

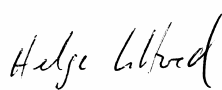
## Technical Report

# Summary of the results of land based and shipboard testing of the OptiMarin Ballast Water Management System



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## 1. General

NIVA has carried out both land based and shipboard testing of the OptiMarin Ballast Water Management System (BWMS) in 2008 and 2009. The testing was conducted in three phases, 1) land based testing in the period of January to May 2008, 2) additional land based testing in June 2009, and 3) shipboard testing in the period from August 2008 to June 2009. A summary of the results from all phases is included in this document.

## 2. Results from the land based trials

Land-based testing of the OptiMarin ballast water management system of OptiMarin AS was completed in the period of January to May 2008. The testing was conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8)*, *Res. MEPC.125(53) Annex 3* and *Procedure for approval of ballast water management systems that make use of active substances (G9)* *Res. MEPC.125(53) Annex 4*, hereafter referred to as G8 and G9. The tests were carried out at NIVA's test site located at Solbergstrand 20 km south of Oslo, with medium salinity and with high salinity water. A total of 12 test cycles have been completed. Each cycle lasted for a period of 5 days and operated with a 330 m<sup>3</sup>/h flow average. An overview of methods used for viable/dead determination of organisms and ecotoxicological effects is given table 1.

### 2.1 Fulfilment of the chemical and biological requirements of the test water

- All IMO requirements of the test water for chemical parameters (total suspended solids, TSS, dissolved organic carbon, DOC, particulate organic carbon, POC), and biological parameters (density and diversity of the  $\geq 50 \mu\text{m}$  group, density and diversity of the  $\geq 10\text{-}50 \mu\text{m}$  group and density of heterotrophic bacteria), were fulfilled in all 12 test cycles, except in test cycle 5, where the concentration of the  $\geq 50 \mu\text{m}$  group was below the IMO requirement.

### 2.2 Biocidal effects of treatment and storage

- The requirement regarding number of organisms  $\geq 50 \mu\text{m}$  in minimum diameter per m<sup>3</sup> in treated water after five days ( $<10/\text{m}^3$ ) and in non-treated control water after five days ( $>100/\text{m}^3$ ) was fulfilled in all test cycles (table 2).
- The requirement regarding number of organisms  $\geq 10 - 50 \mu\text{m}$  in minimum diameter per ml in treated water after five days ( $<10/\text{ml}$ ) and in non treated control water after five days ( $>100/\text{ml}$ ) was fulfilled in all test cycles (table 2).
- Regulation D-2 requires documentation of maximum allowable effluent concentration after five days storage of *Escherichia coli*, *Vibrio cholerae* (toxicogenic serotypes O1 and O139) and Intestinal *enterococci*;  $<250 \text{ cfu}/100\text{ml}$ ,  $<1 \text{ cfu}/100\text{ml}$  and  $<100 \text{ cfu}/100\text{ml}$ , respectively. These requirements were fulfilled in all test cycles.

An overview of the results of the viable/dead determination of organisms and ecotoxicological effects is given in table 2.

**Table 1.** Methods used for viable/dead determination of organisms and ecotoxicological effects. NA= not applicable

Parameter	Instrument/method	Reference	Detection limit
Organisms $\geq 50 \mu\text{m}$	Dissecting microscope 10-40x magnification	OECD Test Guideline (1985)	1/m <sup>3</sup>
Organisms $\geq 10\text{-}50 \mu\text{m}$	Epifluorescence microscope (excitation filter 485 nm; emission filter 530 nm) at 300-480 times magnification. CFDA method.	Ganassin <i>et al.</i> (2000)	1/ml
Organisms $\geq 10\text{-}50 \mu\text{m}$	Serial dilution technique	Thronsen (1978)	0.2/ml
Heterotrophic bacteria	Marine Agar, spread plate	Norwegian Standard NS-EN 6222	1 cfu/ml
Coliform bacteria	m-Endo Broth MF 274930 (Difco) after concentration by filtration.	Norwegian Standard NS 4788	1 cfu/100 ml
<i>Escherichia. coli</i>	m-FC agar after concentration by filtration, 24 h, 44 °C, produce acid from lactose, presumptive <i>E.coli</i> produce gas from lactose or manitol and indol from tryptophan.	NS 4792 or NS-EN ISO 9308-3	1 cfu/100 ml
Enterococcus group	m Enterococcus Agar 274620 (Difco) after concentration by filtration	Norwegian Standard NS-EN ISO 7899-2	1 cfu/100 ml
Intestinal <i>Enterococci</i>	Verification on bile-esculinagar as coloured colonies, 44 °C	NS-EN ISO 7899-2 or 7899-1	1 cfu/100 ml
<i>Vibrio species</i>	T.C.B.S. Cholera-medium Agar CM0333 (Oxoid) after concentration by filtration	NMKL method No. 156, 2. ed 1997, "sykdomsfremkallende Vibrio-arter. Påvisning og bestemmelse i levnedsmidler"	1 cfu/100 ml
<i>Vibrio cholerae</i>	Colonies restriked on TCBS Cholera medium. Physiological and biochemical test kits (API 20E) used for further elimination/identification.	NMKL method No. 156, 2. ed 1997, "sykdomsfremkallende Vibrio-arter. Påvisning og bestemmelse i levnedsmidler"	NA
<i>Vibrio cholerae</i> (serotypes O1 and O139)	PCR, Serotyping	NMKL method No. 156, 2. ed 1997, "sykdomsfremkallende Vibrio-arter. Påvisning og bestemmelse i levnedsmidler"	<1 cfu/100 ml
<i>Algae acute and chronic</i>	Growth inhibition of the marine alga <i>Skeletonema costatum</i>	ISO 10253: Marine algal growth inhibition test	NA
<i>Crustacean acute test</i>	Acute toxicity to the marine crustacean <i>Acartia tonsa</i>	ISO 14669: Determination of acute lethal toxicity to marine copepods ( <i>Copepoda</i> , <i>Crustacea</i> ).	NA
<i>Crustacean chronic test</i>	Reproductive toxicity to the marine crustacean <i>Nitocra spinipes</i>	Forslag til Dansk Standard: "Økotoksikologisk undersøgelse med krepsdyret <i>Nitocra spinipes</i> . Reproduksjonsmetode".	NA
Fish acute test	Acute toxicity to the juvenile turbot ( <i>Scophthalmus maximus</i> )	OECD Guidelines for testing of chemicals (No. 203; Fish, acute toxicity test).	NA
Fish chronic test	Chronic toxicity to the juvenile turbot ( <i>Scophthalmus maximus</i> )	OECD Guidelines for testing of chemicals" (No. 215; Fish, juvenile growth test), adapted for marine species	NA

**Table 2.** Number of viable organisms in treated ballast water after 5 days for all test cycles. The number of the  $\geq 10$ -50  $\mu\text{m}$  group is given by two methods, the CFDA-method and the serial dilution method.

Test cycle	$\geq 50 \mu\text{m}$ per m <sup>3</sup>	$\geq 10$ -50 $\mu\text{m}$ per ml	Heterotrophic bacteria per ml	<i>Vibrio</i> <i>cholerae</i> per 100ml	<i>E.coli</i> per 100ml	Intestinal <i>Enterococci</i> per 100ml
<b>D-2 regulation</b>	<10	<10	-	<1	<250	<100
<b>Seawater</b>						
		CDFA	Dilut.			
<b>1</b>	<1	1	<0.2	12	<1	<1
<b>2</b>	<1	<1	<0.2	61	<1	<1
<b>3</b>	<1	<1	<0.2	59	<1	<1
<b>4</b>	<1	<1	<0.2	57	<1	<1
<b>5</b>	<1	<1	<0.2	20	<1	<1
<b>11</b>	<1	<1	<0.2	150	<1	<1
<b>12</b>	<1	<1	<0.2	31	<1	<1
<b>Brackishwater</b>						
<b>6</b>	1	3	<0.2	210	<1	<1
<b>7</b>	1	5	<0.2	34	<1	<1
<b>8</b>	1	8	<0.2	2000	<1	<1
<b>9</b>	1	5	<0.2	110	<1	<1
<b>10</b>	<1	5	<0.2	4	<1	<1

## 2.3 Toxicity testing

### *Growth inhibition of the marine alga Skeletonema costatum*

A total of 10 algal tests with *Skeletonema costatum* were performed with treated ballast water on day 5. In 6 out of 10 test cycles a low temporary growth inhibition was observed. In these 6 test cycles, reduced growth was observed during the first 24 hours only. After that point, the growth rate became equal to that of the control, indicating no residual toxicity. An EC<sub>10</sub> estimate was made for these tests. The average EC<sub>10</sub> was estimated to a concentration of 81% of undiluted treated ballast water in control water, with a concentration range of 29% to >100%.

### *Crustacean toxicity testing*

Three acute tests with *Acartia tonsa* and two chronic tests with *Nitocra spinipes* were performed on day 5 treated ballast water. In the acute tests, some mortality was only observed in the first test with undiluted treated ballast water. However, mortality was less than 50 %, indicating that the test water was not acute toxic to *Acartia tonsa*. The chronic reproduction tests with *Nitocra spinipes* showed a 32 % reduction in fecundity in the first test at >32 PSU and 20 % reduction of fecundity in the second test at <22 PSU, indicating low chronic effects.

### *Fish toxicity testing*

Two acute and chronic tests with juvenile turbot were performed with undiluted treated ballast water at day 5. No effects were observed.

### *Summary and conclusion with regard to ecotoxicity*

The Optimarin BMWS gave low temporary effects in some of the algal growth inhibition tests and low chronic effect in the invertebrate tests. The observed algae growth inhibition was temporary, indicating that the inhibiting compound will disappear after 24 hours. The causal agent of these effects has not been identified. No significant amounts of organic or inorganic by-products were found by chemical analysis of the treated test water.

**Table 3.** Ecotoxicological effects of treated ballast water after 5 days for all test cycles.

Test cycle	Acute and chronic test	Acute test		Chronic test	
	Algae EC <sub>10</sub> (%)	Crustacea LC <sub>50</sub> (%)	Fish LC <sub>50</sub> (%)	Crustacea NOEC (%)	Fish NOEC (%)
<b>Seawater</b>					
1	29				
2	100	>100	>100	<100	≥100
3	>100				
4	45				
5	81				
11	29	>100			
12	100				
<b>Brackishwater</b>					
6	90				
7	>100		>100	<100	≥100
8	66	>100			
9	>100				
10	>100				

### 3. Summary from the additional land based trial results

Additional land-based tests of the OptiMarin ballast water management system of OptiMarin AS were completed in June 2009 with an alternate filter and an average flow of 500m<sup>3</sup>/h. The report should be regarded as a supplement to previous reported land based testing conducted by NIVA; report no 5643-2008 “Land based testing of the OptiMarin ballast water management system of OptiMarin AS” and report no 5659-2008 “Land based testing of the OptiMarin ballast water management system of OptiMarin AS - treatment effect studies”. Two test cycles were conducted, one with medium salinity water and one with high salinity water. Each cycle lasted for a period of 5 days.

#### 3.1 Fulfilment of the chemical and biological requirements of the test water

- All IMO requirements of the test water for total suspended solids (TSS), dissolved organic carbon (DOC), particulate organic carbon (POC), density and diversity of the ≥50 µm group, density and diversity of the ≥10-50 µm group and density of heterotrophic bacteria were fulfilled in both test cycles.

#### 3.2 Biocidal effects of treatment and storage

- The required less than 10 viable organisms ≥50 µm in minimum diameter per m<sup>3</sup> in the treated water after five days storage was fulfilled in both test cycles. The equivalent requirement of the non-treated control test water, stating that the level of viable organisms after 5 days storage should be higher than 100 per m<sup>3</sup>, was fulfilled in both test cycles.
- The required less than 10 viable organisms ≥10 - 50 µm in minimum diameter per ml in the treated water after five days storage was reached in both test cycles when results were based on the dilution method. By using the microscope CFDA method, the viable count was higher than the requirement on day 5 in test cycle 2. It was concluded that the apparent viability in CFDA-stained cells was due to a methodical problem. The equivalent requirement for the non-treated control test water after 5 days storage states that the level of viable organisms should be higher than 100 per ml. This requirement was fulfilled in both test cycles.
- The regulation D-2 requires documentation of maximum allowable effluent concentration after five days storage of *Escherichia coli*, *Vibrio cholerae* (toxicogenic serotypes O1 and

O139) and Intestinal *enterococci*; which is <250 cfu/100ml, <1 cfu/100ml and <100 cfu/100ml, respectively. These requirements were fulfilled for both test cycles.

In conclusion, treatment system performed in compliance with the D-2 criteria for organisms in the various size groups. The results indicated that ballast water needs to be UV-treated both on ballasting and deballasting, according to the current procedure, to meet the D-2 criteria.

### 3.3 Toxicity testing

In the additional tests, there were no evidence of toxicity in ballast water treated with the OptiMarin BWMS by using the two sensitive tests; 1) the algal growth inhibition test and 2) standard oyster test. In the algae test, the percentage of treated ballast water resulting in 10% and 50% inhibition of the growth rate (i.e. EC<sub>10</sub> and EC<sub>50</sub>) was higher than 100% in all samples from day 0 and day 5, indicating no significant effects by un-diluted treated ballast water. The possible observed effects on oyster larvae when extending the exposure duration for 7 days can not be given too much weight since control mortalities were greater than the normal validity test criteria of 40%. The high mortality in test water and control water can possibly be attributed to high bacteria concentrations.

An overview of the biological and ecotoxicity results for the additional land based test cycles is given in the table 4.

**Table 4.** Number of viable organisms in treated ballast water after 5 days and ecotoxicological effects of treated ballast water after 5 days

Test cycle	Viable organisms						Ecotoxicological tests		
	≥50 µm per m3	≥10-50 µm per ml	Hetero. bacteria per ml	<i>V. cholerae</i> per 100ml	<i>E. coli</i> per 100ml	Intestinal <i>Ent.</i> per 100ml	Acute chronic Alge test EC <sub>10</sub> (%)	Acute Oyster test NOEC (%)	Chronic Oyster test NOEC (%)
D-2 reg	<10	<10	-	<1	<250	<100			
1	<1	1	47	<1	<1	<1	>100	100	56
2	6	40	39	<1	<1	<1	>100	-	-

## 4. Results from the shipboard testing

Shipboard testing of the OptiMarin Ballast System of OptiMarin AS was conducted in the period from August 2008 to June 2009, a period exceeding 6 months. The testing was conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8)*, Res. MEPC.126(53) Annex 3, hereafter referred to as G8. The tests were carried out onboard the vessel KCL Banshee. A total of 8 test cycles (A-H) were completed. An overview of the procedures used for biological verification of treatment efficiency is given in the table 1. The results of the tests cycles in compliance with D-2 (B, G and H) are shown in table 5.

### 4.1 Fulfilment of the biological requirements of the uptake ballast water

- The required density of the ≥50 µm group was met in all 8 tests cycles (number of viable organism concentration exceeding 10 times the values of Regulation D-2.1).
- The requirement in the ≥10-50 µm group was met in 5 test cycles (number of viable organism concentration exceeding 10 times the values of Regulation D-2.1).

#### 4.2 Biocidal effects of treatment

- The required <10 viable organisms  $\geq 50 \mu\text{m}$  in minimum diameter per  $\text{m}^3$  in the treated water was fulfilled in 5 tests cycles. The requirement of the non-treated control water, stating that the level of viable organisms at deballasting should be higher than 10 per  $\text{m}^3$ , was fulfilled in all 8 test cycles.
- The required <10 viable organisms  $\geq 10 - 50 \mu\text{m}$  in minimum diameter per ml in treated water on deballasting was reached in all 8 test cycles. The requirement for the non-treated control water, stating that the level of viable organisms should be higher than 10 per ml, was fulfilled in all test cycles.
- Regulation D-2 requires the maximum concentrations of *Escherichia coli*, *Vibrio cholerae* (toxicogenic serotypes O1 and O139) and Intestinal *Enterococci to be*; <250 cfu/100ml, <1 cfu/100ml and <100 cfu/100ml, respectively, in the treated ballast water upon discharge. These requirements were fulfilled for all the bacteria in all 8 test cycles.

The results of the three cycles in compliance with IMO requirements (B, G and H) are given in Table 5.

Incompliance in test cycles A and C was due to low counts of organisms in uptake water. The reason for incompliance in test cycle D and E was a contamination problem due to faulty ship equipment causing water containing high concentrations of organisms  $\geq 50 \mu\text{m}$  to contaminate the treated water discharge. In test cycle F the failure to meet the requirement was caused by the ships crew not operating the system according to the standard operating procedure between test cycles. These operational problems were not indicative for a faulty BWMS, and as such it can be claimed that three consecutive, valid and successful test cycles in compliance with the IMO requirements were completed.

**Table 5.** Overview of test cycles B-G-H and the concentration of viable organisms in the different size classes. The results are reported as mean value of triplicate samples including standard deviations.

Test cycle	Uptake water for the ballast water to be treated		Uptake water for the control tank		Treated ballast water on discharge		Control ballast water on discharge	
	$\geq 50 \mu\text{m}$	$\geq 10-50 \mu\text{m}$	$\geq 50 \mu\text{m}$	$\geq 10-50 \mu\text{m}$	$\geq 50 \mu\text{m}$	$\geq 10-50 \mu\text{m}$	$\geq 50 \mu\text{m}$	$\geq 10-50 \mu\text{m}$
<b>Requirement</b>	<b>&gt;100</b>	<b>&gt;90</b>	<b>&gt;100</b>	<b>&gt;90</b>	<b>&lt;10</b>	<b>&lt;10</b>	<b>&gt;10</b>	<b>&gt;10</b>
B	10284 ±6261	204±53	14898 ±3762	187±8	8.4±5.5	0.1±0.3	4558 ±3321	135±59
G	68758 ±13305	937±167	57492 ±7349	724±205	0.8±1.6	0.3±0.7	17110 ±4274	365±74
H	96529 ±42838	1052±98	160017 ±18696	1096±143	2.1±2.8	0.7±0.8	84242 ±38845	773±54