: Control	Time (min)						Conc.: 6.25	Time (min)						Conc.: 12.5	Time (min)					
Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180
1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0
3	0	0	0	0	0	0	3	0	0	0	0	0	0	3	0	0	0	0	0	0
Remark							Remark							Remark						

Conc.: 25	Time (min)						Conc.: 50	Time (min)						Conc.: 100	Time (min)					
Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180
1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0
3	0	0	0	0	0	0	3	0	0	0	0	0	0	3	0	0	0	0	0	0
Remark							Remark							Remark						

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
Control	1	0/0	0/0	1/0	1/0	1/0	1/0	1/0	1/0
	2	0/0	1/1	3/3	3/3	4/3	6/3	8/3	8/3
	3	0/0	0/0	0/0	0/0	1/0	2/0	2/0	3/0
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
6.25%	1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	2	0/0	0/0	1/0	1/0	2/0	2/0	3/0	4/0
	3	0/0	0/0	3/0	4/0	5/0	5/0	5/0	5/0
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
12.5%	1	0/0	0/0	0/0	0/0	1/0	1/0	1/0	1/0
	2	0/0	0/0	1/1	1/1	1/1	2/1	2/1	4/1
	3	0/0	2/2	4/3	6/3	7/3	7/3	7/3	10/3
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
25%	1	0/0	1/1	2/1	3/1	3/1	3/1	3/1	3/1
	2	0/0	1/1	3/1	4/1	4/1	4/1	4/1	7/1
	3	0/0	0/0	3/1	3/1	4/1	4/1	4/1	7/1
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
50%	1	0/0	0/0	1/0	6/0	7/0	7/0	8/0	8/0
	2	0/0	0/1	2/1	7/1	9/1	10/1	11/1	12/1
	3	0/0	0/2	7/2	8/2	11/2	12/2	12/2	12/2
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
100%	1	0/0	1/1	2/1	2/1	3/1	4/1	4/1	4/1
	2	0/0	0/0	0/0	0/0	2/0	2/0	2/0	4/0
	3	0/0	1/1	1/1	1/1	2/1	3/1	3/1	5/1
	Remark								

▶ Routine chemical and physical determinations

Conc.:		Control							
Days		1	2	3	4	5	6	7	8
Temp. (℃)	Initial	20.1		19.6		19.5		20.1	
	Final		19.6	19.8	19.7	20.1	20.2	19.9	20.0
Salinity (psu)	Initial	33.3		33.2		33.5		33.9	
	Final		33.2	33.1	33.0	33.4	33.3	33.4	33.8
DO (mg/L)	Initial	6.41		5.01		6.01		6.58	
	Final		5.32	5.24	5.47	5.11	7.03	5.35	6.33
pH	Initial	7.85		7.84		7.70		7.56	
	Final		7.82	7.81	7.89	7.96	7.63	7.75	7.82

Conc.:		6.25							
Days		1	2	3	4	5	6	7	8
Temp. (℃)	Initial	20.0		19.6		19.7		20.0	
	Final		19.5	19.6	19.7	19.7	19.9	19.8	19.9
Salinity (psu)	Initial	33.3		33.2		33.4		33.8	
	Final		33.2	33.1	33.1	33.8	33.3	33.4	33.8
DO (mg/L)	Initial	6.10		5.27		5.70		6.38	
	Final		5.16	5.88	5.44	5.57	6.57	5.11	6.06
pH	Initial	7.75		7.86		7.80		7.59	
	Final		7.84	7.81	7.88	7.90	7.74	7.78	7.84

Conc.:		12.5							
Days		1	2	3	4	5	6	7	8
Temp. (℃)	Initial	19.9		19.6		19.7		20.0	
	Final		19.5	19.5	19.7	19.6	19.8	19.8	19.9
Salinity (psu)	Initial	33.4		33.3		33.6		33.7	
	Final		33.3	33.3	33.3	33.7	33.3	33.3	33.7
DO (mg/L)	Initial	6.20		5.94		5.32		6.08	
	Final		5.72	5.11	5.01	5.09	6.10	5.09	5.40
pH	Initial	7.74		7.74		7.82		7.61	
	Final		7.83	7.81	7.84	7.81	7.74	7.78	7.84

Conc.:		25							
Days		1	2	3	4	5	6	7	8
Temp. (℃)	Initial	19.8		19.6		19.6		19.7	
	Final		19.4	19.5	19.7	19.5	19.8	19.5	19.8
Salinity (psu)	Initial	33.2		33.2		33.5		33.7	
	Final		33.2	33.2	33.3	33.6	33.2	33.3	33.6
DO (mg/L)	Initial	6.17		5.80		5.92		5.86	
	Final		5.60	5.71	5.07	5.14	5.80	5.93	5.82
pH	Initial	7.67		7.81		7.74		7.61	
	Final		7.81	7.86	7.88	7.89	7.75	7.78	7.83

Conc.:		50								100								
Days		1	2	3	4	5	6	7	8	Days	1	2	3	4	5	6	7	8
Temp. (℃)	Initial	19.7		19.5		19.0		19.7		Initial	19.5		19.6		19.3		19.6	
	Final		19.3	19.4	19.6	19.4	19.7	19.6	19.7	Final		19.4	19.4	19.6	19.4	19.6	19.6	19.6
Salinity (psu)	Initial	33.2		33.4		33.4		33.8		Initial	33.2		33.3		33.5		33.7	
	Final		33.2	33.3	33.4	33.7	33.3	33.3	33.7	Final		33.2	33.2	33.1	33.6	33.2	33.2	33.7
DO (mg/L)	Initial	5.39		6.00		5.72		5.85		Initial	5.96		5.02		5.72		5.68	
	Final		5.78	6.10	5.77	5.62	5.56	5.84	5.71	Final		5.00	6.00	5.73	5.30	5.32	5.82	5.73
pH	Initial	7.71		7.81		7.79		7.59		Initial	7.67		7.82		7.75		7.54	
	Final		7.81	7.87	7.86	7.82	7.75	7.77	7.82	Final		7.61	7.85	7.83	7.82	7.72	7.76	7.81

2011. 7. 7

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COC code : BW-DBWT1104-KT-F002

Sheet of Raw Data – Fish – Condition index

Date	110630-110707	Signature	Jin Hee Kim
Lab. manger	Myung-Baek Shon		
Test Observer	Tae-Won Kim		
Test organism	Embryo and larvae of Paralichthys olivaceus	End point	Survival
Test substance	KTM-SSDS-1,2(>32psu)		

▶ **Condition index of test organism**

▷ **Concentration:** Control-1 ▷ **Concentration:** Control-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	3.0	0.1	1	2.8	0.1
2	2.9	0.1	2	2.7	0.1
3	2.6	0.1	3	2.9	0.1
4	2.7	0.1	4	3.0	0.1
5	2.7	0.1	5	3.0	0.1
6	2.7	0.1	6	2.9	0.1
7	2.7	0.1	7	2.9	0.1
8	2.8	0.1	8	2.9	0.1
9	2.6	0.1	9	2.9	0.1
10	2.9	0.1	10	2.7	0.1
11	2.7	0.1	11	2.7	0.1
12	2.8	0.1	12	2.8	0.1
13	2.8	0.1	13	2.9	0.1
14	2.7	0.1	14	2.7	0.1
15	2.8	0.1	15	2.8	0.1
16	2.6	0.1	16	2.8	0.1
17	2.9	0.1	17	2.9	0.1
18	2.7	0.1	18	2.7	0.1
19	2.8	0.1	19	2.8	0.1
20	2.8	0.1	20	2.9	0.1
21	2.8	0.1	21	2.8	0.1
22	2.8	0.1	22	2.9	0.1
23	2.7	0.1	23		
24	2.7	0.1	24		
25	2.8	0.1	25		
26	3.0	0.1	26		
27	2.7	0.1	27		
28	2.7	0.1	28		
29	2.8	0.1	29		
30			30		
Mean	2.8	0.1	Mean	2.8	0.1

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▷Concentration: Control-3

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.7	0.1
3	2.8	0.1
4	2.9	0.1
5	2.7	0.1
6	2.8	0.1
7	2.9	0.1
8	2.8	0.1
9	2.7	0.1
10	2.8	0.1
11	2.9	0.1
12	2.8	0.1
13	2.4	0.1
14	2.6	0.1
15	2.6	0.1
16	2.9	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.7	0.1
21	2.9	0.1
22	2.9	0.1
23	2.8	0.1
24	2.8	0.1
25	2.7	0.1
26	2.7	0.1
27	3.0	0.1
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 6.25%-1

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.6	0.1
3	2.7	0.1
4	2.8	0.1
5	2.8	0.1
6	2.9	0.1
7	2.7	0.1
8	2.8	0.1
9	2.6	0.1
10	2.6	0.1
11	2.6	0.1
12	2.7	0.1
13	2.8	0.1
14	2.9	0.1
15	2.7	0.1
16	2.7	0.1
17	2.8	0.1
18	2.9	0.1
19	2.7	0.1
20	2.7	0.1
21	2.8	0.1
22	2.8	0.1
23	2.9	0.1
24	2.8	0.1
25	2.7	0.1
26	2.8	0.1
27	2.8	0.1
28	2.9	0.1
29	2.8	0.1
30	2.9	0.1
Mean	2.8	0.1

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▷ Concentration: 6.25%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.8	0.1
3	2.8	0.1
4	2.7	0.1
5	2.9	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.8	0.1
10	2.8	0.1
11	2.8	0.1
12	2.9	0.1
13	2.8	0.1
14	2.7	0.1
15	2.9	0.1
16	2.9	0.1
17	2.7	0.1
18	2.7	0.1
19	2.8	0.1
20	2.9	0.1
21	2.9	0.1
22	2.7	0.1
23	2.7	0.1
24	2.7	0.1
25	2.7	0.1
26	2.8	0.1
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 6.25%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.8	0.1
10	2.8	0.1
11	2.7	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.7	0.1
16	2.8	0.1
17	2.9	0.1
18	2.8	0.1
19	2.7	0.1
20	2.8	0.1
21	2.9	0.1
22	2.8	0.1
23	2.8	0.1
24	2.7	0.1
25	2.6	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷Concentration: 12.5%-1

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.8	0.1
4	2.9	0.1
5	2.9	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.8	0.1
10	2.7	0.1
11	2.7	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.6	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.9	0.1
21	2.7	0.1
22	2.8	0.1
23	2.7	0.1
24	2.8	0.1
25	2.8	0.1
26	2.9	0.1
27	2.7	0.1
28	2.8	0.1
29	2.8	0.1
30		
Mean	2.8	0.1

▷ Concentration: 12.5%-2

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.7	0.1
3	2.7	0.1
4	2.7	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.7	0.1
10	2.8	0.1
11	2.9	0.1
12	2.6	0.1
13	2.7	0.1
14	2.8	0.1
15	2.7	0.1
16	2.9	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.7	0.1
21	2.8	0.1
22	2.8	0.1
23	2.9	0.1
24	2.7	0.1
25	2.8	0.1
26	2.8	0.1
27		
28		
29		
30		
Mean	2.8	0.1

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▷ Concentration: 12.5%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.8	0.1
10	2.7	0.1
11	2.7	0.1
12	2.8	0.1
13	2.9	0.1
14	2.7	0.1
15	2.7	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19	2.8	0.1
20	2.8	0.1
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 25%-1

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.7	0.1
4	2.6	0.1
5	2.7	0.1
6	2.8	0.1
7	2.9	0.1
8	2.8	0.1
9	2.8	0.1
10	2.8	0.1
11	2.8	0.1
12	2.9	0.1
13	2.9	0.1
14	2.9	0.1
15	2.9	0.1
16	2.9	0.1
17	3.0	0.1
18	3.0	0.1
19	2.8	0.1
20	2.8	0.1
21	2.6	0.1
22	2.9	0.1
23	2.8	0.1
24	2.7	0.1
25	2.7	0.1
26	2.7	0.1
27	2.8	0.1
28		
29		
30		
Mean	2.8	0.1

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▷Concentration: 25%-2

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.7	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.7	0.1
10	2.7	0.1
11	3.0	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.7	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19	2.8	0.1
20	2.8	0.1
21	2.8	0.1
22	2.8	0.1
23	2.8	0.1
24		
25		
26		
27		
28		
29		
30		
Mean	2.7	0.1

▷ Concentration: 25%-3

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.4	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.9	0.1
10	2.8	0.1
11	2.8	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.8	0.1
17	2.7	0.1
18	2.7	0.1
19	2.7	0.1
20	2.7	0.1
21	2.8	0.1
22	2.8	0.1
23	2.9	0.1
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷ Concentration: 50%-1

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.9	0.1
4	2.9	0.1
5	2.8	0.1
6	2.8	0.1
7	2.7	0.1
8	2.7	0.1
9	2.8	0.1
10	2.7	0.1
11	2.7	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.9	0.1
16	2.8	0.1
17	2.7	0.1
18	2.7	0.1
19	2.7	0.1
20	2.7	0.1
21	2.8	0.1
22	2.8	0.1
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 50%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.7	0.1
3	2.7	0.1
4	2.8	0.1
5	2.8	0.1
6	2.9	0.1
7	2.8	0.1
8	2.7	0.1
9	2.7	0.1
10	2.7	0.1
11	2.8	0.1
12	2.8	0.1
13	2.7	0.1
14	2.7	0.1
15	2.7	0.1
16	2.8	0.1
17	2.8	0.1
18	2.9	0.1
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷Concentration: 50%-3 ▷ Concentration: 100%-1

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.8	0.1	1	2.7	0.1
2	2.7	0.1	2	2.8	0.1
3	2.7	0.1	3	2.7	0.1
4	2.8	0.1	4	2.7	0.1
5	2.8	0.1	5	2.7	0.1
6	2.8	0.1	6	2.7	0.1
7	2.8	0.1	7	2.7	0.1
8	2.7	0.1	8	2.8	0.1
9	2.8	0.1	9	2.7	0.1
10	2.7	0.1	10	2.8	0.1
11	2.8	0.1	11	2.9	0.1
12	2.8	0.1	12	2.7	0.1
13	2.9	0.1	13	2.8	0.1
14	2.7	0.1	14	2.7	0.1
15	2.8	0.1	15	2.8	0.1
16	2.7	0.1	16	2.7	0.1
17	2.7	0.1	17	2.7	0.1
18	2.8	0.1	18	2.8	0.1
19			19	2.8	0.1
20			20	2.8	0.1
21			21	2.9	0.1
22			22	2.8	0.1
23			23	2.7	0.1
24			24	2.7	0.1
25			25	2.7	0.1
26			26	2.8	0.1
27			27		
28			28		
29			29		
30			30		
Mean	2.8	0.1	Mean	2.8	0.1

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▷Concentration: 100%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.7	0.1
3	2.8	0.1
4	2.7	0.1
5	2.8	0.1
6	2.8	0.1
7	2.7	0.1
8	2.7	0.1
9	2.8	0.1
10	2.9	0.1
11	2.8	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.7	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.8	0.1
21	2.9	0.1
22	2.8	0.1
23	2.7	0.1
24	2.7	0.1
25	2.7	0.1
26	2.8	0.1
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 100%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.9	0.1
9	2.7	0.1
10	2.8	0.1
11	2.8	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.7	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.9	0.1
21	2.8	0.1
22	2.7	0.1
23	2.7	0.1
24	2.7	0.1
25	2.8	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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COC code : BW-DBNT1104-KT-F004

Date : 2011 . 9 . 13.

Sheet of Raw Data – Fish – Chronic toxicity

[1] COC Client	KT Marine CO., Ltd.	End point	Survival
Lab. manager	Myung Baek Shon	Observer	Tae Won Kim
Test organism	Eggs of Paralichthys olivaceus	Test duration	11 days
[2] COTS	0, 6.25, 12.5, 25, 50, 100%	Test substance	KTM-SSD5-3 (3-32 psu)

Test Conditions and Methods

Temperature	20±1 ℃	Salinity	22 psu	Light	2,000 Lux, LD cycle = 16 L : 8 D
Medium	Filtered Seawater (/ μm) or other ( )				
Initial biomass	30	Vessel type and volume	1L Circle chamber with mesh (50 μm)		
[3] MV / [4] VV	0.8 L / / L	Method of observation (SOP No.)	SOP#TP-007		

▶ Results (Accumulated Number of Dead Organisms)

Conc.: Control	Time (min)						Conc.: 6.25	Time (min)						Conc.: 12.5	Time (min)					
Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180
1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0
3	0	0	0	0	0	0	3	0	0	0	0	0	0	3	0	0	0	0	0	0
Remark							Remark							Remark						

Conc.: 25	Time (min)						Conc.: 50	Time (min)						Conc.: 100	Time (min)					
Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180	Repl.	30	60	90	120	150	180
1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
2	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0
3	0	0	0	0	0	0	3	0	0	0	0	0	0	3	0	0	0	0	0	0
Remark							Remark							Remark						

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
Control	1	0/0	2/2	2/2	4/2	7/2	7/2	8/2	8/2
	2	0/0	1/1	1/1	6/1	6/1	8/1	8/1	8/1
	3	0/0	3/3	5/3	9/3	9/3	9/3	9/3	9/3
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
6pt	1	0/0	1/1	1/1	8/1	9/1	9/1	9/1	9/1
	2	0/0	3/3	6/3	7/4	7/4	8/4	8/4	8/4
	3	0/0	1/1	2/1	3/1	3/1	3/1	4/1	5/1
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
12pt	1	0/0	3/2	5/2	6/2	7/2	8/2	13/2	13/2
	2	0/0	4/4	7/4	9/5	9/5	9/5	12/5	12/5
	3	0/0	2/2	2/2	6/2	6/2	7/2	8/2	8/2
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
24	1	0/0	2/2	2/2	3/2	3/2	5/2	5/2	5/2
	2	0/0	2/2	2/2	3/2	4/2	4/2	5/2	7/2
	3	0/0	1/1	2/1	2/1	2/1	2/1	3/1	6/1
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
50	1	0/0	2/2	3/2	3/2	3/2	4/2	5/2	5/2
	2	0/0	0/0	0/0	0/0	0/0	1/0	2/0	4/0
	3	0/0	0/0	2/0	4/0	5/0	5/0	5/0	5/0
	Remark								

Conc.	Repl.	Days							
		Initial	1	2	3	4	5	6	7
100	1	0/0	1/1	2/1	4/1	5/1	7/1	7/1	7/1
	2	0/0	0/0	1/0	1/0	3/0	7/0	10/0	10/0
	3	0/0	1/1	2/1	6/1	6/1	8/1	8/1	8/1
	Remark								

▶ Routine chemical and physical determinations

Conc.:		Control							
Days		0	1	2	3	4	5	6	7
Temp. (℃)	Initial	20.6		20.1		19.9		19.6	
	Final		20.2	19.9	19.7	19.7	19.8	19.6	19.6
Salinity (psu)	Initial	20.8		20.7		20.8		20.8	
	Final		20.8	20.9	20.8	20.7	20.7	20.8	20.8
DO (mg/L)	Initial	5.78		6.25		6.00		6.53	
	Final		5.99	6.04	6.13	6.75	6.21	5.51	5.49
pH	Initial	7.72		7.35		7.67		7.82	
	Final		7.77	7.73	7.73	7.77	7.76	7.79	7.82

Conc.:		6.25							
Days		0	1	2	3	4	5	6	7
Temp. (℃)	Initial	20.6		19.9		19.9		19.6	
	Final		19.9	19.5	19.8	19.8	19.6	19.5	19.5
Salinity (psu)	Initial	20.8	1	20.8		20.8		20.8	
	Final		20.9	20.9	20.9	20.9	20.8	20.8	20.8
DO (mg/L)	Initial	5.69		5.57		6.72		6.29	
	Final		6.04	5.97	6.02	5.97	6.36	5.97	5.31
pH	Initial	7.72		7.48		7.69		7.81	
	Final		7.79	7.73	7.73	7.78	7.75	7.77	7.82

Conc.:		12.5							
Days		0	1	2	3	4	5	6	7
Temp. (℃)	Initial	20.6		20.0		19.9		19.5	
	Final		19.7	19.3	19.5	19.9	19.4	19.4	19.4
Salinity (psu)	Initial	20.8		20.8		20.9		20.8	
	Final		20.8	20.8	20.9	20.9	20.9	20.9	20.8
DO (mg/L)	Initial	5.60		6.03		6.14		5.79	
	Final		6.00	5.77	6.71	6.02	5.77	6.17	5.36
pH	Initial	7.76		7.74		7.73		7.78	
	Final		7.80	7.73	7.72	7.76	7.74	7.76	7.80

Conc.:		25							
Days		0	1	2	3	4	5	6	7
Temp. (℃)	Initial	20.4		19.7		20.0		19.4	
	Final		19.6	19.3	19.9	20.0	19.3	19.4	19.4
Salinity (psu)	Initial	20.8		20.8		20.8		20.8	
	Final		20.9	20.8	20.8	20.8	20.9	20.8	20.8
DO (mg/L)	Initial	6.14		5.73		5.97		5.74	
	Final		6.13	5.65	6.24	6.52	6.93	6.18	6.03
pH	Initial	7.77		7.49		7.70		7.79	
	Final		7.81	7.73	7.70	7.73	7.75	7.77	7.81

Conc.:		50								100								
Days		0	1	2	3	4	5	6	7	Days	0	1	2	3	4	5	6	7
Temp. (℃)	Initial	20.4		19.6		20.0		19.4		Initial	20.0		19.6		19.6		19.3	
	Final		19.6	19.2	19.9	20.0	19.3	19.4	19.3	Final		19.5	19.2	19.9	19.4	19.3	19.4	19.3
Salinity (psu)	Initial	20.8		20.8		20.7		20.8		Initial	20.8		20.8		20.7		20.7	
	Final		20.9	20.9	20.9	20.7	20.8	20.8	20.8	Final		20.8	20.8	20.8	20.9	20.8	20.8	20.8
DO (mg/L)	Initial	5.56		5.41		6.92		5.63		Initial	5.48		5.11		6.24		5.49	
	Final		6.40	6.45	6.82	6.21	6.15	6.09	6.03	Final		6.17	6.26	6.02	6.81	6.48	5.94	5.81
pH	Initial	7.77		7.50		7.71		7.79		Initial	7.76		7.41		7.65		7.77	
	Final		7.82	7.72	7.74	7.73	7.75	7.76	7.80	Final		7.82	7.71	7.69	7.73	7.72	7.72	7.78

20 11. 7. 20

Approved by

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COC code : BW-DBNT1104-KT-F004

Sheet of Raw Data – Fish – Condition index

Date	110713-110720	**Signature**	**Jin Hee Kim**
Lab. manger	Myung-Baek Shon		
Test Observer	Tae-Won Kim		
Test organism	Embryo and larvae of Paralichthys olivaceus	**End point**	Survival
Test substance	KTM-SSDS-3 (3-32 psu)		

▶ **Condition index of test organism**

▷ **Concentration:** Control-1 ▷ **Concentration:** Control-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.9	0.1	1	2.7	0.1
2	2.8	0.1	2	2.7	0.1
3	2.8	0.1	3	2.9	0.1
4	2.7	0.1	4	3.0	0.1
5	2.7	0.1	5	3.0	0.1
6	2.7	0.1	6	3.0	0.1
7	2.7	0.1	7	2.7	0.1
8	2.8	0.1	8	2.9	0.1
9	2.7	0.1	9	2.9	0.1
10	2.7	0.1	10	2.7	0.1
11	2.7	0.1	11	2.7	0.1
12	2.7	0.1	12	2.7	0.1
13	2.7	0.1	13	2.9	0.1
14	2.7	0.1	14	2.7	0.1
15	2.8	0.1	15	2.8	0.1
16	2.6	0.1	16	2.7	0.1
17	2.9	0.1	17	2.9	0.1
18	2.7	0.1	18	2.7	0.1
19	2.7	0.1	19	2.7	0.1
20	2.7	0.1	20	2.7	0.1
21	2.7	0.1	21	2.7	0.1
22	2.8	0.1	22	2.9	0.1
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	2.7	0.1	Mean	2.8	0.1

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▷ Concentration: Control-3 ▷ Concentration: 6.25%-1

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	3.0	0.1	1	2.7	0.1
2	2.7	0.1	2	2.6	0.1
3	2.7	0.1	3	2.6	0.1
4	2.9	0.1	4	2.8	0.1
5	2.7	0.1	5	2.8	0.1
6	2.7	0.1	6	3.0	0.1
7	2.9	0.1	7	2.7	0.1
8	2.7	0.1	8	2.8	0.1
9	2.7	0.1	9	2.7	0.1
10	2.8	0.1	10	2.7	0.1
11	2.7	0.1	11	2.7	0.1
12	2.8	0.1	12	2.7	0.1
13	2.4	0.1	13	2.8	0.1
14	2.7	0.1	14	2.9	0.1
15	2.6	0.1	15	2.7	0.1
16	2.7	0.1	16	2.7	0.1
17	2.8	0.1	17	2.8	0.1
18	2.7	0.1	18	2.9	0.1
19	2.8	0.1	19	2.7	0.1
20	2.7	0.1	20	3.0	0.1
21	3.0	0.1	21	3.0	0.1
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	2.7	0.1	Mean	2.8	0.1

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▷Concentration: 6.25%-2

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.9	0.1
4	2.7	0.1
5	2.9	0.1
6	2.8	0.1
7	2.9	0.1
8	2.8	0.1
9	2.9	0.1
10	2.9	0.1
11	2.8	0.1
12	2.9	0.1
13	2.9	0.1
14	2.9	0.1
15	2.7	0.1
16	2.9	0.1
17	2.8	0.1
18	2.8	0.1
19	2.9	0.1
20	2.8	0.1
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 6.25%-3

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.8	0.1
3	2.7	0.1
4	2.8	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.8	0.1
10	2.8	0.1
11	2.8	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.8	0.1
17	2.9	0.1
18	2.7	0.1
19	2.7	0.1
20	2.7	0.1
21	2.9	0.1
22	2.9	0.1
23	2.8	0.1
24	2.7	0.1
25	2.9	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷Concentration: 12.5%-1

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.9	0.1
3	2.9	0.1
4	2.9	0.1
5	2.9	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.8	0.1
10	3.0	0.1
11	2.7	0.1
12	2.7	0.1
13	2.8	0.1
14	3.0	0.1
15	2.8	0.1
16	2.6	0.1
17	3.0	0.1
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 12.5%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.7	0.1
3	2.8	0.1
4	2.7	0.1
5	2.8	0.1
6	2.7	0.1
7	2.8	0.1
8	2.8	0.1
9	3.0	0.1
10	2.8	0.1
11	2.9	0.1
12	2.6	0.1
13	3.0	0.1
14	2.8	0.1
15	2.7	0.1
16	2.9	0.1
17	2.8	0.1
18	3.0	0.1
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷Concentration: 12.5%-3

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.8	0.1
3	2.7	0.1
4	2.8	0.1
5	2.7	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.7	0.1
10	2.7	0.1
11	2.7	0.1
12	2.8	0.1
13	2.7	0.1
14	2.7	0.1
15	2.7	0.1
16	3.0	0.1
17	3.0	0.1
18	2.8	0.1
19	2.8	0.1
20	2.8	0.1
21	3.0	0.1
22	2.7	0.1
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 25%-1

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.7	0.1
3	2.8	0.1
4	2.6	0.1
5	2.9	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.7	0.1
10	2.8	0.1
11	2.6	0.1
12	2.7	0.1
13	2.9	0.1
14	3.0	0.1
15	2.9	0.1
16	2.9	0.1
17	3.0	0.1
18	3.0	0.1
19	2.9	0.1
20	2.9	0.1
21	2.6	0.1
22	2.9	0.1
23	2.8	0.1
24	2.8	0.1
25	2.8	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷Concentration: 25%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.9	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.9	0.1
10	2.7	0.1
11	3.0	0.1
12	2.9	0.1
13	2.7	0.1
14	2.7	0.1
15	2.9	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19	2.7	0.1
20	2.8	0.1
21	2.8	0.1
22	2.8	0.1
23	2.7	0.1
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 25%-3

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.9	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.9	0.1
8	2.8	0.1
9	2.9	0.1
10	2.8	0.1
11	2.9	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.9	0.1
17	2.7	0.1
18	2.7	0.1
19	2.7	0.1
20	2.7	0.1
21	2.9	0.1
22	2.8	0.1
23	2.9	0.1
24	3.0	0.1
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷Concentration: 50%-1 ▷ Concentration: 50%-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.9	0.1	1	3.0	0.1
2	3.0	0.1	2	2.7	0.1
3	2.9	0.1	3	2.7	0.1
4	2.9	0.1	4	2.9	0.1
5	3.0	0.1	5	2.9	0.1
6	2.8	0.1	6	2.9	0.1
7	2.7	0.1	7	2.8	0.1
8	3.0	0.1	8	2.9	0.1
9	2.8	0.1	9	2.7	0.1
10	2.7	0.1	10	2.9	0.1
11	3.0	0.1	11	2.8	0.1
12	2.7	0.1	12	2.9	0.1
13	2.8	0.1	13	2.7	0.1
14	2.7	0.1	14	2.7	0.1
15	3.0	0.1	15	2.9	0.1
16	2.8	0.1	16	2.8	0.1
17	2.9	0.1	17	2.8	0.1
18	2.7	0.1	18	2.9	0.1
19	2.9	0.1	19	2.7	0.1
20	2.7	0.1	20	2.7	0.1
21	2.8	0.1	21	2.9	0.1
22	2.8	0.1	22	2.8	0.1
23	2.7	0.1	23	2.7	0.1
24	2.8	0.1	24	2.6	0.1
25	2.7	0.1	25	2.7	0.1
26			26	2.8	0.1
27			27		
28			28		
29			29		
30			30		
Mean	2.8	0.1	Mean	2.8	0.1

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▷Concentration: 50%-3 ▷ Concentration: 100%-1

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.8	0.1	1	2.8	0.1
2	2.7	0.1	2	2.8	0.1
3	2.7	0.1	3	2.7	0.1
4	2.8	0.1	4	2.7	0.1
5	2.8	0.1	5	2.7	0.1
6	2.8	0.1	6	2.8	0.1
7	2.8	0.1	7	2.7	0.1
8	2.7	0.1	8	2.8	0.1
9	2.8	0.1	9	2.7	0.1
10	2.9	0.1	10	2.8	0.1
11	2.9	0.1	11	2.9	0.1
12	2.8	0.1	12	2.7	0.1
13	2.9	0.1	13	2.8	0.1
14	2.7	0.1	14	2.7	0.1
15	2.9	0.1	15	2.8	0.1
16	2.7	0.1	16	2.8	0.1
17	2.9	0.1	17	2.7	0.1
18	2.8	0.1	18	3.0	0.1
19	2.8	0.1	19	2.8	0.1
20	2.7	0.1	20	3.0	0.1
21	3.0	0.1	21	2.9	0.1
22	3.0	0.1	22	2.8	0.1
23	2.8	0.1	23	2.8	0.1
24	2.6	0.1	24		
25	2.7	0.1	25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	2.8	0.1	Mean	2.8	0.1

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▷ Concentration: 100% -2

No.	Length (mm)	Dry weight (mg)
1	3.0	0.1
2	2.7	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.8	0.1
10	2.9	0.1
11	2.8	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.8	0.1
17	2.8	0.1
18	2.7	0.1
19	2.7	0.1
20	2.9	0.1
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ Concentration: 100% -3

No.	Length (mm)	Dry weight (mg)
1	2.6	0.1
2	2.8	0.1
3	2.7	0.1
4	2.9	0.1
5	2.9	0.1
6	2.8	0.1
7	2.9	0.1
8	2.9	0.1
9	2.7	0.1
10	2.9	0.1
11	2.8	0.1
12	2.7	0.1
13	2.9	0.1
14	2.7	0.1
15	2.8	0.1
16	2.9	0.1
17	2.8	0.1
18	2.7	0.1
19	2.9	0.1
20	2.9	0.1
21	2.8	0.1
22	2.9	0.1
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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COC Code. BW-DBWT1104-KT-F002

Test date	110630-110707	**Test substance**	KTM-S5D5-1,2(>32psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	180min
Test organism	Eggs of *Paralichthys olivaceus*	**Observe duration**	30min
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live in the first 3hrs

Elased time (min)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
30	30	30	30	30	30	30	30	30	30	30	30	30
60	30	30	30	30	30	30	30	30	30	30	30	30
90	30	30	30	30	30	30	30	30	30	30	30	30
120	30	30	30	30	30	30	30	30	30	30	30	30
150	30	30	30	30	30	30	30	30	30	30	30	30
180	30	30	30	30	30	30	30	30	30	30	30	30

Elased time (min)	25%				50%				100%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
30	30	30	30	30	30	30	30	30	30	30	30	30
60	30	30	30	30	30	30	30	30	30	30	30	30
90	30	30	30	30	30	30	30	30	30	30	30	30
120	30	30	30	30	30	30	30	30	30	30	30	30
150	30	30	30	30	30	30	30	30	30	30	30	30
180	30	30	30	30	30	30	30	30	30	30	30	30

▶ Dead in the first 3hrs

Elased time (min)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0	0	0	0
180	0	0	0	0	0	0	0	0	0	0	0	0

Elased time (min)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0	0	0	0
180	0	0	0	0	0	0	0	0	0	0	0	0

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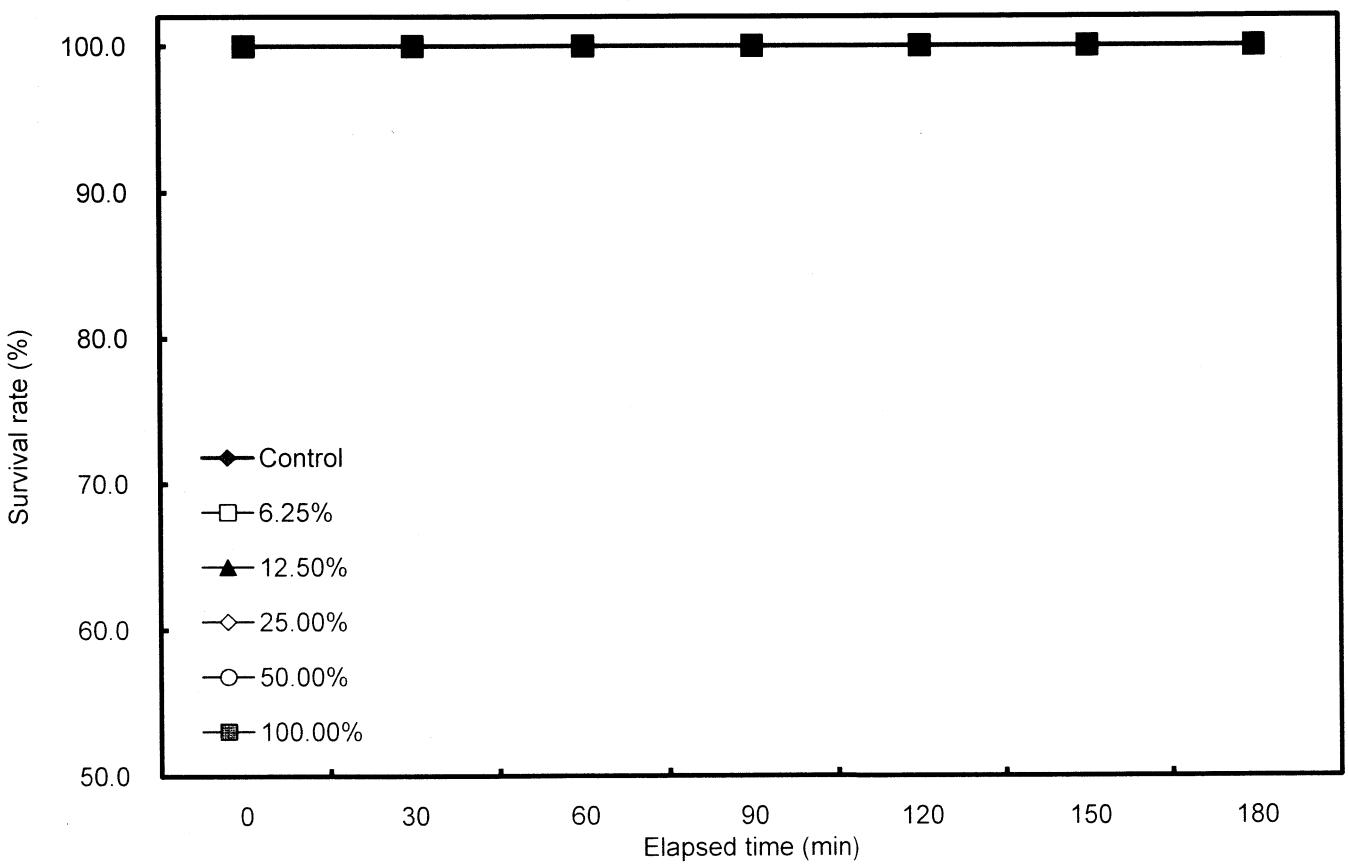
▶ **Survival rate (%)**

Elased time (min)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
60	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
120	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
150	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
180	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Elased time (min)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
60	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
120	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
150	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
180	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0



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COC Code. BW-DBWT1104-KT-F002

Test date	110630-110707	**Test substance**	KTM-S5D5-1,2(>32psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	3days
Test organism	Embryo of *Paralichthys olivaceus*	**Observe duration**	daily
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	30	29	30	30	30	30	30	30	30	30	28	29
2	30	27	30	29	30	30	30	30	30	29	27	29
3	30	27	30	29	30	30	30	30	30	29	27	29

Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	29	29	30	29	30	29	28	29	29	30	29	29
2	29	29	29	29	30	29	28	29	29	30	29	29
3	29	29	29	29	30	29	28	29	29	30	29	29

▶ Dead

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0	2	1
2	0	3	0	1	0	0	0	0	0	1	3	1
3	0	3	0	1	0	0	0	0	0	1	3	1

Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	0	1	0	1	2	1	1	0	1	1
2	1	1	1	1	0	1	2	1	1	0	1	1
3	1	1	1	1	0	1	2	1	1	0	1	1

▶ Survival rate (%)

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	100.0	96.7	100.0	98.9	100.0	100.0	100.0	100.0	100.0	100.0	93.3	97.8
2	100.0	90.0	100.0	96.7	100.0	100.0	100.0	100.0	100.0	96.7	90.0	95.6
3	100.0	90.0	100.0	96.7	100.0	100.0	100.0	100.0	100.0	96.7	90.0	95.6

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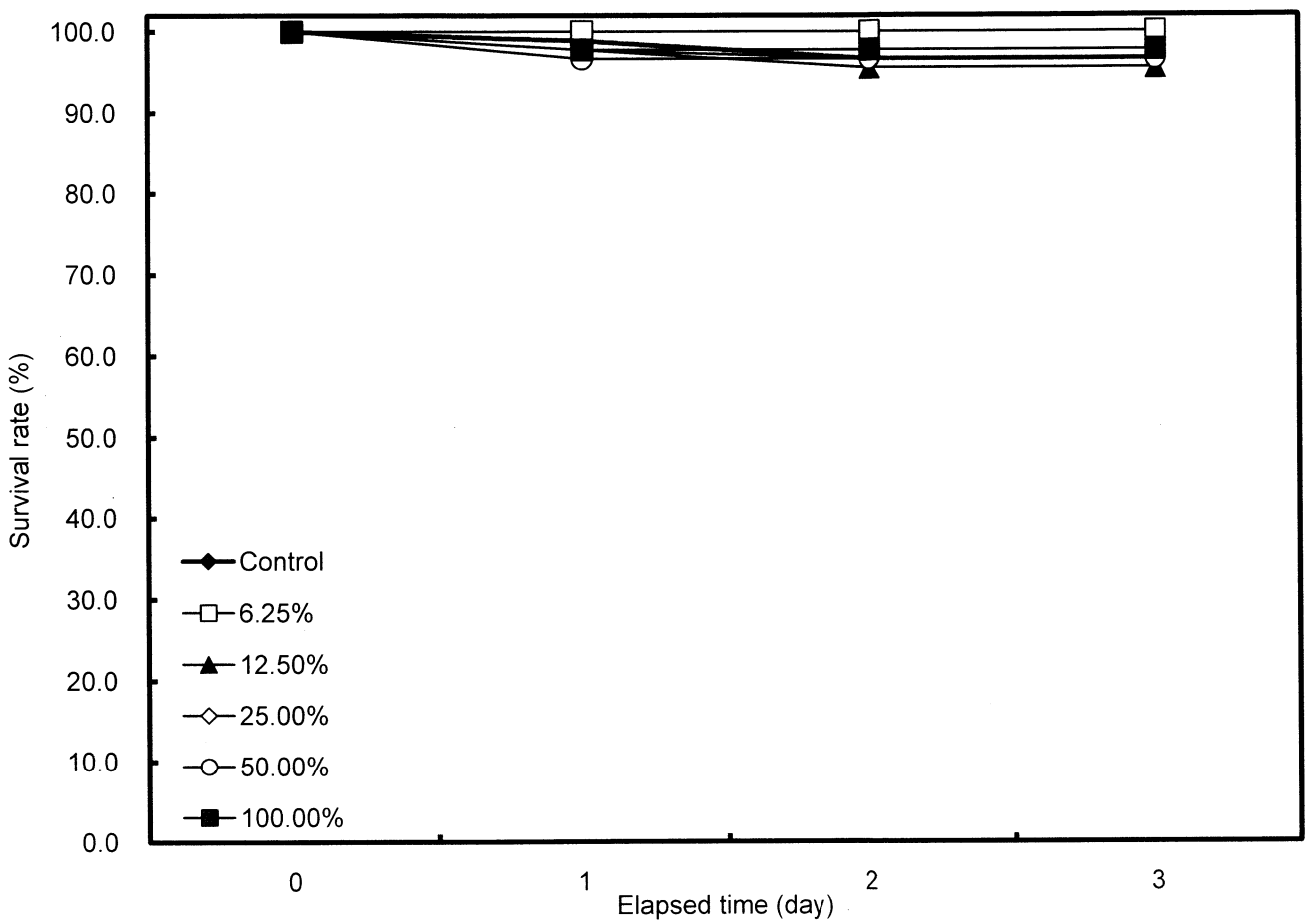
Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	96.7	96.7	100.0	97.8	100.0	96.7	93.3	96.7	96.7	100.0	96.7	97.8
2	96.7	96.7	96.7	96.7	100.0	96.7	93.3	96.7	96.7	100.0	96.7	97.8
3	96.7	96.7	96.7	96.7	100.0	96.7	93.3	96.7	96.7	100.0	96.7	97.8



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COC Code. BW-DBWT1104-KT-F002

Test date	110630-110707	**Test substance**	KTM-S5D5-1,2(>32psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	7days
Test organism	Embryo and larvae of *Paralichthys olivaceus*	**Observe duration**	daily
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	30	29	30	30	30	30	30	30	30	30	28	29
2	29	27	30	29	30	29	27	29	30	29	26	28
3	29	27	30	29	30	29	26	28	30	29	24	28
4	29	26	29	28	30	28	25	28	29	29	23	27
5	29	24	28	27	30	28	25	28	29	28	23	27
6	29	22	28	26	30	27	25	27	29	28	23	27
7	29	22	27	26	30	26	25	27	29	26	20	25

Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	29	29	30	29	30	30	30	30	29	30	29	29
2	28	27	27	27	29	28	23	27	28	30	29	29
3	27	26	27	27	24	23	22	23	28	30	29	29
4	27	26	26	26	23	21	19	21	27	28	28	28
5	27	26	26	26	23	20	18	20	26	28	27	27
6	27	26	26	26	22	19	18	20	26	28	27	27
7	27	23	23	24	22	18	18	19	26	26	25	26

▶ Dead

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0	2	1
2	1	3	0	1	0	1	3	1	0	1	4	2
3	1	3	0	1	0	1	4	2	0	1	6	2
4	1	4	1	2	0	2	5	2	1	1	7	3
5	1	6	2	3	0	2	5	2	1	2	7	3
6	1	8	2	4	0	3	5	3	1	2	7	3
7	1	8	3	4	0	4	5	3	1	4	10	5

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Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	0	1	0	0	0	0	1	0	1	1
2	2	3	3	3	1	2	7	3	2	0	1	1
3	3	4	3	3	6	7	8	7	2	0	1	1
4	3	4	4	4	7	9	11	9	3	2	2	2
5	3	4	4	4	7	10	12	10	4	2	3	3
6	3	4	4	4	8	11	12	10	4	2	3	3
7	3	7	7	6	8	12	12	11	4	4	5	4

▶ **Survival rate (%)**

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	100.0	96.7	100.0	98.9	100.0	100.0	100.0	100.0	100.0	100.0	93.3	97.8
2	96.7	90.0	100.0	95.6	100.0	96.7	90.0	95.6	100.0	96.7	86.7	94.4
3	96.7	90.0	100.0	95.6	100.0	96.7	86.7	94.4	100.0	96.7	80.0	92.2
4	96.7	86.7	96.7	93.3	100.0	93.3	83.3	92.2	96.7	96.7	76.7	90.0
5	96.7	80.0	93.3	90.0	100.0	93.3	83.3	92.2	96.7	93.3	76.7	88.9
6	96.7	73.3	93.3	87.8	100.0	90.0	83.3	91.1	96.7	93.3	76.7	88.9
7	96.7	73.3	90.0	86.7	100.0	86.7	83.3	90.0	96.7	86.7	66.7	83.3

Elased time (day)	25%				50%				100%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	96.7	96.7	100.0	97.8	100.0	100.0	100.0	100.0	96.7	100.0	96.7	97.8
2	93.3	90.0	90.0	91.1	96.7	93.3	76.7	88.9	93.3	100.0	96.7	96.7
3	90.0	86.7	90.0	88.9	80.0	76.7	73.3	76.7	93.3	100.0	96.7	96.7
4	90.0	86.7	86.7	87.8	76.7	70.0	63.3	70.0	90.0	93.3	93.3	92.2
5	90.0	86.7	86.7	87.8	76.7	66.7	60.0	67.8	86.7	93.3	90.0	90.0
6	90.0	86.7	86.7	87.8	73.3	63.3	60.0	65.6	86.7	93.3	90.0	90.0
7	90.0	76.7	76.7	81.1	73.3	60.0	60.0	64.4	86.7	86.7	83.3	85.6

▶ **Mortality (%)**

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.0	3.3	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	6.7	2.2
2	3.3	10.0	0.0	4.4	0.0	3.3	10.0	4.4	0.0	3.3	13.3	5.6
3	3.3	10.0	0.0	4.4	0.0	3.3	13.3	5.6	0.0	3.3	20.0	7.8
4	3.3	13.3	3.3	6.7	0.0	6.7	16.7	7.8	3.3	3.3	23.3	10.0
5	3.3	20.0	6.7	10.0	0.0	6.7	16.7	7.8	3.3	6.7	23.3	11.1
6	3.3	26.7	6.7	12.2	0.0	10.0	16.7	8.9	3.3	6.7	23.3	11.1
7	3.3	26.7	10.0	13.3	0.0	13.3	16.7	10.0	3.3	13.3	33.3	16.7

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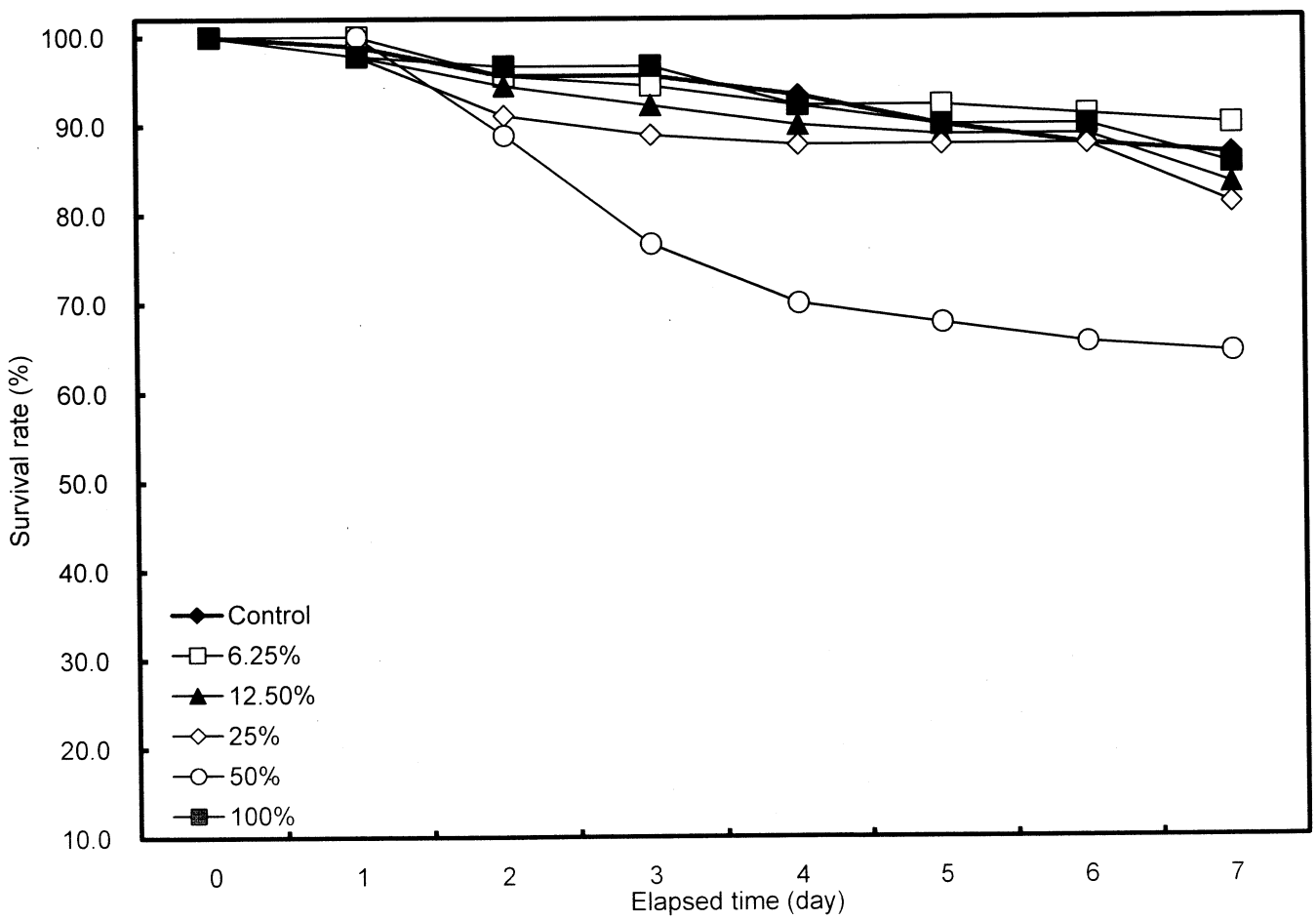
Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	3.3	3.3	0.0	2.2	0.0	0.0	0.0	0.0	3.3	0.0	3.3	2.2
2	6.7	10.0	10.0	8.9	3.3	6.7	23.3	11.1	6.7	0.0	3.3	3.3
3	10.0	13.3	10.0	11.1	20.0	23.3	26.7	23.3	6.7	0.0	3.3	3.3
4	10.0	13.3	13.3	12.2	23.3	30.0	36.7	30.0	10.0	6.7	6.7	7.8
5	10.0	13.3	13.3	12.2	23.3	33.3	40.0	32.2	13.3	6.7	10.0	10.0
6	10.0	13.3	13.3	12.2	26.7	36.7	40.0	34.4	13.3	6.7	10.0	10.0
7	10.0	23.3	23.3	18.9	26.7	40.0	40.0	35.6	13.3	13.3	16.7	14.4



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Test date	110630-110707	**Test substance**	KTM-S5D5-1,2(>32psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	7days
Test organism	Embryo and larvae of *Paralichthys olivaceus*	**Observe duration**	daily
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

▶ **Temperature (℃)**

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	20.10	-	20.00	-	19.90	-	19.80	-	19.70	-	19.50	-
1	-	19.60	-	19.50	-	19.50	-	19.40	-	19.30	-	19.40
2	19.60	19.80	19.60	19.60	19.60	19.50	19.60	19.50	19.50	19.40	19.60	19.40
3	-	19.90	-	19.70	-	19.70	-	19.70	-	19.60	-	19.60
4	19.50	20.10	19.70	19.70	19.70	19.60	19.60	19.50	19.00	19.40	19.30	19.40
5	-	20.20	-	19.90	-	19.80	-	19.80	-	19.70	-	19.60
6	20.10	19.90	20.00	19.80	20.00	19.80	19.70	19.50	19.70	19.60	19.60	19.60
7	-	20.00	-	19.90	-	19.90	-	19.80	-	19.70	-	19.60
8	-	-	-	-	-	-	-	-	-	-	-	-

▶ **Salinity (psu)**

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	33.30	-	33.30	-	33.40	-	33.20	-	33.20	-	33.20	-
1	-	33.20	-	33.10	-	33.30	-	33.20	-	33.20	-	33.20
2	33.20	33.10	33.30	33.30	33.30	33.30	33.20	33.20	33.40	33.30	33.30	33.20
3	-	33.00	-	33.10	-	33.30	-	33.30	-	33.40	-	33.10
4	33.50	33.40	33.40	33.80	33.60	33.70	33.50	33.60	33.40	33.70	33.50	33.60
5	-	33.30	-	33.30	-	33.30	-	33.20	-	33.30	-	33.20
6	33.90	33.40	33.80	33.40	33.70	33.30	33.70	33.30	33.80	33.30	33.70	33.20
7	-	33.80	-	33.80	-	33.70	-	33.60	-	33.70	-	33.70
8	-	-	-	-	-	-	-	-	-	-	-	-

▶ **DO (mg/L)**

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	6.41	-	6.10	-	6.20	-	6.17	-	5.39	-	5.96	-
1	-	5.32	-	5.16	-	5.72	-	5.48	-	5.78	-	5.77
2	5.01	5.24	5.27	5.88	5.94	5.11	5.80	5.61	6.00	6.10	5.02	6.00
3	-	5.47	-	5.44	-	5.01	-	5.07	-	5.77	-	5.93
4	6.01	5.11	5.70	5.57	5.32	5.09	5.92	5.14	5.72	5.62	5.72	5.30
5	-	7.03	-	6.53	-	6.10	-	5.80	-	5.56	-	5.32
6	6.58	5.35	6.38	5.11	6.08	5.09	5.96	5.93	5.85	5.84	5.68	5.82
7	-	6.33	-	6.06	-	5.40	-	5.82	-	5.71	-	5.73
8	-	-	-	-	-	-	-	-	-	-	-	-

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▶ pH

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	7.85	-	7.75	-	7.74	-	7.67	-	7.71	-	7.67	-
1	-	7.82	-	7.84	-	7.83	-	7.81	-	7.81	-	7.67
2	7.84	7.87	7.86	7.87	7.84	7.87	7.81	7.86	7.81	7.87	7.82	7.85
3	-	7.89	-	7.88	-	7.89	-	7.88	-	7.86	-	7.83
4	7.70	7.96	7.80	7.90	7.82	7.89	7.74	7.89	7.79	7.82	7.75	7.82
5	-	7.63	-	7.74	-	7.74	-	7.75	-	7.75	-	7.72
6	7.56	7.78	7.59	7.78	7.61	7.90	7.61	7.78	7.59	7.77	7.54	7.76
7	-	7.82	-	7.84	-	7.84	-	7.83	-	7.82	-	7.81
8	-	-	-	-	-	-	-	-	-	-	-	-

20/1. 7. 8 Approved by

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■ COC code: BW-DBWT1104-KT-F002

Date	110630-110707		**Signature**	**Jin Hee Kim**
Lab. manger	Myung-Baek Shon			
Test Observer	Tae-Won Kim			
Test organism	Embryo and larvae of *Paralichthys olivaceus*		**End point**	Survival
Test substance	KTM-S5D5-1,2(>32 psu)			

▶ **Condition index of test organism**

▷ **Concentration:** Control-1 ▷ **Concentration:** Control-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	3.0	0.1	1	2.8	0.1
2	2.9	0.1	2	2.7	0.1
3	2.6	0.1	3	2.9	0.1
4	2.7	0.1	4	3.0	0.1
5	2.7	0.1	5	3.0	0.1
6	2.7	0.1	6	2.9	0.1
7	2.7	0.1	7	2.9	0.1
8	2.8	0.1	8	2.9	0.1
9	2.6	0.1	9	2.9	0.1
10	2.9	0.1	10	2.7	0.1
11	2.7	0.1	11	2.7	0.1
12	2.8	0.1	12	2.8	0.1
13	2.8	0.1	13	2.9	0.1
14	2.7	0.1	14	2.7	0.1
15	2.8	0.1	15	2.8	0.1
16	2.6	0.1	16	2.8	0.1
17	2.9	0.1	17	2.9	0.1
18	2.7	0.1	18	2.7	0.1
19	2.8	0.1	19	2.8	0.1
20	2.8	0.1	20	2.9	0.1
21	2.8	0.1	21	2.8	0.1
22	2.8	0.1	22	2.9	0.1
23	2.7	0.1	23		
24	2.7	0.1	24		
25	2.8	0.1	25		
26	3.0	0.1	26		
27	2.7	0.1	27		
28	2.7	0.1	28		
29	2.8	0.1	29		
30			30		
Mean	2.8	0.1	Mean	2.8	0.1

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▷ **Concentration:** Control-3 ▷ **Concentration:** 6.25%-1

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.9	0.1	1	2.8	0.1
2	2.7	0.1	2	2.6	0.1
3	2.8	0.1	3	2.7	0.1
4	2.9	0.1	4	2.8	0.1
5	2.7	0.1	5	2.8	0.1
6	2.8	0.1	6	2.9	0.1
7	2.9	0.1	7	2.7	0.1
8	2.8	0.1	8	2.8	0.1
9	2.7	0.1	9	2.6	0.1
10	2.8	0.1	10	2.6	0.1
11	2.9	0.1	11	2.6	0.1
12	2.8	0.1	12	2.7	0.1
13	2.4	0.1	13	2.8	0.1
14	2.6	0.1	14	2.9	0.1
15	2.6	0.1	15	2.7	0.1
16	2.9	0.1	16	2.7	0.1
17	2.8	0.1	17	2.8	0.1
18	2.7	0.1	18	2.9	0.1
19	2.8	0.1	19	2.7	0.1
20	2.7	0.1	20	2.7	0.1
21	2.9	0.1	21	2.8	0.1
22	2.9	0.1	22	2.8	0.1
23	2.8	0.1	23	2.9	0.1
24	2.8	0.1	24	2.8	0.1
25	2.7	0.1	25	2.7	0.1
26	2.7	0.1	26	2.8	0.1
27	3.0	0.1	27	2.8	0.1
28			28	2.9	0.1
29			29	2.8	0.1
30			30	2.9	0.1
Mean	2.8	0.1	Mean	2.8	0.1

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▷ **Concentration:** 6.25%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.8	0.1
3	2.8	0.1
4	2.7	0.1
5	2.9	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.8	0.1
10	2.8	0.1
11	2.8	0.1
12	2.9	0.1
13	2.8	0.1
14	2.7	0.1
15	2.9	0.1
16	2.9	0.1
17	2.7	0.1
18	2.7	0.1
19	2.8	0.1
20	2.9	0.1
21	2.9	0.1
22	2.7	0.1
23	2.7	0.1
24	2.7	0.1
25	2.7	0.1
26	2.8	0.1
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 6.25%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.8	0.1
10	2.8	0.1
11	2.7	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.7	0.1
16	2.8	0.1
17	2.9	0.1
18	2.8	0.1
19	2.7	0.1
20	2.8	0.1
21	2.9	0.1
22	2.8	0.1
23	2.8	0.1
24	2.7	0.1
25	2.6	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 12.5%-1

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.8	0.1
4	2.9	0.1
5	2.9	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.8	0.1
10	2.7	0.1
11	2.7	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.6	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.9	0.1
21	2.7	0.1
22	2.8	0.1
23	2.7	0.1
24	2.8	0.1
25	2.8	0.1
26	2.9	0.1
27	2.7	0.1
28	2.8	0.1
29	2.8	0.1
30		
mean	2.8	0.1

▷ **Concentration:** 12.5%-2

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.7	0.1
3	2.7	0.1
4	2.7	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.7	0.1
10	2.8	0.1
11	2.9	0.1
12	2.6	0.1
13	2.7	0.1
14	2.8	0.1
15	2.7	0.1
16	2.9	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.7	0.1
21	2.8	0.1
22	2.8	0.1
23	2.9	0.1
24	2.7	0.1
25	2.8	0.1
26	2.8	0.1
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 12.5%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.8	0.1
10	2.7	0.1
11	2.7	0.1
12	2.8	0.1
13	2.9	0.1
14	2.7	0.1
15	2.7	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19	2.8	0.1
20	2.8	0.1
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 25%-1

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.7	0.1
4	2.6	0.1
5	2.7	0.1
6	2.8	0.1
7	2.9	0.1
8	2.8	0.1
9	2.8	0.1
10	2.8	0.1
11	2.8	0.1
12	2.9	0.1
13	2.9	0.1
14	2.9	0.1
15	2.9	0.1
16	2.9	0.1
17	3.0	0.1
18	3.0	0.1
19	2.8	0.1
20	2.8	0.1
21	2.6	0.1
22	2.9	0.1
23	2.8	0.1
24	2.7	0.1
25	2.7	0.1
26	2.7	0.1
27	2.8	0.1
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 25%-2

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.7	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.7	0.1
10	2.7	0.1
11	3.0	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.7	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19	2.8	0.1
20	2.8	0.1
21	2.8	0.1
22	2.8	0.1
23	2.8	0.1
24		
25		
26		
27		
28		
29		
30		
Mean	2.7	0.1

▷ **Concentration:** 25%-3

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.4	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.8	0.1
9	2.9	0.1
10	2.8	0.1
11	2.8	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.8	0.1
17	2.7	0.1
18	2.7	0.1
19	2.7	0.1
20	2.7	0.1
21	2.8	0.1
22	2.8	0.1
23	2.9	0.1
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 50%-1 ▷ **Concentration:** 50%-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.8	0.1	1	2.9	0.1
2	2.8	0.1	2	2.7	0.1
3	2.9	0.1	3	2.7	0.1
4	2.9	0.1	4	2.8	0.1
5	2.8	0.1	5	2.8	0.1
6	2.8	0.1	6	2.9	0.1
7	2.7	0.1	7	2.8	0.1
8	2.7	0.1	8	2.7	0.1
9	2.8	0.1	9	2.7	0.1
10	2.7	0.1	10	2.7	0.1
11	2.7	0.1	11	2.8	0.1
12	2.7	0.1	12	2.8	0.1
13	2.8	0.1	13	2.7	0.1
14	2.7	0.1	14	2.7	0.1
15	2.9	0.1	15	2.7	0.1
16	2.8	0.1	16	2.8	0.1
17	2.7	0.1	17	2.8	0.1
18	2.7	0.1	18	2.9	0.1
19	2.7	0.1	19		
20	2.7	0.1	20		
21	2.8	0.1	21		
22	2.8	0.1	22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	2.8	0.1	Mean	2.8	0.1

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▷ **Concentration:** 50%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.7	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.8	0.1
10	2.7	0.1
11	2.8	0.1
12	2.8	0.1
13	2.9	0.1
14	2.7	0.1
15	2.8	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 100%-1

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.8	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.7	0.1
7	2.7	0.1
8	2.8	0.1
9	2.7	0.1
10	2.8	0.1
11	2.9	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19	2.8	0.1
20	2.8	0.1
21	2.9	0.1
22	2.8	0.1
23	2.7	0.1
24	2.7	0.1
25	2.7	0.1
26	2.8	0.1
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 100%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.7	0.1
3	2.8	0.1
4	2.7	0.1
5	2.8	0.1
6	2.8	0.1
7	2.7	0.1
8	2.7	0.1
9	2.8	0.1
10	2.9	0.1
11	2.8	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.7	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.8	0.1
21	2.9	0.1
22	2.8	0.1
23	2.7	0.1
24	2.7	0.1
25	2.7	0.1
26	2.8	0.1
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 100%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.9	0.1
9	2.7	0.1
10	2.8	0.1
11	2.8	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.7	0.1
17	2.8	0.1
18	2.7	0.1
19	2.8	0.1
20	2.9	0.1
21	2.8	0.1
22	2.7	0.1
23	2.7	0.1
24	2.7	0.1
25	2.8	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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COC Code. BW-DBWT1104-KT-F004

Test date	110713-110720	**Test substance**	KTM-S5D5-3(3-32 psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	180min
Test organism	Eggs of *Paralichthys olivaceus*	**Observe duration**	30min
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live in the first 3hrs

Elased time (min)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
30	30	30	30	30	30	30	30	30	30	30	30	30
60	30	30	30	30	30	30	30	30	30	30	30	30
90	30	30	30	30	30	30	30	30	30	30	30	30
120	30	30	30	30	30	30	30	30	30	30	30	30
150	30	30	30	30	30	30	30	30	30	30	30	30
180	30	30	30	30	30	30	30	30	30	30	30	30

Elased time (min)	25%				50%				100%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
30	30	30	30	30	30	30	30	30	30	30	30	30
60	30	30	30	30	30	30	30	30	30	30	30	30
90	30	30	30	30	30	30	30	30	30	30	30	30
120	30	30	30	30	30	30	30	30	30	30	30	30
150	30	30	30	30	30	30	30	30	30	30	30	30
180	30	30	30	30	30	30	30	30	30	30	30	30

▶ Dead in the first 3hrs

Elased time (min)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0	0	0	0
180	0	0	0	0	0	0	0	0	0	0	0	0

Elased time (min)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0	0	0	0
180	0	0	0	0	0	0	0	0	0	0	0	0

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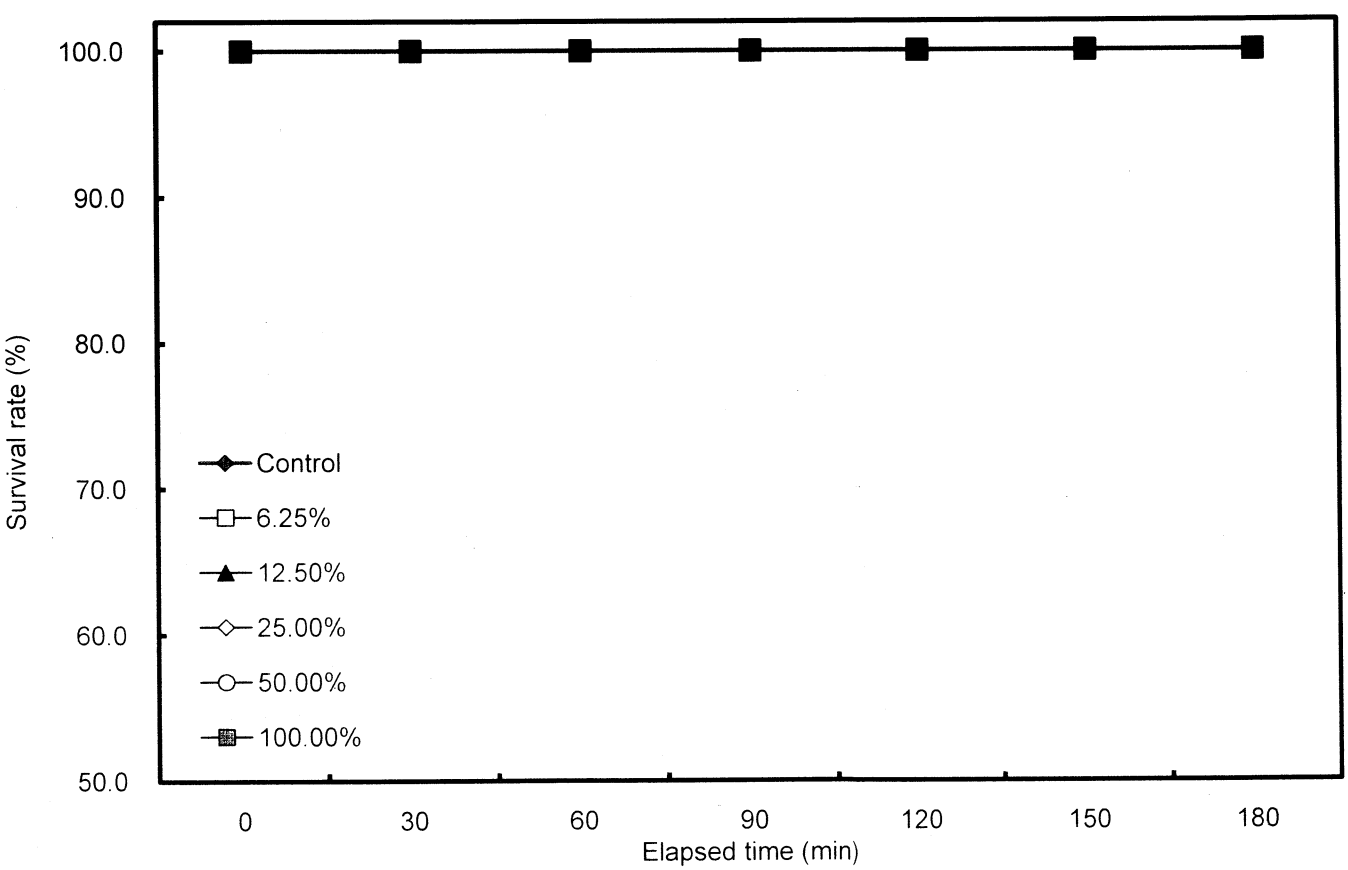
▶ **Survival rate (%)**

Elased time (min)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
60	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
120	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
150	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
180	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Elased time (min)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
30	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
60	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
90	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
120	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
150	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
180	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0



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COC Code. BW-DBWT1104-KT-F004

Test date	110713-110720	**Test substance**	KTM-S5D5-3(3-32 psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	3days
Test organism	Embryo of *Paralichthys olivaceus*	**Observe duration**	daily
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	28	29	27	28	29	27	29	28	28	26	28	27
2	28	29	27	28	29	27	29	28	28	26	28	27
3	28	29	27	28	29	26	29	28	28	25	28	27

Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	28	28	29	28	28	30	30	29	29	30	29	29
2	28	28	29	28	28	30	30	29	29	30	29	29
3	28	28	29	28	28	30	30	29	29	30	29	29

▶ Dead

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	1	3	2	1	3	1	2	2	4	2	3
2	2	1	3	2	1	3	1	2	2	4	2	3
3	2	1	3	2	1	4	1	2	2	5	2	3

Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	2	1	2	2	0	0	1	1	0	1	1
2	2	2	1	2	2	0	0	1	1	0	1	1
3	2	2	1	2	2	0	0	1	1	0	1	1

▶ Survival rate (%)

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.3	96.7	90.0	93.3	96.7	90.0	96.7	94.4	93.3	86.7	93.3	91.1
2	93.3	96.7	90.0	93.3	96.7	90.0	96.7	94.4	93.3	86.7	93.3	91.1
3	93.3	96.7	90.0	93.3	96.7	86.7	96.7	93.3	93.3	83.3	93.3	90.0

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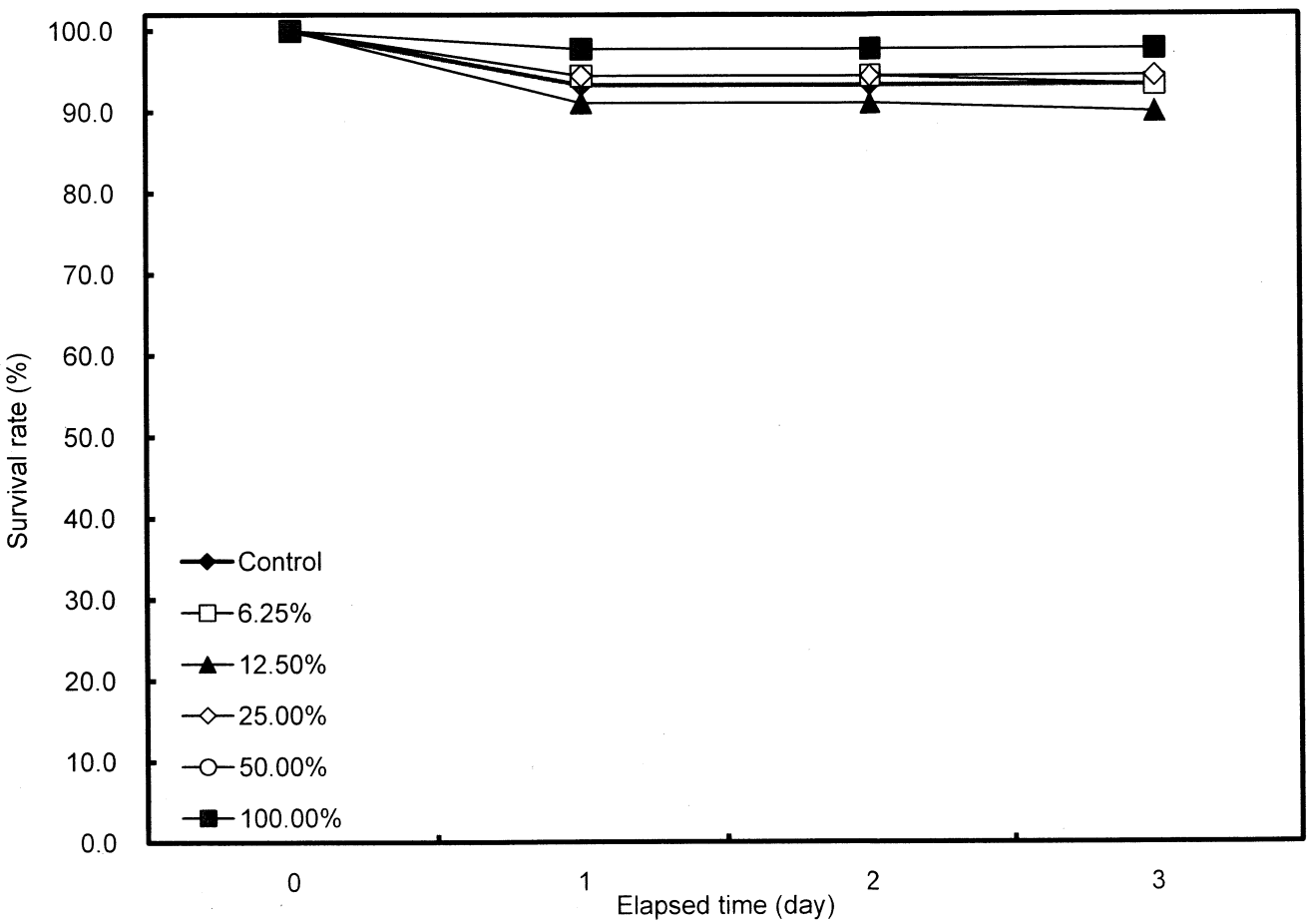
Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.3	93.3	96.7	94.4	93.3	100.0	100.0	97.8	96.7	100.0	96.7	97.8
2	93.3	93.3	96.7	94.4	93.3	100.0	100.0	97.8	96.7	100.0	96.7	97.8
3	93.3	93.3	96.7	94.4	93.3	100.0	100.0	97.8	96.7	100.0	96.7	97.8



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COC Code. BW-DBWT1104-KT-F004

Test date	110713-110720	**Test substance**	KTM-S5D5-3(3-32 psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	7days
Test organism	Embryo and larvae of *Paralichthys olivaceus*	**Observe duration**	daily
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

■ Raw Data

▶ Live

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	28	29	27	28	29	27	29	28	27	26	28	27
2	28	29	25	27	29	24	28	27	25	23	28	25
3	26	24	21	24	22	23	27	24	24	21	24	23
4	23	24	21	23	21	23	27	24	23	21	24	23
5	23	22	21	22	21	22	27	23	22	21	23	22
6	22	22	21	22	21	22	26	23	17	18	22	19
7	22	22	21	22	21	22	25	23	17	18	22	19

Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	30	30	30	30	30	30	30	30	30	30	30	30
1	28	28	29	28	28	30	30	29	29	30	29	29
2	28	28	28	28	27	30	28	28	28	29	28	28
3	27	27	28	27	27	30	26	28	26	29	24	26
4	27	26	28	27	27	30	25	27	25	27	24	25
5	25	26	28	26	26	29	25	27	23	23	22	23
6	25	25	27	26	25	28	25	26	23	20	22	22
7	25	23	24	24	25	26	25	25	23	20	22	22

▶ Dead

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	1	3	2	1	3	1	2	3	4	2	3
2	2	1	5	3	1	6	2	3	5	7	2	5
3	4	6	9	6	8	7	3	6	6	9	6	7
4	7	6	9	7	9	7	3	6	7	9	6	7
5	7	8	9	8	9	8	3	7	8	9	7	8
6	8	8	9	8	9	8	4	7	13	12	8	11
7	8	8	9	8	9	8	5	7	13	12	8	11

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Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	2	1	2	2	0	0	1	1	0	1	1
2	2	2	2	2	3	0	2	2	2	1	2	2
3	3	3	2	3	3	0	4	2	4	1	6	4
4	3	4	2	3	3	0	5	3	5	3	6	5
5	5	4	2	4	4	1	5	3	7	7	8	7
6	5	5	3	4	5	2	5	4	7	10	8	8
7	5	7	6	6	5	4	5	5	7	10	8	8

▶ Survival rate (%)

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.3	96.7	90.0	93.3	96.7	90.0	96.7	94.4	90.0	86.7	93.3	90.0
2	93.3	96.7	83.3	91.1	96.7	80.0	93.3	90.0	83.3	76.7	93.3	84.4
3	86.7	80.0	70.0	78.9	73.3	76.7	90.0	80.0	80.0	70.0	80.0	76.7
4	76.7	80.0	70.0	75.6	70.0	76.7	90.0	78.9	76.7	70.0	80.0	75.6
5	76.7	73.3	70.0	73.3	70.0	73.3	90.0	77.8	73.3	70.0	76.7	73.3
6	73.3	73.3	70.0	72.2	70.0	73.3	86.7	76.7	56.7	60.0	73.3	63.3
7	73.3	73.3	70.0	72.2	70.0	73.3	83.3	75.6	56.7	60.0	73.3	63.3

Elased time (day)	25%				50%				100%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.3	93.3	96.7	94.4	93.3	100.0	100.0	97.8	96.7	100.0	96.7	97.8
2	93.3	93.3	93.3	93.3	90.0	100.0	93.3	94.4	93.3	96.7	93.3	94.4
3	90.0	90.0	93.3	91.1	90.0	100.0	86.7	92.2	86.7	96.7	80.0	87.8
4	90.0	86.7	93.3	90.0	90.0	100.0	83.3	91.1	83.3	90.0	80.0	84.4
5	83.3	86.7	93.3	87.8	86.7	96.7	83.3	88.9	76.7	76.7	73.3	75.6
6	83.3	83.3	90.0	85.6	83.3	93.3	83.3	86.7	76.7	66.7	73.3	72.2
7	83.3	76.7	80.0	80.0	83.3	86.7	83.3	84.4	76.7	66.7	73.3	72.2

▶ Mortality (%)

Elased time (day)	Control				6.25%				12.50%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	6.7	3.3	10.0	6.7	3.3	10.0	3.3	5.6	10.0	13.3	6.7	10.0
2	6.7	3.3	16.7	8.9	3.3	20.0	6.7	10.0	16.7	23.3	6.7	15.6
3	13.3	20.0	30.0	21.1	26.7	23.3	10.0	20.0	20.0	30.0	20.0	23.3
4	23.3	20.0	30.0	24.4	30.0	23.3	10.0	21.1	23.3	30.0	20.0	24.4
5	23.3	26.7	30.0	26.7	30.0	26.7	10.0	22.2	26.7	30.0	23.3	26.7
6	26.7	26.7	30.0	27.8	30.0	26.7	13.3	23.3	43.3	40.0	26.7	36.7
7	26.7	26.7	30.0	27.8	30.0	26.7	16.7	24.4	43.3	40.0	26.7	36.7

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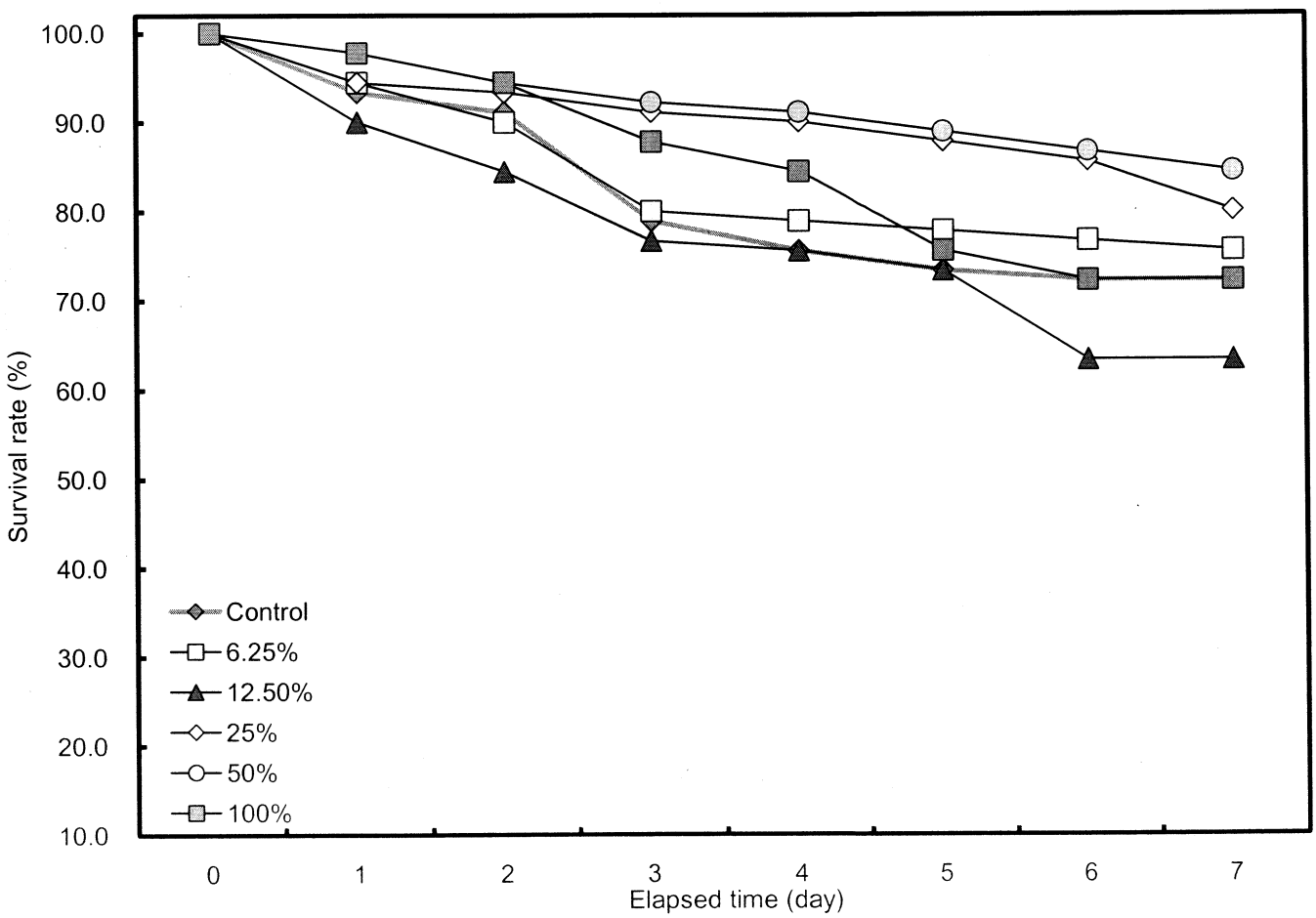
Elased time (day)	25.00%				50.00%				100.00%			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	6.7	6.7	3.3	5.6	6.7	0.0	0.0	2.2	3.3	0.0	3.3	2.2
2	6.7	6.7	6.7	6.7	10.0	0.0	6.7	5.6	6.7	3.3	6.7	5.6
3	10.0	10.0	6.7	8.9	10.0	0.0	13.3	7.8	13.3	3.3	20.0	12.2
4	10.0	13.3	6.7	10.0	10.0	0.0	16.7	8.9	16.7	10.0	20.0	15.6
5	16.7	13.3	6.7	12.2	13.3	3.3	16.7	11.1	23.3	23.3	26.7	24.4
6	16.7	16.7	10.0	14.4	16.7	6.7	16.7	13.3	23.3	33.3	26.7	27.8
7	16.7	23.3	20.0	20.0	16.7	13.3	16.7	15.6	23.3	33.3	26.7	27.8



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Test date	110713-110720	**Test substance**	KTM-S5D5-3(3-32 psu)
End point	Survival	**Numbers of Initial individual**	30 inds. / Repl.
Test manager	Jin Hee Kim	**Numbers of replication**	3 replication
Test Observer	Tae Won Kim	**Test duration**	7days
Test organism	Embryo and larvae of *Paralichthys olivaceus*	**Observe duration**	daily
Light	16L:8D, Cool white, 2000lux	**Temperature**	20±1℃

▶ **Temperature (℃)**

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	20.60	-	20.60	-	20.60	-	20.40	-	20.40	-	20.00	-
1	-	20.20	-	19.90	-	19.70	-	19.60	-	19.60	-	19.50
2	20.10	19.90	19.90	19.50	20.00	19.30	19.70	19.30	19.60	19.20	19.60	19.20
3	-	19.70	-	19.80	-	19.50	-	19.90	-	19.90	-	19.90
4	19.90	19.70	19.90	19.80	19.90	19.90	20.00	19.90	20.00	20.00	19.60	19.40
5	-	19.80	-	19.60	-	19.40	-	19.40	-	19.30	-	19.30
6	19.60	19.60	19.60	19.50	19.50	19.40	19.50	19.40	19.40	19.40	19.30	19.40
7	-	19.60	-	19.50	-	19.40	-	19.40	-	19.40	-	19.30
8	-	-	-	-	-	-	-	-	-	-	-	-

▶ **Salinity (psu)**

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	20.80	-	20.80	-	20.80	-	20.80	-	20.80	-	20.80	-
1	-	20.80	-	20.90	-	20.80	-	20.90	-	20.90	-	20.80
2	20.70	20.90	20.80	20.90	20.80	20.80	20.80	20.80	20.80	20.90	20.80	20.80
3	-	20.80	-	20.90	-	20.90	-	20.80	-	20.90	-	20.80
4	20.80	20.70	20.80	20.90	20.90	20.90	20.80	20.80	20.70	20.70	20.70	20.90
5	-	20.70	-	20.80	-	20.80	-	20.90	-	20.80	-	20.80
6	20.80	20.80	20.80	20.80	20.80	20.90	20.80	20.80	20.80	20.80	20.70	20.80
7	-	20.80	-	20.80	-	20.80	-	20.80	-	20.80	-	20.80
8	-	-	-	-	-	-	-	-	-	-	-	-

▶ **DO (mg/L)**

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	5.78	-	5.69	-	5.60	-	6.14	-	5.56	-	5.48	-
1	-	5.99	-	6.04	-	6.00	-	6.13	-	6.40	-	6.17
2	6.25	6.04	5.57	5.97	6.03	5.77	5.73	5.65	5.47	6.45	5.77	6.26
3	-	6.13	-	6.02	-	6.71	-	6.24	-	6.82	-	6.02
4	6.00	6.75	6.72	5.97	6.14	6.02	5.97	6.52	6.92	6.21	6.24	6.81
5	-	6.21	-	6.36	-	5.77	-	6.93	-	6.75	-	6.48
6	6.53	5.51	6.24	5.97	5.79	6.17	5.74	6.18	5.63	6.09	5.47	5.94
7	-	5.49	-	5.31	-	5.36	-	6.03	-	5.85	-	5.81
8	-	-	-	-	-	-	-	-	-	-	-	-

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▶ pH

Elased time (day)	Control		6.25%		12.50%		25.00%		50.00%		100.00%	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0	7.72	-	7.74	-	7.76	-	7.77	-	7.77	-	7.76	-
1	-	7.77	-	7.79	-	7.80	-	7.81	-	7.82	-	7.82
2	7.35	7.73	7.48	7.73	7.44	7.73	7.49	7.73	7.50	7.72	7.41	7.71
3	-	7.73	-	7.73	-	7.72	-	7.70	-	7.74	-	7.69
4	7.67	7.77	7.69	7.78	7.73	7.76	7.70	7.73	7.71	7.73	7.65	7.73
5	-	7.76	-	7.75	-	7.74	-	7.75	-	7.75	-	7.72
6	7.82	7.79	7.81	7.77	7.78	7.76	7.79	7.77	7.79	7.76	7.77	7.72
7	-	7.82	-	7.82	-	7.80	-	7.81	-	7.80	-	7.78
8	-	-	-	-	-	-	-	-	-	-	-	-

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■ COC code: BW-DBWT1104-KT-F004

Date	110713-110720		**Signature**	**Jin Hee Kim**
Lab. manger	Myung-Baek Shon			
Test Observer	Tae-Won Kim			
Test organism	Embryo and larvae of *Paralichthys olivaceus*		**End point**	Survival
Test substance	KTM-S5D5-3(3-32 psu)			

▶ **Condition index of test organism**

▷ **Concentration:** Control-1 ▷ **Concentration:** Control-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.9	0.1	1	2.7	0.1
2	2.8	0.1	2	2.7	0.1
3	2.8	0.1	3	2.9	0.1
4	2.7	0.1	4	3.0	0.1
5	2.7	0.1	5	3.0	0.1
6	2.7	0.1	6	3.0	0.1
7	2.7	0.1	7	2.7	0.1
8	2.8	0.1	8	2.9	0.1
9	2.7	0.1	9	2.9	0.1
10	2.7	0.1	10	2.7	0.1
11	2.7	0.1	11	2.7	0.1
12	2.7	0.1	12	2.7	0.1
13	2.7	0.1	13	2.9	0.1
14	2.7	0.1	14	2.7	0.1
15	2.8	0.1	15	2.8	0.1
16	2.6	0.1	16	2.7	0.1
17	2.9	0.1	17	2.9	0.1
18	2.7	0.1	18	2.7	0.1
19	2.7	0.1	19	2.7	0.1
20	2.7	0.1	20	2.7	0.1
21	2.7	0.1	21	2.7	0.1
22	2.8	0.1	22	2.9	0.1
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	2.7	0.1	Mean	2.8	0.1

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▷ **Concentration:** Control-3 ▷ **Concentration:** 6.25%-1

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	3.0	0.1	1	2.7	0.1
2	2.7	0.1	2	2.6	0.1
3	2.7	0.1	3	2.6	0.1
4	2.9	0.1	4	2.8	0.1
5	2.7	0.1	5	2.8	0.1
6	2.7	0.1	6	3.0	0.1
7	2.9	0.1	7	2.7	0.1
8	2.7	0.1	8	2.8	0.1
9	2.7	0.1	9	2.7	0.1
10	2.8	0.1	10	2.7	0.1
11	2.7	0.1	11	2.7	0.1
12	2.8	0.1	12	2.7	0.1
13	2.4	0.1	13	2.8	0.1
14	2.7	0.1	14	2.9	0.1
15	2.6	0.1	15	2.7	0.1
16	2.7	0.1	16	2.7	0.1
17	2.8	0.1	17	2.8	0.1
18	2.7	0.1	18	2.9	0.1
19	2.8	0.1	19	2.7	0.1
20	2.7	0.1	20	3.0	0.1
21	3.0	0.1	21	3.0	0.1
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
Mean	2.7	0.1	Mean	2.8	0.1

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▷ **Concentration:** 6.25%-2

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.9	0.1
4	2.7	0.1
5	2.9	0.1
6	2.8	0.1
7	2.9	0.1
8	2.8	0.1
9	2.9	0.1
10	2.8	0.1
11	2.9	0.1
12	2.9	0.1
13	2.8	0.1
14	2.9	0.1
15	2.9	0.1
16	2.9	0.1
17	2.7	0.1
18	2.9	0.1
19	2.8	0.1
20	2.8	0.1
21	2.9	0.1
22	2.8	0.1
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 6.25%-3

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.8	0.1
3	2.7	0.1
4	2.8	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.8	0.1
10	2.8	0.1
11	2.8	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.8	0.1
17	2.9	0.1
18	2.7	0.1
19	2.7	0.1
20	2.7	0.1
21	2.9	0.1
22	2.9	0.1
23	2.8	0.1
24	2.7	0.1
25	2.9	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 12.5%-1 ▷ **Concentration:** 12.5%-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.9	0.1	1	2.9	0.1
2	2.9	0.1	2	2.7	0.1
3	2.9	0.1	3	2.8	0.1
4	2.9	0.1	4	2.7	0.1
5	2.9	0.1	5	2.8	0.1
6	2.8	0.1	6	2.7	0.1
7	2.7	0.1	7	2.8	0.1
8	2.8	0.1	8	2.8	0.1
9	2.8	0.1	9	3.0	0.1
10	3.0	0.1	10	2.8	0.1
11	2.7	0.1	11	2.9	0.1
12	2.7	0.1	12	2.6	0.1
13	2.8	0.1	13	3.0	0.1
14	3.0	0.1	14	2.8	0.1
15	2.8	0.1	15	2.7	0.1
16	2.6	0.1	16	2.9	0.1
17	3.0	0.1	17	2.8	0.1
18			18	3.0	0.1
19			19		
20			20		
21			21		
22			22		
23			23		
24			24		
25			25		
26			26		
27			27		
28			28		
29			29		
30			30		
mean	2.8	0.1	Mean	2.8	0.1

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▷ **Concentration:** 12.5%-3

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.8	0.1
3	2.7	0.1
4	2.8	0.1
5	2.7	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.7	0.1
10	2.7	0.1
11	2.7	0.1
12	2.8	0.1
13	2.7	0.1
14	2.7	0.1
15	2.7	0.1
16	3.0	0.1
17	3.0	0.1
18	2.8	0.1
19	2.8	0.1
20	2.8	0.1
21	3.0	0.1
22	2.7	0.1
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 25%-1

No.	Length (mm)	Dry weight (mg)
1	2.7	0.1
2	2.7	0.1
3	2.8	0.1
4	2.6	0.1
5	2.9	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.7	0.1
10	2.8	0.1
11	2.6	0.1
12	2.7	0.1
13	2.9	0.1
14	3.0	0.1
15	2.9	0.1
16	2.9	0.1
17	3.0	0.1
18	3.0	0.1
19	2.9	0.1
20	2.9	0.1
21	2.6	0.1
22	2.9	0.1
23	2.8	0.1
24	2.8	0.1
25	2.8	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 25%-2

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.9	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.9	0.1
10	2.7	0.1
11	3.0	0.1
12	2.9	0.1
13	2.7	0.1
14	2.7	0.1
15	2.9	0.1
16	2.7	0.1
17	2.7	0.1
18	2.8	0.1
19	2.7	0.1
20	2.8	0.1
21	2.8	0.1
22	2.8	0.1
23	2.7	0.1
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 25%-3

No.	Length (mm)	Dry weight (mg)
1	2.9	0.1
2	2.9	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.9	0.1
8	2.8	0.1
9	2.9	0.1
10	2.8	0.1
11	2.9	0.1
12	2.7	0.1
13	2.7	0.1
14	2.7	0.1
15	2.8	0.1
16	2.9	0.1
17	2.7	0.1
18	2.7	0.1
19	2.7	0.1
20	2.7	0.1
21	2.9	0.1
22	2.8	0.1
23	2.9	0.1
24	3.0	0.1
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 50%-1 ▷ **Concentration:** 50%-2

No.	Length (mm)	Dry weight (mg)	No.	Length (mm)	Dry weight (mg)
1	2.9	0.1	1	3.0	0.1
2	3.0	0.1	2	2.7	0.1
3	2.9	0.1	3	2.7	0.1
4	2.9	0.1	4	2.9	0.1
5	3.0	0.1	5	2.9	0.1
6	2.8	0.1	6	2.9	0.1
7	2.7	0.1	7	2.8	0.1
8	3.0	0.1	8	2.9	0.1
9	2.8	0.1	9	2.7	0.1
10	2.7	0.1	10	2.9	0.1
11	3.0	0.1	11	2.8	0.1
12	2.7	0.1	12	2.9	0.1
13	2.8	0.1	13	2.7	0.1
14	2.7	0.1	14	2.7	0.1
15	3.0	0.1	15	2.9	0.1
16	2.8	0.1	16	2.8	0.1
17	2.9	0.1	17	2.8	0.1
18	2.7	0.1	18	2.9	0.1
19	2.9	0.1	19	2.7	0.1
20	2.7	0.1	20	2.7	0.1
21	2.8	0.1	21	2.9	0.1
22	2.8	0.1	22	2.8	0.1
23	2.7	0.1	23	2.7	0.1
24	2.8	0.1	24	2.6	0.1
25	2.7	0.1	25	2.7	0.1
26			26	2.8	0.1
27			27		
28			28		
29			29		
30			30		
Mean	2.8	0.1	Mean	2.8	0.1

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▷ **Concentration:** 50%-3

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.7	0.1
3	2.7	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.8	0.1
10	2.9	0.1
11	2.9	0.1
12	2.8	0.1
13	2.9	0.1
14	2.7	0.1
15	2.9	0.1
16	2.7	0.1
17	2.9	0.1
18	2.8	0.1
19	2.8	0.1
20	2.7	0.1
21	3.0	0.1
22	3.0	0.1
23	2.8	0.1
24	2.6	0.1
25	2.7	0.1
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 100%-1

No.	Length (mm)	Dry weight (mg)
1	2.8	0.1
2	2.8	0.1
3	2.7	0.1
4	2.7	0.1
5	2.7	0.1
6	2.8	0.1
7	2.7	0.1
8	2.8	0.1
9	2.7	0.1
10	2.8	0.1
11	2.9	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.8	0.1
17	2.7	0.1
18	3.0	0.1
19	2.8	0.1
20	3.0	0.1
21	2.9	0.1
22	2.8	0.1
23	2.8	0.1
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

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▷ **Concentration:** 100%-2

No.	Length (mm)	Dry weight (mg)
1	3.0	0.1
2	2.7	0.1
3	2.8	0.1
4	2.8	0.1
5	2.8	0.1
6	2.8	0.1
7	2.8	0.1
8	2.7	0.1
9	2.8	0.1
10	2.9	0.1
11	2.8	0.1
12	2.7	0.1
13	2.8	0.1
14	2.7	0.1
15	2.8	0.1
16	2.8	0.1
17	2.8	0.1
18	2.7	0.1
19	2.7	0.1
20	2.9	0.1
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

▷ **Concentration:** 100%-3

No.	Length (mm)	Dry weight (mg)
1	2.6	0.1
2	2.8	0.1
3	2.7	0.1
4	2.9	0.1
5	2.9	0.1
6	2.8	0.1
7	2.9	0.1
8	2.9	0.1
9	2.7	0.1
10	2.9	0.1
11	2.8	0.1
12	2.7	0.1
13	2.9	0.1
14	2.7	0.1
15	2.8	0.1
16	2.9	0.1
17	2.8	0.1
18	2.7	0.1
19	2.9	0.1
20	2.9	0.1
21	2.8	0.1
22	2.9	0.1
23		
24		
25		
26		
27		
28		
29		
30		
Mean	2.8	0.1

4.2 Sampling locations and sampling methods

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■ Sampling Locations and Sampling Method/SOPs

▶ COC Code : BW-DBWT1104- KT

Sampling Date	2011. 6. 29
Field team leader	Tae Won Kim
Sampling area	Busan, Sinho
Sampling location	S3, S5
# Samples	2

Condition of transit	
Vehicle	Equipped with ✓ Refrigerator Oxygen Water tank etc. ( )
Temp.	✓ <-4℃ -4~0℃ 0~4℃ ✓ etc. 60℃)
Transfer time	✓ 1h 2h 3h 4h 5h 6h 1day etc. ( )

Location ID Number	Treated seawater (KTM-S3D5-1				
Matrix	IMO soup	**Depth**	0 m	**Sample vol.**	600 L
Sampling SOP	SOP#TP-011				
Containers					
#	1	**Size**	100L 200L 500L ✓ 1,000L etc. (L)		
Type	✓ Cubic Circle	**Material**	Glass ✓ PE PP etc. ( )		
Preservation					
Salinity	33.6 psu	**pH**	7.67	**Temp.**	23.2 ℃
Light protected	✓ Y N				
Maximum Holding Time at 4℃	24h 48h 72h 96h ✓ a week etc. (days)				

Location ID Number	Control seawater (KTM-S3D5-1				
Matrix	IMO soup	**Depth**	0 m	**Sample vol.**	1,000 L
Sampling SOP	SOP#TP-011				
Containers					
#	1	**Size**	100L 200L 500L ✓ 1,000L etc. (L)		
Type	✓ Cubic Circle	**Material**	Glass ✓ PE PP etc. ( )		
Preservation					
Salinity	33.9 psu	**pH**	7.50	**Temp.**	23.1 ℃
Light protected	✓ Y N				
Maximum Holding Time at 4℃	24h 48h 72h 96h ✓ a week etc. (days)				

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▶ Check list of apparatus for field sampling (BW-DBWT1109-01

No.	Division	Standard	Quantity	Check	Remark
1	Water Tank	1000L, PE	2	OK	
2	Water quality checker	YSI 650MDS	1	ok	
3	Refrigerator car	2.4 ton, Equipped with refrigerator	1	ok	
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
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21					
22					
23					
24					
25					
26					
27					
28					
29					
30					

2011. 6. 29

Certified by

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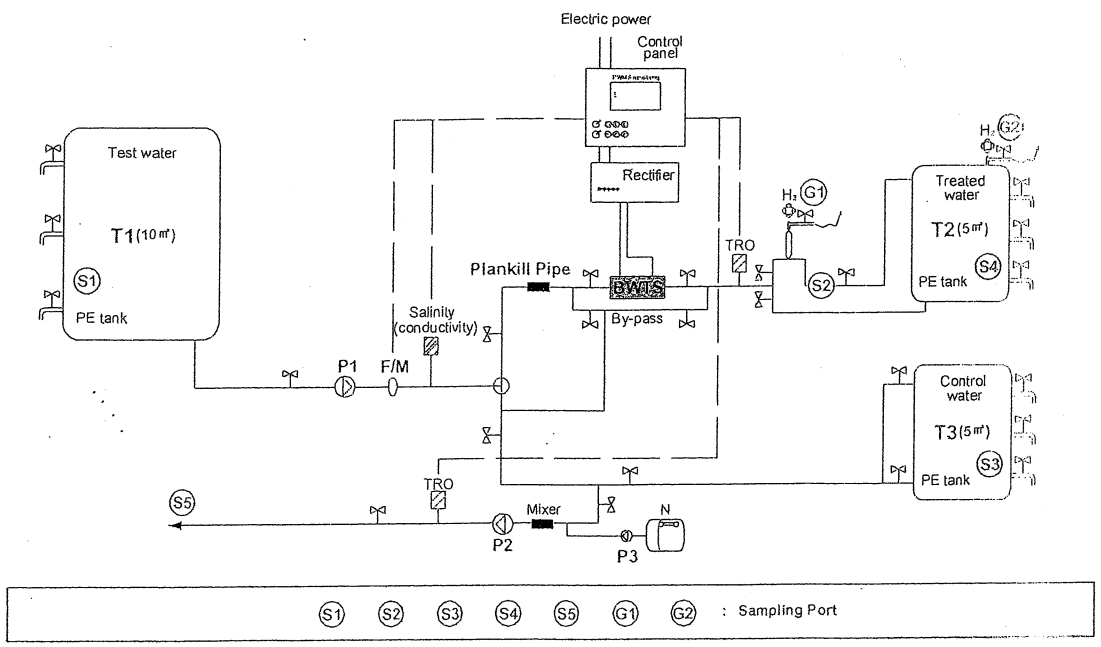
Annex-1



T1: Test water
⋈: Butterfly Valve
P2: De-Ballast Pump
TRO: Sensor (Total residual oxidant)
T2: Treatedwater hoding tank
F/M: Flow meter (magnetic type)
P3: Controlled Volume Pump
H_2: Drain valve (hydrogen)
T3: Control (untreated) water holding tank
P1: Ballast Pump
Sal.: Sensor (Salinity by electrical conductivity)
N: Neutralizing Tank

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■ Sampling Locations and Sampling Method/SOPs

▶ COC Code : BW-DBWT1104-KT

Sampling Date	2011, 1, 5
Field team leader	Tae Won Kim
Sampling area	Busan, Sinho
Sampling location	S3, S5
# Samples	2

Condition of transit	
Vehicle	Equipped with ✓Refrigerator Oxygen Water tank etc. ( )
Temp.	<-4℃ -4~0℃ 0~4℃ ✓etc.(10℃)
Transfer time	✓1h 2h 3h 4h 5h 6h 1day etc. ( )

Location ID Number	Treated seawater (KTM-S5D5-2				
Matrix	IMO soup	**Depth**	0 m	**Sample vol.**	400 L
Sampling SOP	SOP#TP-011				
Containers					
#	1	**Size**	100L 200L 500L ✓1,000L etc. (L)		
Type	✓Cubic Circle	**Material**	Glass ✓PE PP etc. ( )		
Preservation					
Salinity	34.7 psu	**pH**	7.67	**Temp.**	25.5 ℃
Light protected	✓Y N				
Maximum Holding Time at 4℃	24h 48h 72h 96h ✓a week etc. (days)				

Location ID Number	Control seawater (KTM-S3D5-2				
Matrix	IMO soup	**Depth**	0 m	**Sample vol.**	800 L
Sampling SOP	SOP#TP-011				
Containers					
#	1	**Size**	100L 200L 500L ✓1,000L etc. (L)		
Type	✓Cubic Circle	**Material**	Glass ✓PE PP etc. ( )		
Preservation					
Salinity	34.8 psu	**pH**	7.60	**Temp.**	25.6 ℃
Light protected	✓Y N				
Maximum Holding Time at 4℃	24h 48h 72h 96h ✓a week etc. (days)				

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▶ Check list of apparatus for field sampling (BW-DBWT 1104KT

No.	Division	Standard	Quantity	Check	Remark
1	Water Tank	1000L, PE	2	OK	
2	Water quality checker	YSI 650MDS	1	OK	
3	Refrigerator car	2.4 ton, Equipped with refrigerator	1	OK	
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
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26					
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28					
29					
30					

2011. 7. 5.

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485-1 Yongdang, Namgu, Busan 608-830, Korea
Tel. +82-51-611-6200
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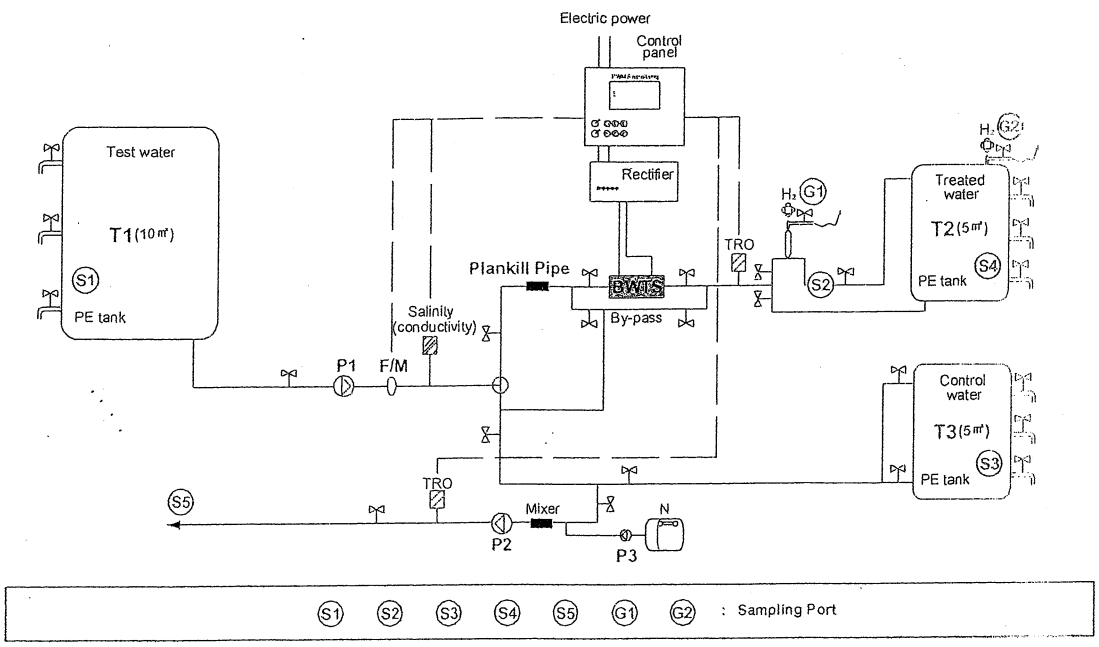
Annex-1



T1: Test water
⋈: Butterfly Valve
P2: De-Ballast Pump
TRO: Sensor (Total residual oxidant)
T2: Treatedwater hoding tank
F/M: Flow meter (magnetic type)
P3: Controlled Volume Pump
H_2: Drain valve (hydrogen)
T3: Control (untreated) water holding tank
P1: Ballast Pump
Sal.: Sensor (Salinity by electrical conductivity)
N: Neutralizing Tank

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■ Sampling Locations and Sampling Method/SOPs

▶ COC Code : BW-DBWT1104-KT

Sampling Date	2011, 7, 12
Field team leader	Tae Won Kim
Sampling area	Busan. Sin ho
Sampling location	S2, S5
# Samples	2

Condition of transit	
Vehicle	Equipped with ✓Refrigerator Oxygen Water tank etc. ( )
Temp.	<-4℃ -4~0℃ 0~4℃ ✓etc.(10℃)
Transfer time	✓1h 2h 3h 4h 5h 6h 1day etc. ( )

Location ID Number	Treated seawater (KTMS5D5-3				
Matrix	IMO soup	**Depth**	0 m	**Sample vol.**	600 L
Sampling SOP	SOP#TP-011				
Containers					
#	1	**Size**	100L 200L 500L ✓1,000L etc. (L)		
Type	✓Cubic Circle	**Material**	Glass ✓PE PP etc. ( )		
Preservation					
Salinity	21.1 psu	**pH**	7.46	**Temp.**	24.6 ℃
Light protected	✓Y N				
Maximum Holding Time at 4℃	24h 48h 72h 96h ✓a week etc. (days)				

Location ID Number	Control seawater (KTMS3D5-3				
Matrix	IMO soup	**Depth**	0 m	**Sample vol.**	1,000 L
Sampling SOP	SOP#TP-011				
Containers					
#	1	**Size**	100L 200L 500L ✓1,000L etc. (L)		
Type	✓Cubic Circle	**Material**	Glass ✓PE PP etc. ( )		
Preservation					
Salinity	22.1 psu	**pH**	7.34	**Temp.**	24.8 ℃
Light protected	✓Y N				
Maximum Holding Time at 4℃	24h 48h 72h 96h ✓a week etc. (days)				

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▶ Check list of apparatus for field sampling (BW-DBWT1104-KT

No.	Division	Standard	Quantity	Check	Remark
1	Water Tank	1000L, PE	2	ok	
2	Water quality checker	YSI 650MDS	1	ok	
3	Refrigerator car	2.4 ton, Equipped with refrigerator	1	ok	
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
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20 11. 9. 12.

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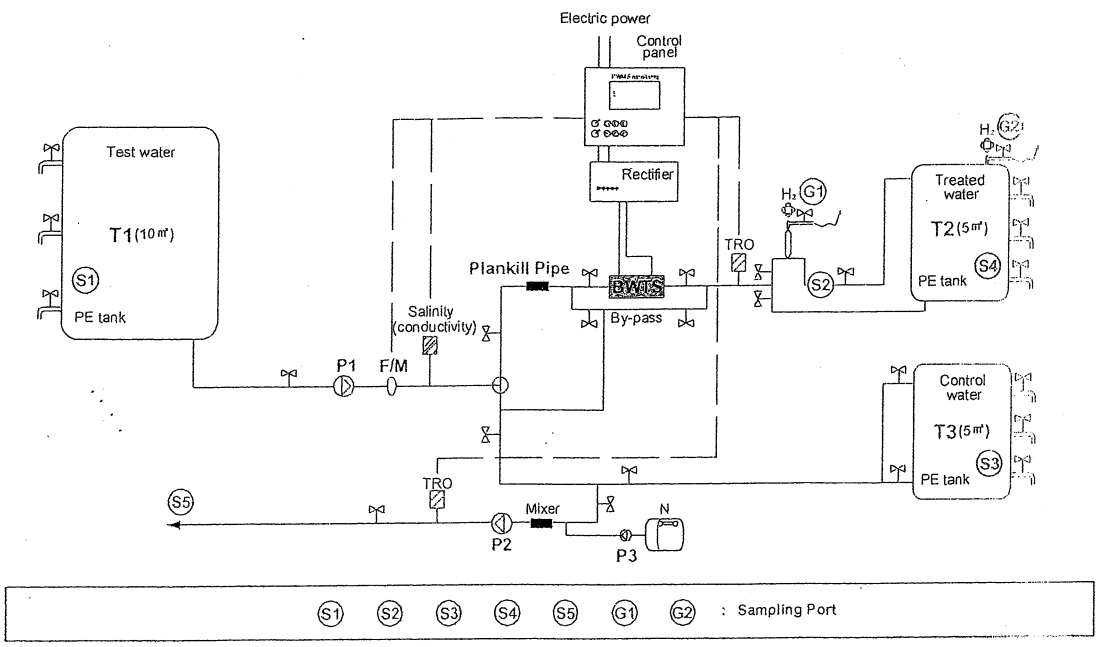
Annex-1



T1: Test water
⋈: Butterfly Valve
P2: De-Ballast Pump
TRO: Sensor (Total residual oxidant)
T2: Treatedwater hoding tank
F/M: Flow meter (magnetic type)
P3: Controlled Volume Pump
H_2: Drain valve (hydrogen)
T3: Control (untreated) water holding tank
P1: Ballast Pump
Sal.: Sensor (Salinity by electrical conductivity)
N: Neutralizing Tank

4.3 Sample handling and custody

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Sample handling and custody

▶ COC Code : BW-DBWT 1104-KT

Date	110629
Lab. manager	Myung-Baek Shon
Sampling area	Busan, Sin ho,

Sample Collection, Packaging and Shipment	
Sample collection	Bio-assay Team
Sample Packing	Bio-assay Team
Coordination of Shipment	None
Type of Shipment	Direct transit Bus Train Airplane Ship ✓other (Vehicle with refrigerator
Sample Receipt and Analysis	
Responsible Organization	MEI
Sample Receipt	Myung Baek Shon
Sample Custody and Storage	Tae Won Kim
Sample preparation	Tae Won Kim and So Eun Jo
Sample Determinative Analysis	Tae Won Kim
Sample archival	
Field Sample Storage (# days from sample collection):	1 2 3 4 5 6 ✓7 etc. (days)
Sample Extract/Digestate Storage (# days from extraction/digestion)	1 2 3 4 5 6 ✓7 etc. (days)
Sample disposal	
Responsible organization	MEI
Responsible personnel	Myung Baek Shon

20 11. 6. 29.

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■ Sample handling and custody

▶ COC Code : BW-DBWT 1104-KT

Date	2011.07.05
Lab. manager	Myung-Baek Shon
Sampling area	Busan, Sinho,

Sample Collection, Packaging and Shipment	
Sample collection	Bio-assay Team
Sample Packing	Bio-assay Team
Coordination of Shipment	None
Type of Shipment	Direct transit Bus Train Airplane Ship ✓ other (Vehicle with refrigerator
Sample Receipt and Analysis	
Responsible Organization	MEI
Sample Receipt	Myung Baek Shon
Sample Custody and Storage	Tae Won Kim
Sample preparation	Tae Won Kim and So Eun Jo
Sample Determinative Analysis	Tae Won Kim
Sample archival	
Field Sample Storage (# days from sample collection):	1 2 3 4 5 6 ✓7 etc. (days)
Sample Extract/Digestate Storage (# days from extraction/digestion)	1 2 3 4 5 6 ✓7 etc. (days)
Sample disposal	
Responsible organization	MEI
Responsible personnel	Myung Baek Shon

2011. 7. 5.

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■ Sample handling and custody

▶ COC Code : BW-DBWT 1104-KT

Date	2011. 7. 12
Lab. manager	Myung-Baek Shon
Sampling area	Busan, Sinho

Sample Collection, Packaging and Shipment	
Sample collection	Bio-assay Team
Sample Packing	Bio-assay Team
Coordination of Shipment	None
Type of Shipment	Direct transit Bus Train Airplane Ship ✓ other (Vehicle with refrigerator
Sample Receipt and Analysis	
Responsible Organization	MEI
Sample Receipt	Myung Baek Shon
Sample Custody and Storage	Tae Won Kim
Sample preparation	Tae Won Kim and So Eun Jo
Sample Determinative Analysis	Tae Won Kim
Sample archival	
Field Sample Storage (# days from sample collection):	1 2 3 4 5 6 ✓ 7 etc. (days)
Sample Extract/Digestate Storage (# days from extraction/digestion)	1 2 3 4 5 6 ✓ 7 etc. (days)
Sample disposal	
Responsible organization	MEI
Responsible personnel	Myung Baek Shon

2011. 7. 12.

Certified by [signature]

Approved by [signature]

4.4 Receipt of test organisms

Marine Eco-Technology Institute Co., Ltd.
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Tel +82-51-611-6200
Fax +82-51-611-0588

■ Receipt of Test Organism

▶ COC Code: BW-DBWT1104-KT

Date / Time	2011 . 6 . 16 13:00
Lab. manager	Myung-Baek Shon
Received by	Tae Won Kim

TEST ORGANISM INFORMATION			
Common Name or Scientific Name	Paralichthys olivaceus		
Ages of the Test Organism	30~50 (hours, days, years)		
Chain of Custody (COC) form	Shipped with sample ✓ No Forms		
Shipped by	MEI (th team) other (Kyeong Yang Fisheries)		
Type of Shipment	Direct transit ✓ Bus Train Airplane Ship other ( )		
TEST ORGANISM RECEIPT			
Responsible Team	BAT		
Temp. for Test Organism	16.0 °C	**Salinity for Test organism**	34 psu
Light source, Intensity L: D cycle	"cool white" fluorecent Lamp, 2000 lux, Light: Dark = 16 h: 8 h		
Mortality (Dead / Total)	10 / 103 , ca. 1.0 %		
Acclimation Period for Use	more than 14 days		
SAMPLE DISPOSAL			
Responsible Team	BAT		
Responsible Personnel	Myung Baek Shon		
REMARKS			

2011 . 6 . 16.

Certified by _____

Approved by _____

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Fax +82-51-611-0588

■ Receipt of Test Organism

▶ COC Code: BW-DBWT 1104-KT

Date / Time	2011 . 6 . 30 14:00
Lab. manager	Myung-Baek Shon
Received by	Tae Won Kim

TEST ORGANISM INFORMATION			
Common Name or Scientific Name	Paralichthys olivaceus		
Ages of the Test Organism	5 (hours, days, years)		
Chain of Custody (COC) form	Shipped with sample ✓No Forms		
Shipped by	MEI (th team) other (Kyeong Yang Fisheries		
Type of Shipment	Direct transit ✓Bus Train Airplane Ship other ( )		
TEST ORGANISM RECEIPT			
Responsible Team	BAT		
Temp. for Test Organism	17.2 °C	**Salinity for Test organism**	33 psu
Light source, Intensity L: D cycle	"cool white" fluorecent Lamp, 2000 lux, Light: Dark = 16 h: 8 h		
Mortality (Dead / Total)	/ , ca. %		
Acclimation Period for Use			
SAMPLE DISPOSAL			
Responsible Team	BAT		
Responsible Personnel	Myung Baek Shon		
REMARKS			

2011 . 6 . 30.

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■ Receipt of Test Organism

▶ COC Code: BW-DBWT 1104 KT

Date / Time	20 11 . 11 . 13 13: 30
Lab. manager	Myung-Baek Shon
Received by	Tae Won Kim

TEST ORGANISM INFORMATION			
Common Name or Scientific Name	Paralichthys olivaceus		
Ages of the Test Organism	6 (hours, days, years)		
Chain of Custody (COC) form	Shipped with sample ✓ No Forms		
Shipped by	MEI (th team) ✓ other (Kyeong Yang Fisheries		
Type of Shipment	Direct transit ✓ Bus Train Airplane Ship other ( )		
TEST ORGANISM RECEIPT			
Responsible Team	BAT		
Temp. for Test Organism	17.2 °C	**Salinity for Test organism**	33 psu
Light source, Intensity L: D cycle	"Cool white" Fluorecent Lamp, 2,000 lux, Light: Dark = 16 h: 8 h		
Mortality (Dead / Total)	/ , ca. %		
Acclimation Period for Use			
SAMPLE DISPOSAL			
Responsible Team	BAT		
Responsible Personnel	Myung Baek Shon		
REMARKS			

20 11 . 1 . 13 .

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4.5 Results of the statistical analysis

Marine Algal Growth Inhibition Test-Specific Growth Rate

Start Date:	110630	Test ID:	BW-DBWT1104-KT-P001	Sample ID:	KTM-S5D5-1(>32 psu)
End Date:	110703	Lab ID:	BAT-Bioassay Team	Sample Type:	34 PSU treated de-ballasting water
Sample Date:	110629	Protocol:	OECD 201	Test Species:	SK-*Skeletonema costatum*
Comments:	72h				

Conc-%	1	2	3
B-Control	0.0674	0.0678	0.0683
6.25	0.0690	0.0703	0.0672
12.5	0.0680	0.0689	0.0689
25	0.0669	0.0690	0.0687
50	0.0640	0.0649	0.0661
100	0.0296	0.0295	0.0308

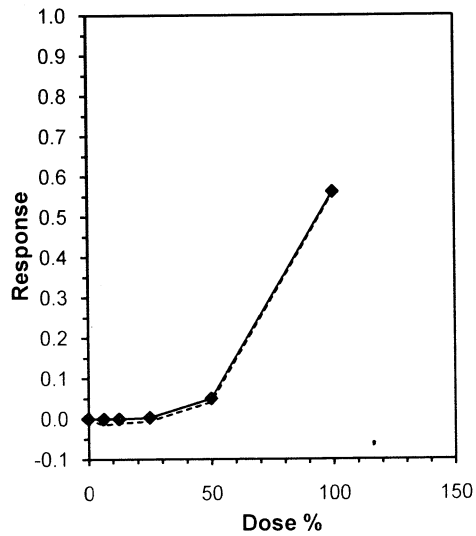
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
B-Control	0.0679	1.0000	0.0679	0.0674	0.0683	0.673	3				0.0684	1.0000
6.25	0.0688	1.0146	0.0688	0.0672	0.0703	2.299	3	-1.201	2.500	0.0021	0.0684	1.0000
12.5	0.0686	1.0108	0.0686	0.0680	0.0689	0.774	3	-0.894	2.500	0.0021	0.0684	1.0000
25	0.0682	1.0051	0.0682	0.0669	0.0690	1.730	3	-0.418	2.500	0.0021	0.0682	0.9966
*50	0.0650	0.9582	0.0650	0.0640	0.0661	1.646	3	3.446	2.500	0.0021	0.0650	0.9501
*100	0.0300	0.4419	0.0300	0.0295	0.0308	2.503	3	46.012	2.500	0.0021	0.0300	0.4382

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.98517	0.897	-0.2623	-0.3646
Bartlett's Test indicates equal variances (p = 0.62)	3.54713	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	25	50	35.3553	4	0.00206	0.03032	0.00072	1E-06	2.2E-14	5, 12
Treatments vs B-Control										

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	50.015	2.556	34.127	52.650	-0.9364
IC10	54.898	0.712	51.811	57.379	0.0738
IC15	59.781	0.647	56.986	61.974	0.0350
IC20	64.665	0.589	62.165	66.608	-0.0047
IC25	69.548	0.537	67.199	71.351	-0.0373
IC40	84.198	0.451	82.145	85.799	0.0314
IC50	93.965	0.470	92.077	95.773	0.1702



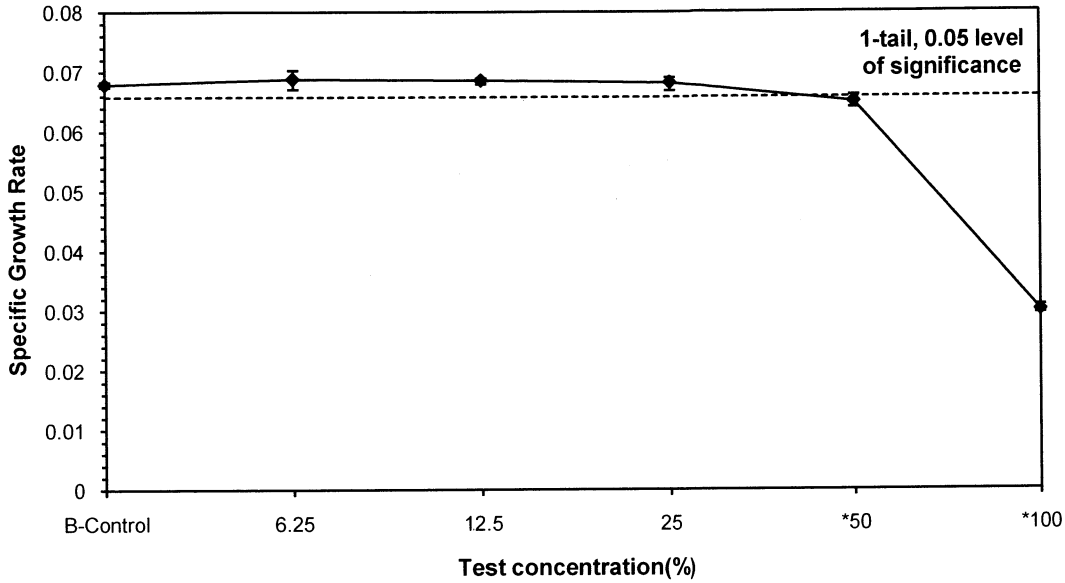
ToxCalc v5.0.23

Reviewed by: 478

Marine Algal Growth Inhibition Test-Specific Growth Rate

Start Date:	110630	Test ID:	BW-DBWT1104-KT-P001	Sample ID:	KTM-S5D5-1(>32 psu)
End Date:	110703	Lab ID:	BAT-Bioassay Team	Sample Type:	34 PSU treated de-ballasting water
Sample Date:	110629	Protocol:	OECD 201	Test Species:	SK-*Skeletonema costatum*
Comments:	72h				

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by:

Marine Algal Growth Inhibition Test-Specific Growth Rate

Start Date:	110713	Test ID:	BW-DBWT1104-KT-P003	Sample ID:	KT-S5D5-3(3-32 PSU)
End Date:	110716	Lab ID:	BAT-Bioassay Team	Sample Type:	20 PSU treated de-ballasting water
Sample Date:	110712	Protocol:	OECD 201	Test Species:	SK-*Skeletonema costatum*
Comments:	72h				

Conc-%	1	2	3
B-Control	0.0658	0.0737	0.0663
6.25	0.0746	0.0757	0.0753
12.5	0.0631	0.0644	0.0680
25	0.0587	0.0616	0.0621
50	0.0444	0.0470	0.0433
100	0.0319	0.0319	0.0306

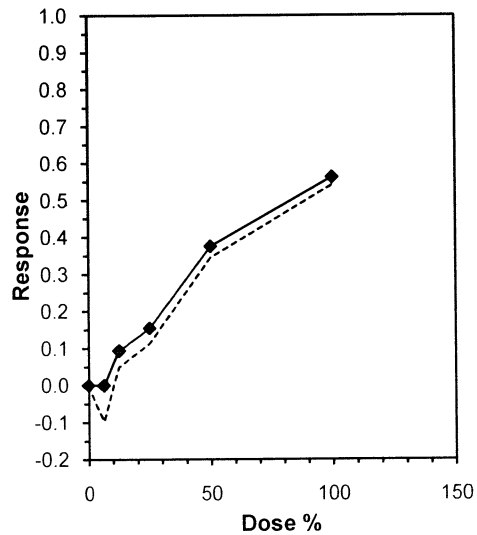
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
B-Control	0.0686	1.0000	0.0686	0.0658	0.0737	6.478	3				0.0719	1.0000
6.25	0.0752	1.0965	0.0752	0.0746	0.0757	0.726	3	-3.410	2.500	0.0049	0.0719	1.0000
12.5	0.0652	0.9506	0.0652	0.0631	0.0680	3.895	3	1.748	2.500	0.0049	0.0652	0.9068
*25	0.0608	0.8869	0.0608	0.0587	0.0621	3.002	3	3.999	2.500	0.0049	0.0608	0.8461
*50	0.0449	0.6550	0.0449	0.0433	0.0470	4.163	3	12.197	2.500	0.0049	0.0449	0.6248
*100	0.0315	0.4586	0.0315	0.0306	0.0319	2.373	3	19.138	2.500	0.0049	0.0315	0.4375

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.94382	0.897	0.92211	1.19009
Bartlett's Test indicates equal variances (p = 0.15)	8.16716	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.6777	8	0.00485	0.07072	0.00081	5.6E-06	2.9E-10	5, 12
Treatments vs B-Control										

Linear Interpolation (200 Resamples)

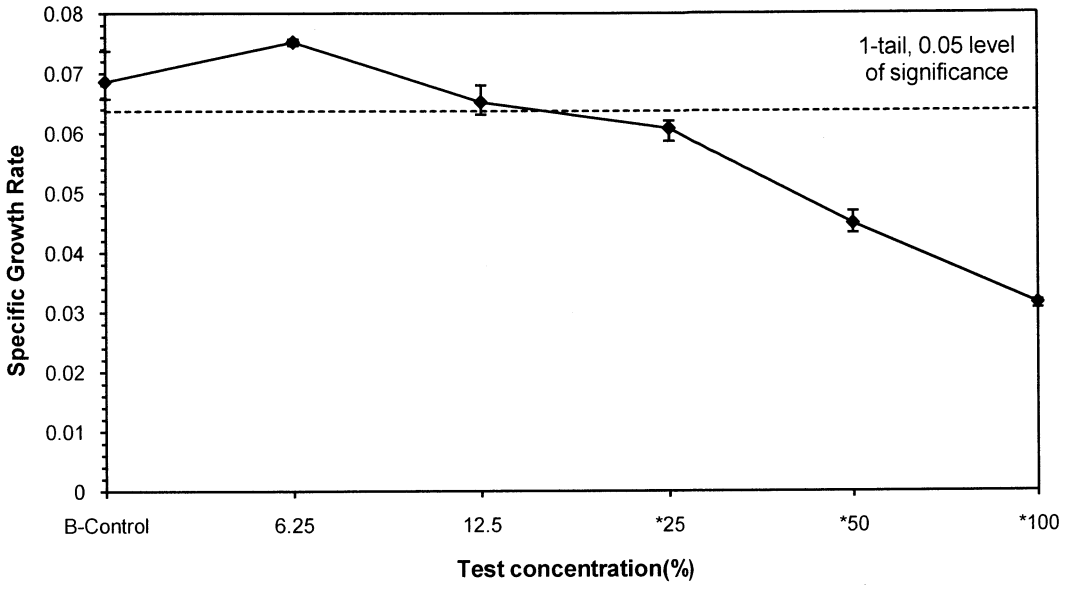
Point	%	SD	95% CL(Exp)		Skew
IC05	9.603	1.052	7.332	15.065	1.6221
IC10	13.902	2.731	7.375	26.870	0.5944
IC15	24.191	3.081	8.411	31.616	-0.9284
IC20	30.205	1.921	20.245	36.465	-0.4083
IC25	35.855	1.778	28.635	42.512	-0.1186
IC40	56.625	3.910	41.906	72.582	0.0436
IC50	83.319	2.489	72.131	92.162	-0.2769



Marine Algal Growth Inhibition Test-Specific Growth Rate

Start Date:	110713	Test ID:	BW-DBWT1104-KT-P003	Sample ID:	KT-S5D5-3(3-32 PSU)
End Date:	110716	Lab ID:	BAT-Bioassay Team	Sample Type:	20 PSU treated de-ballasting water
Sample Date:	110712	Protocol:	OECD 201	Test Species:	SK-*Skeletonema costatum*
Comments:	72h				

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by: 481

Invertebrate Growth Inhibition Test-Population growth

Start Date:	110630	Test ID:	BW-DBWT1104-KT-I002	Sample ID:	KTM-S5D5-1 (>32 psu)
End Date:	110704	Lab ID:	BAT-Bioassay Team	Sample Type:	34 PSU treated de-ballasting water
Sample Date:	110629	Protocol:	ASTM E1440-91	Test Species:	BP-*Brachionus plicatilis*
Comments:	96h reproduction		Janssen et al., 1994		

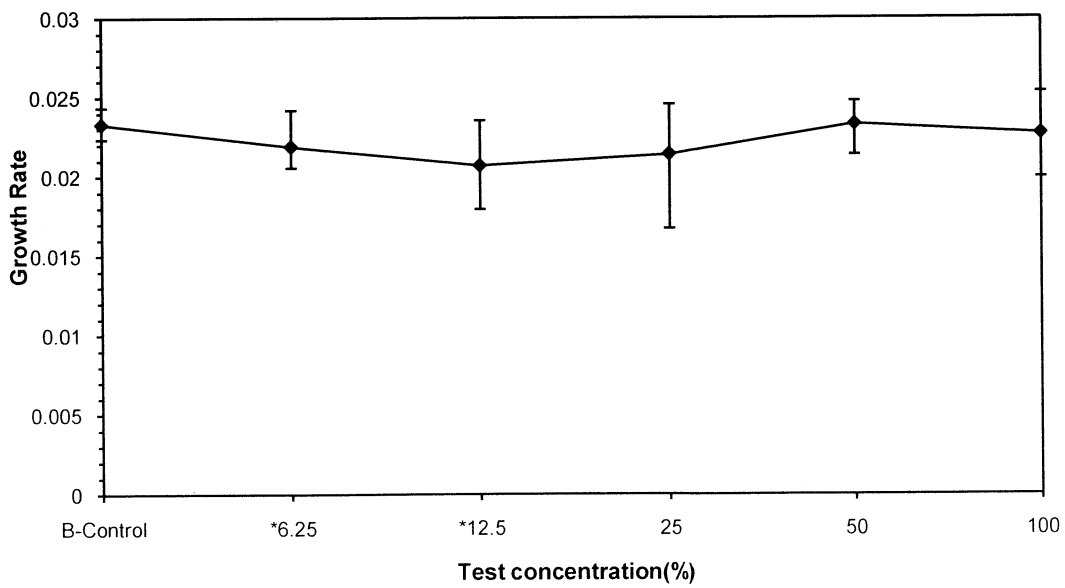
Conc-%	1	2	3	4	5	6	7	8	9	10
B-Control	0.0242	0.0227	0.0227	0.0238	0.0229	0.0240	0.0244	0.0240	0.0233	0.0224
B-Control	0.0227	0.0231								
6.25	0.0214	0.0242	0.0217	0.0211	0.0206	0.0206	0.0238	0.0227	0.0227	0.0222
6.25	0.0214	0.0206								
12.5	0.0233	0.0236	0.0236	0.0193	0.0219	0.0214	0.0183	0.0187	0.0200	0.0214
12.5	0.0190	0.0179								
25	0.0227	0.0244	0.0236	0.0246	0.0244	0.0224	0.0214	0.0176	0.0168	0.0183
25	0.0203	0.0208								
50	0.0238	0.0233	0.0224	0.0248	0.0214	0.0219	0.0222	0.0240	0.0246	0.0229
50	0.0248	0.0238								
100	0.0254	0.0240	0.0248	0.0236	0.0211	0.0231	0.0240	0.0236	0.0206	0.0217
100	0.0200	0.0208								

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
B-Control	0.0233	1.0000	0.0233	0.0224	0.0244	2.997	12			0.0233	1.0000
*6.25	0.0219	0.9381	0.0219	0.0206	0.0242	5.593	12	101.00	111.00	0.0220	0.9431
*12.5	0.0207	0.8870	0.0207	0.0179	0.0236	10.143	12	98.50	111.00	0.0220	0.9431
25	0.0214	0.9183	0.0214	0.0168	0.0246	12.792	12	123.00	111.00	0.0220	0.9431
50	0.0233	0.9992	0.0233	0.0214	0.0248	4.970	12	149.50	111.00	0.0220	0.9431
100	0.0227	0.9731	0.0227	0.0200	0.0254	7.894	12	139.50	111.00	0.0220	0.9431

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.57781	0.895	-0.2346	-0.074
Bartlett's Test indicates unequal variances (p = 4.72E-04)	22.2367	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs B-Control				

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by: 482

Invertebrate Acute Test-24 Hr Survival

Start Date:	110713	Test ID:	BW-DBWT1104-KT-I003	Sample ID:	KT-S5D5-3(3-32 psu)
End Date:	110714	Lab ID:	BAT-Bioassay Team	Sample Type:	3-32 psu treated de-ballasting water
Sample Date:	110712	Protocol:	ASTM E1440-91	Test Species:	BP-*Brachionus plicatilis*
Comments:	End point-24h survival				

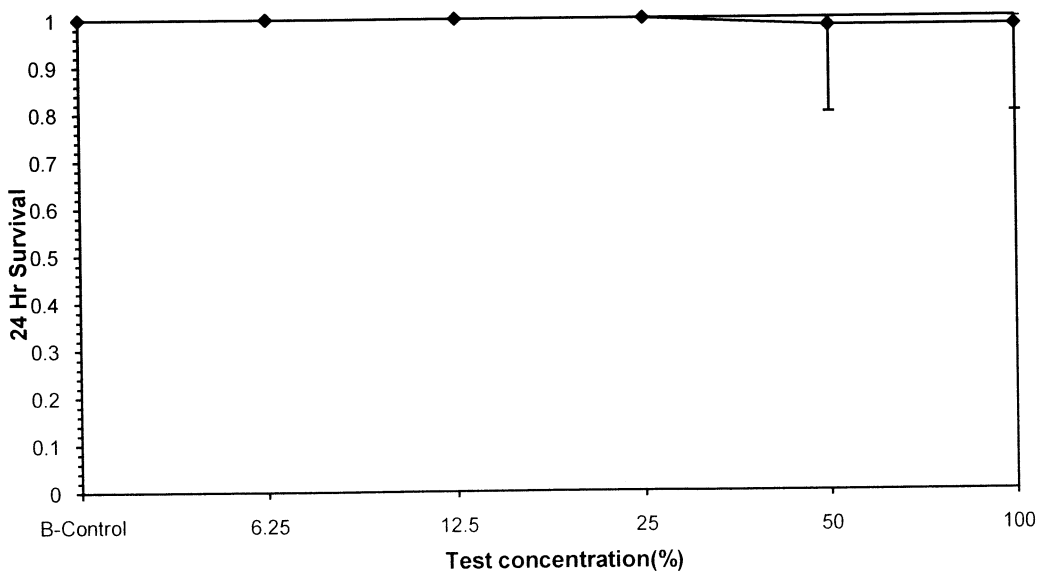
Conc-%	1	2	3	4	5	6	7	8	9	10
B-Control	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
B-Control	1.0000	1.0000								
6.25	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000								
12.5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000								
25	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000								
50	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000	1.0000	1.0000
50	1.0000	1.0000								
100	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
100	0.8000	1.0000								

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
B-Control	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	12		
6.25	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	12	150.00	111.00
12.5	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	12	150.00	111.00
25	1.0000	1.0000	1.5208	1.5208	1.5208	0.000	12	150.00	111.00
50	0.9833	0.9833	1.4863	1.1071	1.5208	8.034	12	144.00	111.00
100	0.9833	0.9833	1.4863	1.1071	1.5208	8.034	12	144.00	111.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	4.04952	0.895	-5.3341	29.3542
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs B-Control				

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by: 483

Invertebrate Growth Inhibition Test-Population growth

Start Date:	110713	Test ID:	BW-DBWT1104-KT-I004	Sample ID:	KTM-S5D5-3(3-32 psu)
End Date:	110717	Lab ID:	BAT-Bioassay Team	Sample Type:	3-32 psu treated de-ballasting water
Sample Date:	110712	Protocol:	ASTM E1440-91	Test Species:	BP-*Brachionus plicatilis*
Comments:	96h reproduction		Janssen et al., 1994		

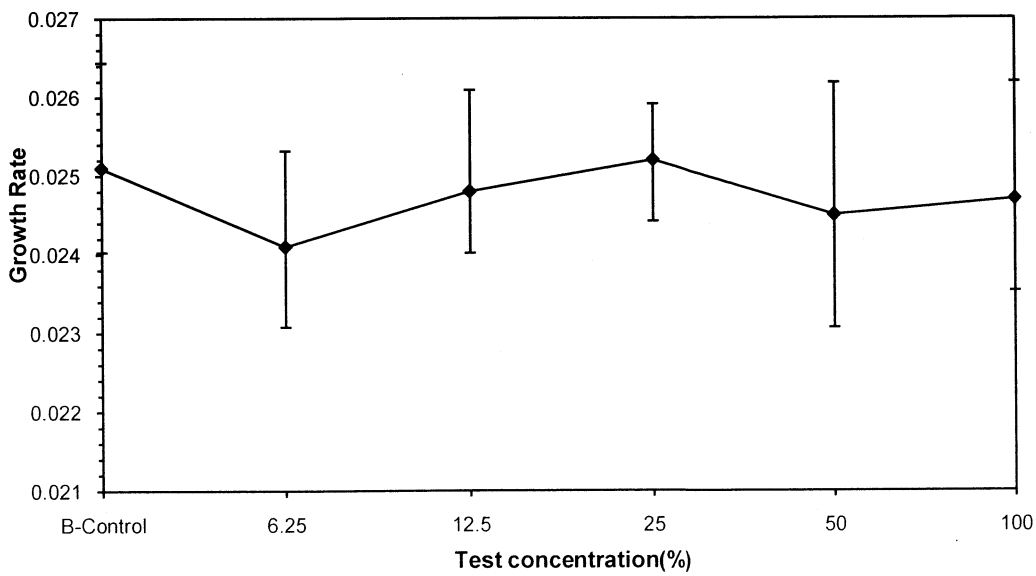
Conc-%	1	2	3	4	5	6	7	8	9	10
B-Control	0.0240	0.0244	0.0255	0.0240	0.0257	0.0262	0.0264	0.0252	0.0240	0.0244
B-Control	0.0252	0.0257								
6.25	0.0231	0.0254	0.0233	0.0240	0.0254	0.0242	0.0231	0.0246	0.0231	0.0231
6.25	0.0254	0.0250								
12.5	0.0248	0.0244	0.0261	0.0244	0.0254	0.0242	0.0240	0.0240	0.0240	0.0255
12.5	0.0254	0.0252								
25	0.0254	0.0252	0.0255	0.0254	0.0250	0.0259	0.0259	0.0252	0.0244	0.0250
25	0.0246	0.0248								
50	0.0238	0.0259	0.0244	0.0233	0.0261	0.0248	0.0246	0.0240	0.0262	0.0231
50	0.0236	0.0248								
100	0.0250	0.0242	0.0244	0.0242	0.0261	0.0238	0.0236	0.0246	0.0238	0.0252
100	0.0262	0.0259								

			Transform: Untransformed					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
B-Control	0.0251	1.0000	0.0251	0.0240	0.0264	3.524	12		
6.25	0.0241	0.9633	0.0241	0.0231	0.0254	3.981	12	113.50	111.00
12.5	0.0248	0.9885	0.0248	0.0240	0.0261	2.863	12	136.00	111.00
25	0.0252	1.0047	0.0252	0.0244	0.0259	1.845	12	155.50	111.00
50	0.0245	0.9796	0.0245	0.0231	0.0262	4.329	12	129.00	111.00
100	0.0247	0.9872	0.0247	0.0236	0.0262	3.755	12	134.50	111.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	0.89734	0.895	0.28955	-0.9977
Bartlett's Test indicates equal variances (p = 0.17)	7.78891	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	100	>100		1
Treatments vs B-Control				

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by: 484

Acute Fish Test-96 Hr Survival

Start Date:	110630	Test ID:	BW-DBWT1104-KT-F001	Sample ID:	KTM-S5D5-1(>32 psu)
End Date:	110704	Lab ID:	BAT-Bioassay Team	Sample Type:	>32 psu de-ballasting water
Sample Date:	110629	Protocol:	OECD 203	Test Species:	PO-Paralichthys olivaceus
Comments:	End point-96hr survival				

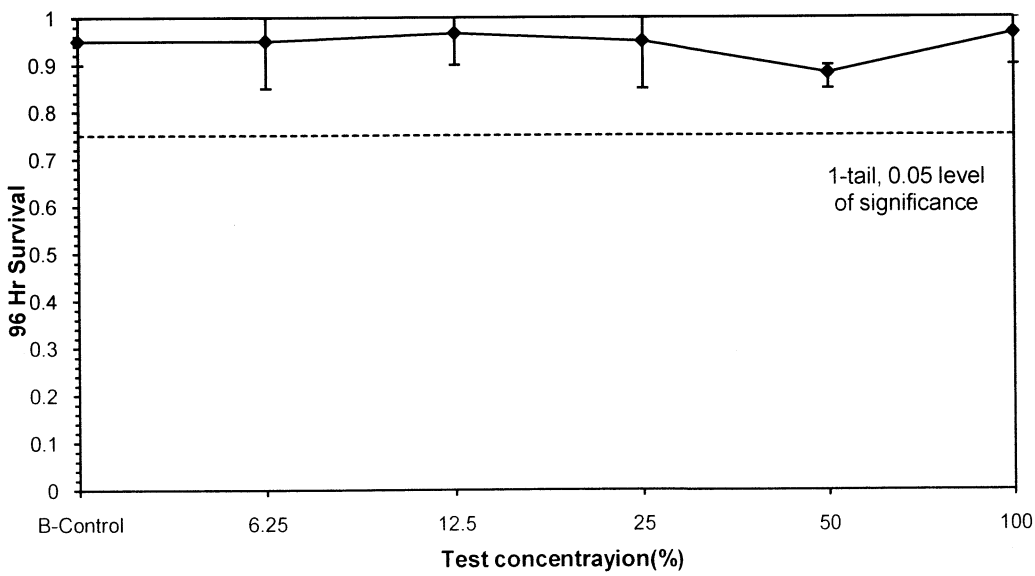
Conc-%	1	2	3
B-Control	1.0000	0.9500	0.9000
6.25	0.8500	1.0000	1.0000
12.5	1.0000	0.9000	1.0000
25	0.8500	1.0000	1.0000
50	0.8500	0.9000	0.9000
100	1.0000	0.9000	1.0000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
B-Control	0.9500	1.0000	1.3717	1.2490	1.5208	10.044	3			
6.25	0.9500	1.0000	1.4049	1.1731	1.5208	14.288	3	-0.256	2.500	0.3235
12.5	0.9667	1.0175	1.4302	1.2490	1.5208	10.969	3	-0.452	2.500	0.3235
25	0.9500	1.0000	1.4049	1.1731	1.5208	14.288	3	-0.256	2.500	0.3235
50	0.8833	0.9298	1.2237	1.1731	1.2490	3.583	3	1.143	2.500	0.3235
100	0.9667	1.0175	1.4302	1.2490	1.5208	10.969	3	-0.452	2.500	0.3235

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.84299	0.897	-0.7356	-1.0409
Bartlett's Test indicates equal variances (p = 0.66)	3.25794	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.21003	0.21858	0.01844	0.02512	0.61185	5, 12
Treatments vs B-Control										

Dose-Response Plot



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Reviewed by: 485

Larval Fish Survival Test-7 Day Survival

Start Date:	110630	Test ID:	BW-DBWT1104-KT-F002	Sample ID:	KTM-S5D5-1,2(>32 psu)
End Date:	110707	Lab ID:	BAT-Bioassay Team	Sample Type:	>32 psu de-ballasting water
Sample Date:	110629	Protocol:	OECD 212	Test Species:	PO-Paralichthys olivaceus

Comments: End point-7days survival

Conc-%	1	2	3
B-Control	0.9667	0.7333	0.9000
6.25	1.0000	0.8667	0.8333
12.5	0.9667	0.8667	0.6667
25	0.9000	0.7667	0.7667
50	0.7333	0.6000	0.6000
100	0.8667	0.8667	0.8333

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.8667	1.0000	1.2215	1.0282	1.3872	14.826	3				40	300
6.25	0.9000	1.0385	1.2893	1.1503	1.5208	15.650	3	-0.546	2.500	0.3106	30	300
12.5	0.8333	0.9615	1.1798	0.9553	1.3872	18.346	3	0.335	2.500	0.3106	49	300
25	0.8111	0.9359	1.1275	1.0667	1.2490	9.339	3	0.757	2.500	0.3106	56	300
50	0.6444	0.7436	0.9334	0.8861	1.0282	8.788	3	2.318	2.500	0.3106	107	300
100	0.8556	0.9872	1.1814	1.1503	1.1970	2.284	3	0.322	2.500	0.3106	43	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.96896	0.897	0.18472	-0.4128
Bartlett's Test indicates equal variances (p = 0.27)	6.38495	15.0863		

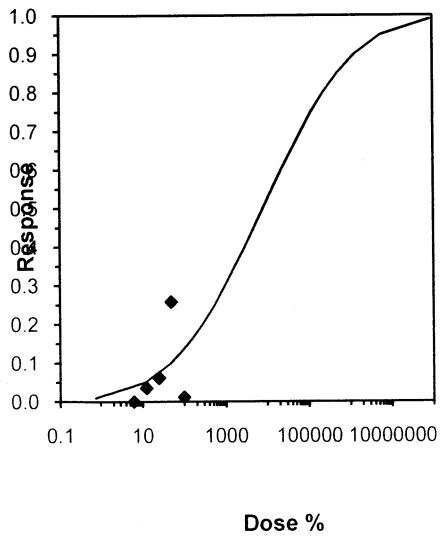
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.25868	0.29301	0.04418	0.02315	0.16657	5, 12
Treatments vs B-Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	0.57699	0.99669	-2.5949	3.7489	0.13333	60.6045	7.81473	4.4E-13	3.90442	1.73312	50
Intercept	2.74717	1.71554	-2.7124	8.20677							
TSCR	0.12203	0.08317	-0.1427	0.38672							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.74574		
EC05	3.355	11.3161		
EC10	3.718	48.2328		
EC15	3.964	128.281		
EC20	4.158	279.122		
EC25	4.326	543.816		
EC40	4.747	2918.72		
EC50	5.000	8024.58		
EC60	5.253	22054.8		
EC75	5.674	118411		
EC80	5.842	230702		
EC85	6.036	501977		
EC90	6.282	1335063		
EC95	6.645	5690457		
EC99	7.326	8.6E+07		

Significant heterogeneity detected (p = 4.37E-13)



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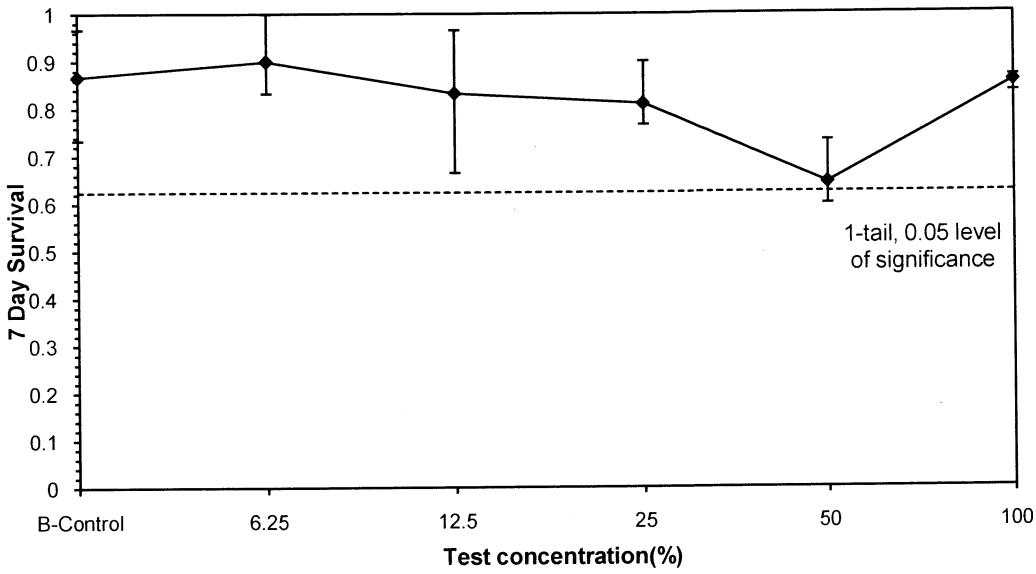
Reviewed by: 486

Larval Fish Survival Test-7 Day Survival

Start Date:	110630	Test ID:	BW-DBWT1104-KT-F002	Sample ID:	KTM-S5D5-1,2(>32 psu)
End Date:	110707	Lab ID:	BAT-Bioassay Team	Sample Type:	>32 psu de-ballasting water
Sample Date:	110629	Protocol:	OECD 212	Test Species:	PO-Paralichthys olivaceus
Comments:	End point-7days survival				

Dose-Response Plot



ToxCalc v5.0.23

Reviewed by: 487

Larval Fish Survival Test-7 Day Survival

Start Date:	110713	Test ID:	BW-DBWT1104-KT-F004	Sample ID:	KTM-S5D5-3(3-32 psu)
End Date:	110720	Lab ID:	BAT-Bioassay Team	Sample Type:	3-32 psu de-ballasting water
Sample Date:	110712	Protocol:	OECD 212	Test Species:	PO-*Paralichthys olivaceus*
Comments:	End point-7days survival				

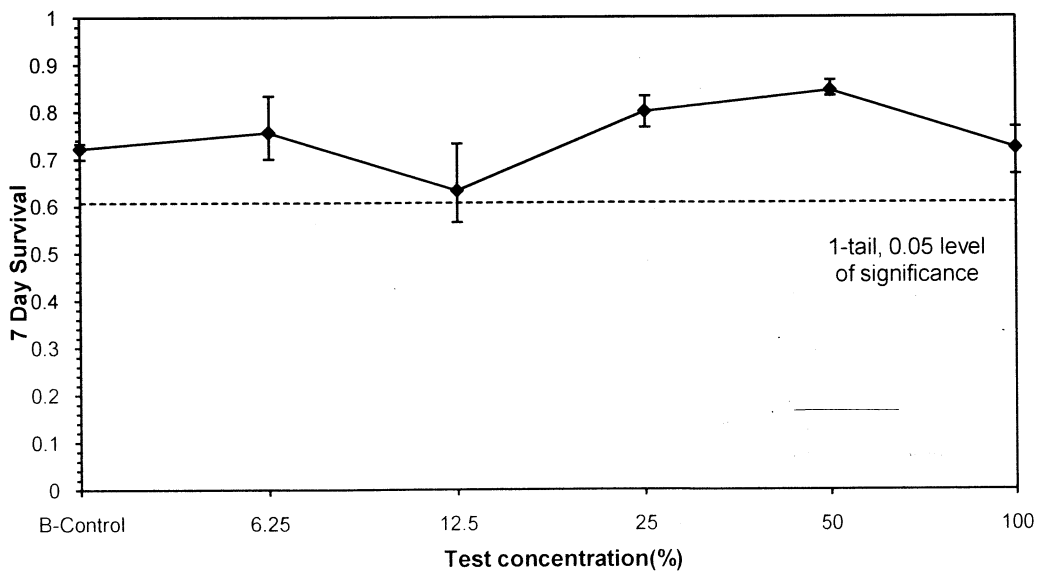
Conc-%	1	2	3
B-Control	0.7333	0.7333	0.7000
6.25	0.7000	0.7333	0.8333
12.5	0.5667	0.6000	0.7333
25	0.8333	0.7667	0.8000
50	0.8333	0.8667	0.8333
100	0.7667	0.6667	0.7333

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
B-Control	0.7222	1.0000	1.0158	0.9912	1.0282	2.103	3			
6.25	0.7556	1.0462	1.0565	0.9912	1.1503	7.881	3	-0.827	2.500	0.1230
12.5	0.6333	0.8769	0.9222	0.8523	1.0282	10.121	3	1.904	2.500	0.1230
25	0.8000	1.1077	1.1080	1.0667	1.1503	3.773	3	-1.875	2.500	0.1230
50	0.8444	1.1692	1.1658	1.1503	1.1970	2.315	3	-3.050	2.500	0.1230
100	0.7222	1.0000	1.0167	0.9553	1.0667	5.562	3	-0.018	2.500	0.1230

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.94903	0.897	0.63005	-0.0877
Bartlett's Test indicates equal variances (p = 0.41)	5.04617	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.11572	0.1602	0.02125	0.00363	0.00577	5, 12
Treatments vs B-Control										

Dose-Response Plot



 ToxCalc v5.0.23 Reviewed by: 488

4.6 Standard Operation Procedure list

Major SOP (Standard Operation Procedure) list for this study is shown as follow:

.1 SOP of TP (Test Procedures)

a. SOP#TP-001: Phytoplankton, Growth Inhibition Test.
b. SOP#TP-006: Fish, Acute Toxicity.
c. SOP#TP-007: Fish, Short-term Toxicity Test on Embryo and Sac-fry Stage.
d. SOP#TP-008: Phytoplankton, Inoculation Methods.
e. SOP#TP-009: Phytoplankton, Counting of Cell numbers.
f. SOP#TP-010: Fish, Measurement of Length and Weight with Larva and Juvenile.
g. SOP#TP-011: Test Water, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests.
h. SOP#TP-012: Measurement of Chlorophyll a and Phaeopigment by fluorometric analysis.
i. SOP#TP-013: Rotifer, Acute Toxicity Test.
j. SOP#TP-014: Rotifer, Population Growth Test.

.2 SOP of TO (Test Organisms)

a. SOP#TO-001: Phytoplankton, Isolation and Culture.
b. SOP#TO-005: Fish, Transportation and Laboratory Culturing.
c. SOP#TO-006: Disposal of the Resting Test Organisms.
d. SOP#TO-007: Rotifer, Cyst Hatching Method.

.3 SOP of TE (Test Equipments)

a. SOP#TE-002: Water Quality Checker

.4 SOP of SP (Statistical Procedure)

a. SOP#SP-101: Toxicity data analysis with Toxcalc software
b. SOP#SP-102: Linear regression analysis with Excel program

5. REFERENCE

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Freshwater Alga and Cyanobacteria, Growth Inhibition Test

INTRODUCTION

1. OECD Guidelines for Testing of Chemicals are periodically reviewed and updated in the light of scientific progress. With respect to Guideline 201, Alga, Growth Inhibition Test (adopted June 1984), the need to extend the Guideline to include additional species and update it to meet the requirements for hazard assessment and classification of chemicals has been identified. The revision has been completed on the basis of extensive practical experience, scientific progress in the field of algal toxicity studies, and extensive regulatory use, which has occurred since the original adoption.

2. Definitions used are given in Annex 1.

PRINCIPLE OF THE TEST

3. The purpose of this test is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria. Exponentially growing test organisms are exposed to the test substance in batch cultures over a period of normally 72 hours. In spite of the relatively brief test duration, effects over several generations can be assessed.

4. The system response is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations of a test substance. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures. For full expression of the system response to toxic effects (optimal sensitivity), the cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate.

5. Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time. Algal biomass is defined as the dry weight per volume, e.g. mg algae/litre test solution. However, dry weight is difficult to measure and therefore surrogate parameters are used. Of these surrogates, cell counts are most often used. Other surrogate parameters include cell volume, fluorescence, optical density, etc. A conversion factor between the measured surrogate parameter and biomass should be known.

6. The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass (average specific growth rate) during the exposure period. From the average specific growth rates recorded in a series of test solutions, the concentration bringing about a specified x % inhibition of growth rate (e.g. 50%) is determined and expressed as the E_rC_x (e.g. E_rC_{50}).

7. An additional response variable used in this Guideline is yield, which may be needed to fulfil specific regulatory requirements in some countries. It is defined as the biomass at the end of the exposure period minus the biomass at the start of the exposure period. From the yield recorded in a series of test solutions, the concentration bringing about a specified x % inhibition of yield (e.g., 50 %) is calculated and expressed as the E_yC_x (e.g. E_yC_{50}).

OECD GUIDELINE FOR TESTING OF CHEMICALS

Adopted by the Council on 17th July 1992

Fish, Acute Toxicity Test

INTRODUCTION

1. This new version of the guideline, originally adopted in 1981 and first updated in 1984, is based on a proposal from the United Kingdom to reduce the numbers of fish in tests of acute aquatic toxicity. The proposal was discussed at a meeting of OECD experts convened at Medmenham (United Kingdom) in November 1988.

2. The main differences in comparison with the earlier versions are the reduction in group-size allowing the use of seven fish per group, the extension of the concentration range by allowing a spacing factor of 2.2 instead of 2 and the introduction of a limit test at 100 mg/l of test substance.

PRINCIPLE OF THE TEST

3. The fish are exposed to the test substance preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 per cent of the fish (LC50) are determined where possible.

INFORMATION ON THE TEST SUBSTANCE

4. It is necessary to know the water solubility of the substance under the conditions of the test. A reliable analytical method for the quantification of the substance in the test solutions must also be available.

5. Useful information includes the structural formula, purity of the substance, stability in water and light, pK_a, P_{ow}, vapour pressure and results of a test for ready biodegradability (see Guideline 301). Solubility and vapour pressure can be used to calculate Henry's constant which will indicate if losses of the test substance may occur.

VALIDITY OF THE TEST

6. For a test to be valid the following conditions should be fulfilled:

- the mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test;

212

Adopted:
21st September 1998

OECD GUIDELINE FOR TESTING OF CHEMICALS

Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages

INTRODUCTION

1. This Short-term Toxicity Test on Fish Embryo and Sac-Fry Stages is a short-term test in which the life stages from the newly fertilized egg to the end of the sac-fry stage are exposed. No feeding is provided in the embryo and sac-fry test, and the test should thus be terminated while the sac-fry are still nourished from the yolk-sac. It is based on a proposal from Denmark which was discussed at an OECD meeting of experts convened at Medmenham (United Kingdom) in December 1991.

2. This guideline is intended to define lethal, and to a limited extent, sublethal effects of chemicals on the specific stages and species tested.

3. This Guideline does not replace Guideline 210 but it would provide useful information in that it could (a) form a bridge between lethal and sublethal tests, (b) be used as a screening test for either a Full Early Life Stage test (Guideline 210) or for chronic toxicity and (c) be used for testing species where husbandry techniques are not sufficiently advanced to cover the period of change from endogenous to exogenous feeding.

4. It should be borne in mind that only tests incorporating all stages of the life-cycle of fish are generally liable to give an accurate estimate of the chronic toxicity of chemicals to fish, and that any reduced exposure with respect to life stages may reduce the sensitivity and thus underestimate the chronic toxicity. It is therefore expected that the embryo and sac-fry test would be less sensitive than the Full Early Life Stage test (Guideline 210), particularly with respect to chemicals with high lipophilicity ($\log P_{ow} > 4$) and chemicals with a specific mode of toxic action. However smaller differences in sensitivity between the two tests would be expected for chemicals with a non-specific, narcotic mode of action (1).

5. Prior to the publication of this Guideline, most experience with this embryo and sac-fry test has been with the freshwater fish *Danio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae - common name zebrafish). More detailed guidance on test performance for this species is therefore given in Annex 1. This does not preclude the use of other species for which experience is also available (Table 1).

Designation: E 1440 – 91 (Reapproved 2004)

Standard Guide for Acute Toxicity Test with the Rotifer Brachionus[1]

This standard is issued under the fixed designation E 1440; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide describes procedures for obtaining laboratory data concerning the acute toxicity of chemicals and aqueous effluents released into fresh, estuarine, or marine waters. Acute toxicity is measured by exposing *Brachionus* newly hatched from cysts to a series of toxicant concentrations under controlled conditions. This guide describes a test for using *B. calyciflorus*, a fresh water rotifer, and the Appendix describes modifications of this test for estuarine and marine waters using *B. plicatilis*. These procedures lead to an estimation of acute toxicity, including the concentration expected to kill 50 % of the test rotifers (LC50) in 24 h. Procedures not specifically stated in this guide should be conducted in accordance with Guide E 729 and Guide E 1192.

1.2 Modifications of these procedures might be justified by special needs or circumstances. Although using appropriate procedures is more important than following prescribed procedures, the results of tests conducted using modified procedures might not be comparable to rotifer acute tests that follow the protocol described here. Comparison of the results using modified procedures might provide useful information concerning new concepts and procedures for conducting acute toxicity tests on chemicals and aqueous effluents.

1.3 This guide is organized as follows:

	Section
Scope	1
Referenced Documents	2
Terminology	3
Summary of Guide	4
Significance and Use	5
Apparatus	6
Dilution Water	7
Hazards	8
Test Material	9
Test Organisms	10
Test Procedure	11
Calculation of Results	12
Acceptability of the Test	13
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1.4 These procedures are applicable to most chemicals, either individually or in formulations, commercial products, or mixtures. This guide can also be used to conduct investigations of the effects on rotifer survival of pH, hardness, and salinity and on materials such as aqueous effluents, leachates, oils, particulate matter, sediments, and surface waters. This guide might not be appropriate for materials with high oxygen demand, with high volatility, subject to rapid biological or chemical transformation, or that readily sorb to test chambers.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazards statements, see Section 8.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

E 380 Practice for Use of the International System of Units (SI) (the Modernized Metric System)

E 729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians[3]

E 943 Terminology Relating to Biological Effects and Environmental Fate[3]

E 1192 Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *rotifer cyst*— a rotifer embryo arrested at an early stage in development, enclosed in an envelope and resistant to desiccation and temperature extremes. Rotifer cysts are often incorrectly referred to as resting eggs. Upon hydration, embryonic development resumes until a neonate female emerges from the cyst.

[1] This guide is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.01 on Aquatic Assessment and Toxicology.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Withdrawn.

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AQUATIC TOXICOLOGY

Cyst-based toxicity tests. VIII. Short-chronic toxicity tests with the freshwater rotifer *Brachionus calyciflorus*

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Abstract

The development and potential use of a 4-day static renewal test [4-day Life Table (LT) test] and a 3-day static test [3-day Population Growth (PG) test] with the freshwater rotifers *Brachionus calyciflorus* are described. For both bioassays, test animals are obtained by hatching cysts which eliminates the need for the culturing and maintenance of the organisms. The toxicity of copper (Cu), pentachlorophenol (PCP), 3,4-dichloroaniline (DCA) and lindane was assessed using the developed methods. The NOEC's, based on the test endpoint r_m, obtained with the 4-day LT test were 0.0025, 0.4, 5 and 20 mg/l for Cu, PCP, DCA and lindane, respectively. Similar results were obtained with the 3-day PG tests for which NOEC's of 0.005, 0.8, 20 and 10 mg/l, respectively, were recorded. The mean cv between replicated 3-day PG tests was 10%, indicating a good intra-laboratory reproducibility of the test results. For Cu and PCP, the sensitivity of *B. calyciflorus* compared favourably to chronic toxicity tests with *Daphnia magna*, while for the other two compounds *B. calyciflorus* proved to be rather insensitive. Considering the increasing need for relatively short toxicity tests, the two described short- chronic bioassays could be valuable new tools for routine toxicity evaluations. The major advantages associated with these tests are: they are less labour-intensive than existing chronic tests, they can be completed within one work week, and do not require stock culturing of test organisms.

Key words: Rotifer; *Brachionus calyciflorus*; Chronic; Life table; Population growth

1. Introduction

Invertebrate short-term chronic toxicity tests have been widely applied in the evaluation of toxic hazards. The two main freshwater chronic toxicity tests with inverte-

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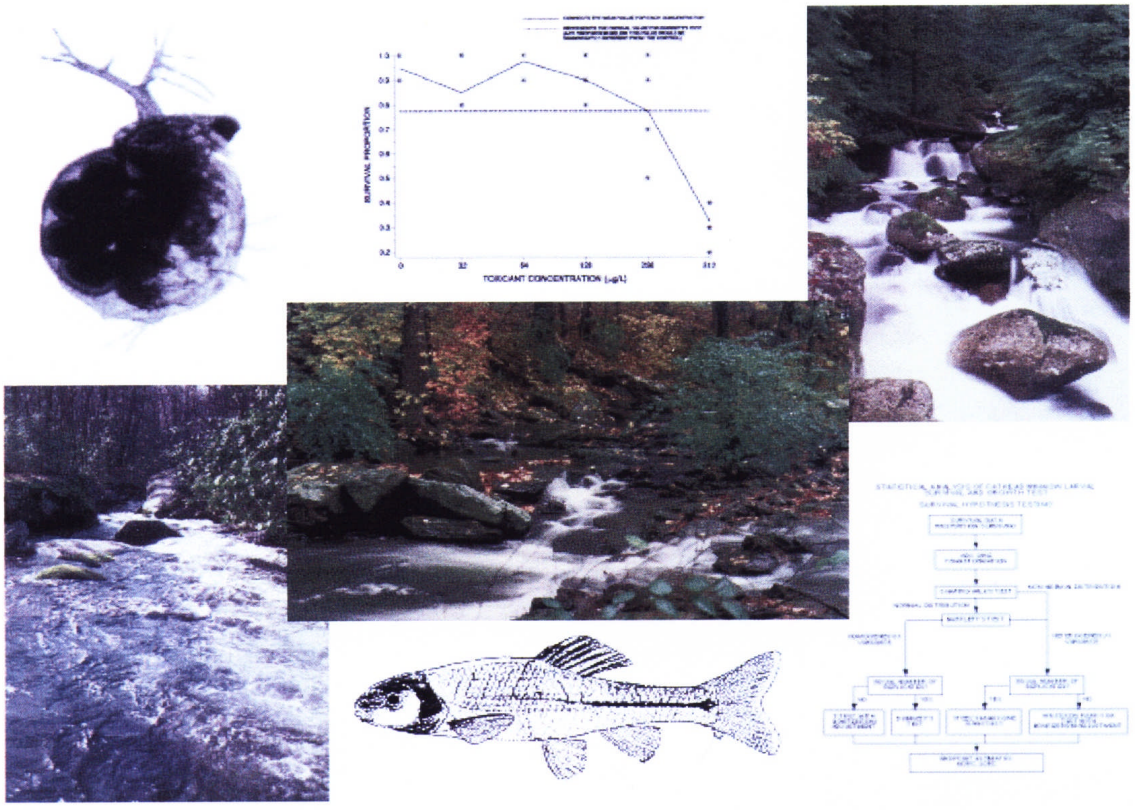
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