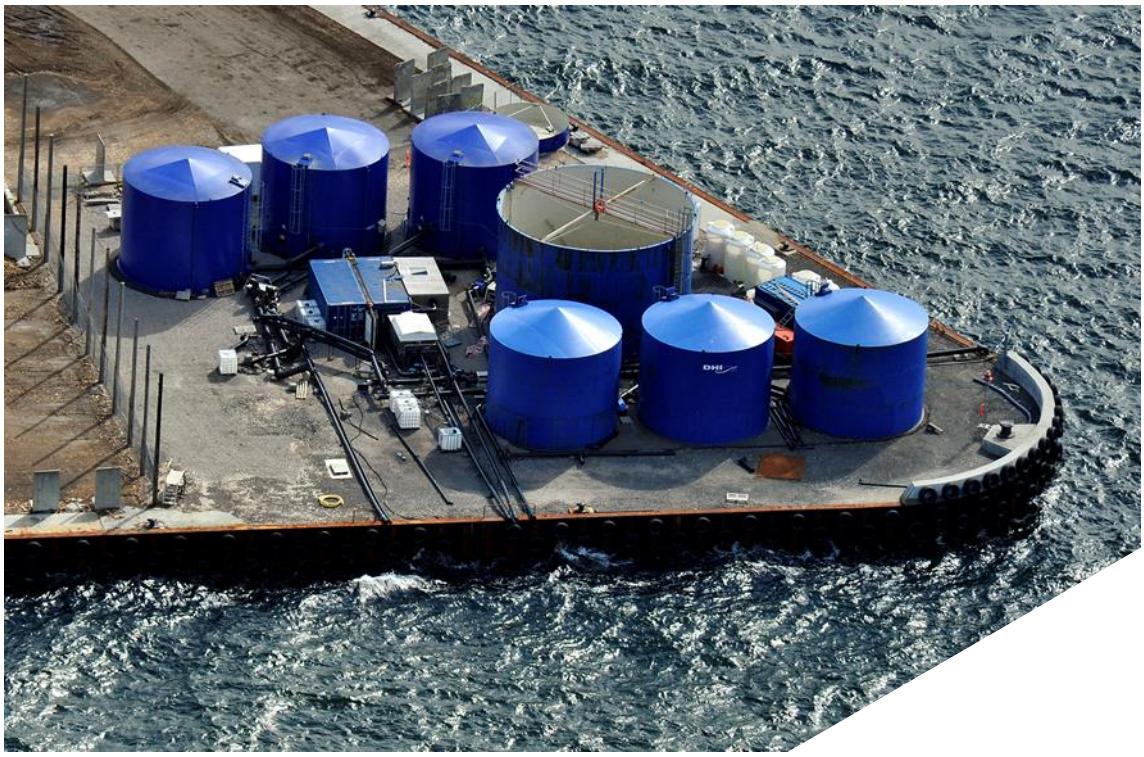
Ecotoxicity testing and risk assessment of wash water from open loop scrubbers



Exhaust Gas Cleaning Systems Association (EGCSA)

Final report

June 2021

The expert in WATER ENVIRONMENTS

This report has been prepared under the DHI Business Management System certified by Bureau Veritas to comply with ISO 9001 (Quality Management)

Approved by

08-06-2021

X

Approved by

Signed by: Torben Madsen

Ecotoxicity testing and risk assessment of wash water from open loop scrubbers

Prepared for	Exhaust Gas Cleaning Systems Association (EGCSA)
Represented by	Mr Donald Gregory, Director

DHI Maritime Technology Evaluation Facility in Hundested, Denmark

Project manager	Anja Kamper
Quality supervisor	Torben Madsen

Project number	Final report / 11826102
Approval date	2021-06-08
Revision	Final report / 11826102 / 2021-06-08
Classification	Confidential

DHI A/S • Agern Allé 5 • DK-2970 Hørsholm • Denmark
Telephone: +45 4516 9200 • dhi@dhigroup.com • www.dhigroup.com • Company Reg. No. DK36466871

CONTENTS

FIGURES

TABLES

APPENDICES

Ecotoxicity testing and risk assessment of wash water from open loop scrubbers

Final report / 11826102 / 2021-06-08

Approved by
08-06-2021 X  Torben Madsen Vice President, BWL Signed by: Torben Madsen

Quality Compliance Statement

This report shall not be reproduced except in full, without written approval of DHI A/S (hereinafter referred to as “DHI”).

The report contains no known errors, omissions or false statements

The on-board sampling of scrubber water was not performed under the DHI quality system. The testing and reporting were prepared in compliance with the international standard ISO/IEC 17025.

Abbreviations

Abbreviation	Description
EC10	The concentration of a chemical or effluent at which other adverse effects than death are observed on 10 % of the test organisms
EC50	The concentration of a chemical or effluent at which other adverse effects than death are observed on 50 % of the test organisms
DANAK	Danish Accreditation Fund
EGCS	Exhaust Gas Cleaning System
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
LC10	The concentration of a chemical or effluent at which lethal effects are observed on 10 % of the test organisms
LC50	The concentration of a chemical or effluent at which lethal effects are observed on 50 % of the test organisms
LDR	Larval development ratio: fraction of animals that have turned into a copepodite stage compared to the total number of surviving nauplii and copepodite
LOEC	Lowest observed effect concentration. The lowest concentration of a chemical or effluent causing statistically significant adverse effects on the test organisms
NOEC	No observed effect concentration. The highest concentration of a chemical or effluent at which no statistically significant adverse effects on the test organisms are observed
P&T	Purge and trap
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
PSU	Practical salinity unit
RCR	Risk characterization ratio
TC	Threshold concentration
WET	Whole effluent toxicity

1 Executive summary

When exhaust gas cleaning systems, also known as scrubbers, are operated in open-loop mode, the scrubber discharge water is discharged into the sea, sometimes after filtering and buffering with seawater. Concerns have been raised about the environmental impact of scrubber discharges, especially in port areas.

The present report includes a risk assessment based on a series of ecotoxicological studies of a composite sample from four different open loop scrubbers. All on-board sampling was organised by the Exhaust Gas Cleaning Systems Association (EGCSA) and performed by the vessel crew according to the detailed procedures provided by EGCSA and DHI.

The scope of the project was to assess the environmental impact of the scrubber discharge water. The project included the following activities:

- Aquatic toxicity tests performed with the whole effluent of the scrubber discharge water
- Chemical analyses of selected substances in the inlet and discharge scrubber water
- Calculation of predicted environmental concentrations (PEC) of the scrubber discharge water by use of MAMPEC modelling
- Environmental risk assessment based on the results of the eco-toxicity tests

A summary of the results of the aquatic toxicity tests with scrubber discharge water is presented in Table 1.1. No significant negative effects of inlet water were observed in any of the tests compared to the laboratory control.

Table 1.1 Results of aquatic toxicity tests in millilitres of scrubber discharge water per litre of test medium. Last column is the effect of inlet scrubber water compared to the laboratory control. Numbers in parentheses are the 95% confidence intervals.

Test organism	Endpoint	NOEC (mL/L)	LOEC (mL/L)	EC/LC10 (mL/L)	EC/LC25 (mL/L)	EC/LC50 (mL/L)
Alga (*Skeletonema sp.*) ISO 10253	Growth	500	625	740 (480->910)	>910	>910
Crustacean (*Acartia tonsa*), acute ISO 14669	Mortality	-	-	720 (580-870)	>1,000	>1,000
Fish (*Dicentrarchus labrax*) OECD 203	Mortality and behaviour	-	-	>910	>910	>910
Crustacean (*Acartia tonsa*), chronic ISO 16778	Hatching success	800	>800	>800	>800	>800
	Mortality	800	>800	>800	>800	>800
	Larval development ratio (LDR)	200	400	200 (110-280)	340 (250-430)	540 (470-610)

The aquatic toxicity tests were performed as whole effluent toxicity (WET) tests, and the results were included in the environmental risk assessment of the scrubber discharge water. The risk assessment is based on the WET approach and the assessment factors for marine risk assessment used in the ballast water regulations (BWM.2/Circ.13/Rev.4 20 July 2017). Furthermore, the recommendations set by the GESAMP Task Team with performance of a set of four to five well-established, good quality WET tests, including short term and long-term endpoints on species of three trophic levels (GESAMP task team, 13. December 2019) were followed.

The PEC was calculated by use of the marine antifoulant model to predict environmental concentrations (MAMPEC). The discharge of the scrubber discharge water was assumed to occur in three different scenarios: The GESAMP-BWWG Model Harbour, the OECD-EU Commercial Harbour, and the OECD-EU Shipping Lane.

The scrubber discharge water was handled as an inert substance that remain dissolved in the water phase, as the exact properties of the substances in the discharge water are not known. This conservative approach was used to calculate volume-based WET thresholds for open-loop scrubber water discharges in the specific scenarios.

The risk of the scrubber discharge water to the aquatic environment is characterized by use of:

- The predicted environmental concentration (PEC) in the aquatic environment which was calculated by use of MAMPEC
- The predicted no effect concentration (PNEC), i.e., the concentration of the scrubber discharge water in the aquatic environment below which unacceptable effects will most likely not occur

The risk of the scrubber discharge water to the aquatic environment is expressed by use of the risk characterization ratio (RCR) which is the ratio between PEC and PNEC. Thus, the RCR = PEC/PNEC, and unacceptable effects to the environment are unlikely, when RCR is below 1.

In the present risk assessment of the scrubber discharge water the RCR was below 1, and this means that the risk to the aquatic environment can be considered acceptable.

2 Introduction

DHI A/S (hereinafter referred to as “DHI”) is an independent, international consulting and research organisation established in Denmark and today represented in all regions of the world with a total of more than 1,000 employees. Our objectives are to advance technological development, governance and competence in the fields of water, environment and health. DHI works with governmental agencies and authorities, contractors, consultants and numerous industries.

DHI’s services include independent type approval testing and risk evaluation of ballast water management systems (BWMS), ecotoxicological studies, and risk assessment of chemicals and effluents from vessels, offshore installations and wastewater treatment plants for regulatory purposes. DHI has no involvement, intellectual or financial, in the mechanics, design or marketing of the products and technologies that are being evaluated. To ensure that DHI’s tests are uncompromised by any real or perceived individual or team bias relative to test outcomes, DHI’s activities are subject to rigorous quality assurance (QA), quality control (QC) and documentation. DHI’s quality management system is certified according to ISO 9001. Furthermore, the ecotoxicological studies were performed in accordance with ISO/IEC 17025:2017.

The objective of this study was to evaluate the potential effects on the aquatic environment of discharge water from Exhaust Gas Cleaning Systems (EGCS), also known as scrubbers, operated in open loop mode. A scrubber system reduces Sulphur oxides (SOx) emissions from combustion engines to air by spraying alkaline water into the exhaust gas flow. An open loop scrubber uses the natural alkalinity of seawater for neutralizing the exhaust emissions, and the drained wash water containing the washed-out substances and particles are discharged into the sea, sometimes after filtering and buffering with seawater. Scrubber water monitoring equipment is used to verify that the discharge water complies with discharge standards for PAH, turbidity and pH.

Concerns have been raised about the environmental impact of scrubber discharges, especially in shipping lanes and ports. This report includes a risk assessment based on a series of ecotoxicological studies of scrubber inlet and discharge water collected from four different test vessels during open-loop scrubber operation at sea. The focus was to provide well documented and sound scientific ecotoxicity studies of discharge water from open loop systems on merchant vessels and not to study a specific cleaning technology, specific types of vessels or engines.

The scope of the project was to assess the ecotoxicological effects and potential environmental risks of the whole effluent from open loop scrubbers. The following main topics were evaluated:

- Whole effluent toxicity (WET) tests carried out with marine species
- Chemical analyses of selected substances
- Calculation of predicted environmental concentrations (PEC) of the whole effluent water by use of MAMPEC
- Environmental risk assessment based on whole effluent toxicity tests

3 Test vessels and sampling

All coordination regarding test vessels, scrubber system operation, data logging on-board, shipment of samples etc. was managed by the Exhaust Gas Cleaning Systems Association (EGCSA).

The four test vessels were a container ship, a Ro-Ro and two bulk carriers with maximum main engine power outputs ranging from 6.0 to 8.4 MW covering both 2-stroke and 4-stroke engines. Samples of scrubber water were taken during voyage with the scrubber system operating in open loop configuration at main engine loads of 33, 73, 78 and 81% for the four test vessels, respectively, representing nominal continuous ratings ranging from 2.0 to 6.8 MW. The scrubber wash water flow rates varied from 225 to 320 m^3/h with an average normalised wash water flow rate during sampling of 74 m^3/MWh.

As the scope of the study was to assess the whole effluent toxicity of discharges from normal operations of open loop systems in general, and not to examine specific technologies or vessels, the ecotoxicological testing was performed on composite inlet and discharge samples. The discharge samples from the four different open loop systems were sent to DHI, and equal volumes of the individual samples were mixed to obtain a composite sample.

3.1 Sampling procedures onboard

All on-board sampling was performed by the vessel crew according to a detailed procedure prepared in collaboration between DHI and EGCSA. DHI provided sampling containers for both WET samples and for samples for chemical analyses for each ship, but DHI was not involved in the practical sampling onboard. The overall principles applied for the sampling are described below.

The samples were collected during steady state conditions for both main engine load and open loop scrubber operation. To limit the time from sampling to processing of samples at DHI, the samples were taken as close to estimated time of berthing of the vessel as practically possible.

Samples for WET testing

For sampling of inlet and discharge water for WET testing the following points were provided:

- Avoid touching the inside of the 5 and 10 L polyethylene (PE) containers or lids during handling
- Flush the sampling point thoroughly by running water at a steady flow for a few minutes before sampling
- The PE containers must be flushed with sample water (inlet or discharge) before the final sampling. Flush the PE containers by filling them to the top and pour out the water once before the actual sample is taken. Fill the PE container to the top with the sample, leaving negligible air headspace, and tightly close the lid
- Collect the samples sequentially to represent steady state main engine load and scrubber operating conditions.

Samples for chemical analyses

For sampling of inlet and discharge water for chemical analyses (60 mL plastic bottles, purge and trap (P&T) bottles, and amber glass bottles) the following points were provided:

- Avoid touching the inside of the sample bottles or lids during handling
- Flush the sampling point thoroughly by running water at a steady flow for a few minutes before sampling
- The sample bottles for chemical analyses should be handled as follows:
    - 60 mL plastic bottles: The bottles should be filled to the top (some air is allowed)

  - 40 mL P&T bottles should be filled to the top, and air bubbles should be avoided (or limited to a minimum) by overflow of sample water before closing the bottles
  - 1 L amber glass bottles must be flushed with sample water (inlet or discharge) before the final sampling. Flush the bottles by filling them to the top and pour out the water once before the actual sample is taken. Fill the bottle to the top with the sample (no air) and tightly close the lid.
- Collect the samples sequentially to represent steady state main engine load and scrubber operating conditions.

Upon completed sampling, all samples should be kept dark and cool until shipment. Cooling boxes with frozen cooling elements were used for the shipment.

3.2 Mixing of samples and sampling for chemical analyses

Upon arrival at DHI, the following parameters were measured and recorded in one inlet and one discharge sample from each ship:

- pH
- Salinity
- Temperature
- Oxygen

The samples for chemical analyses were sent by DHI to the analytical laboratory (ALS Global). The samples for WET testing were kept cold at DHI at 4ºC until all samples had arrived.

Table 3.1 Characterisation of samples received by DHI.

Ship No.	Sample type and DHI ID	Date of arrival at DHI	pH	Salinity (PSU)	Temperature (ºC)	Oxygen (%)
Ship 1	INLET DHI No.: 21-1258	15-03-2021	8.1	33.9	17.4	100
Ship 1	DISCHARGE DHI No.: 21-1257	15-03-2021	3.6	33.3	18.1	7.4
Ship 2	INLET DHI No.: 21-1260	15-03-2021	8.0	32.6	17.6	100
Ship 2	DISCHARGE DHI No.: 21-1259	15-03-2021	5.1	33.0	17.6	0.5
Ship 3	INLET DHI No.: 21-1264	17/03-2021	8.0	33.9	13.0	100
Ship 3	DISCHARGE DHI No.: 21-1263	17/03-2021	6.3	34.3	15.0	76
Ship 4	INLET DHI No.: 21-1262	16/03-2021	8.0	33.6	5.9	99
Ship 4	DISCHARGE DHI No.: 21-1261	16/03-2021	5.6	33.6	6.9	2.4
Composite sample	INLET DHI No.: 21-1266	17/03-2021	8.1	33.9	-	100
Composite sample	DISCHARGE DHI No.: 21-1265	17/03-2021	5.4	33.7	-	27

Table 3.2 illustrates the mixing of the individual samples.

Table 3.2 Mixing of individual samples to generate composite samples.

Type of water samples	Ship No.	Sample volume (L)
Scrubber inlet water (mixed in 120 L tank)	Ship 1	30
	Ship 2	30
	Ship 3	30
	Ship 4	30
Inlet water composite sample		120
Scrubber discharge water (mixed in 200 L tank)	Ship 1	40
	Ship 2	40
	Ship 3	40
	Ship 4	40
Discharge water composite sample		160

After thorough mixing, sub-samples for chemical analyses of the composite samples were collected. Description of chemical analyses is presented in section 4.5, . All samples were kept dark and cool (4ºC) until shipment to the analytical laboratory (ALS Global).

3.3 Sub-sampling for WET test

Subsamples of the two composite samples (inlet and discharge) were withdrawn after thorough mixing of the composite sample according to the specifications in Table 3.4.

To avoid that particles in the samples should be the cause of effects in the ecotoxicological tests, the samples were filtered using a Whatman GF/C filter (approx. 1.2 µm) and, subsequently, divided into subsamples for the different WET tests. WET tests with fish were performed with unfiltered samples.

Table 3.3 Sub-sampling of composite samples (one set inlet, and one set discharge) used for ecotoxicological tests (WET tests).

Organism	Filtration	Amount
Alga growth (*Skeletonema* sp.). ISO 10253, considered both acute and chronic	Yes	1 L
Crustacean acute (*Acartia tonsa*). ISO 14669	Yes	1 L
Early-life stage test with *Acartia tonsa.*	Yes	2 L
Fish, acute toxicity, European sea bass, limit test (renewal at 48h) OECD 203	No	36 L (3 x 10L and 1 x 5L)

Organism	Filtration	Amount
Fish, acute toxicity, European sea bass, (renewal at 48h). Only necessary if mortality is observed in the limit test OECD 203	No	INLET: 60 L (6 x 10L) DISCHARGE: 80 L (8 x 10L)

4 Methodology for ecotoxicological tests

WET tests were conducted with inlet water and discharge water composite samples obtained from open loop scrubber systems.

The ecotoxicological tests were conducted in accordance with OECD Test Guidelines or ISO standards. For each toxicity test, dilution series were made by mixing scrubber discharge water with the prescribed laboratory medium.

When possible, the WET tests were conducted with undiluted scrubber discharge water as the highest test concentration. Addition of nutrients was required in some of the tests, and, thus, undiluted samples could not be tested. The test concentrations of scrubber discharge water and scrubber inlet water in each toxicity test are shown in Table 4.1.

The pH, salinity and dissolved oxygen concentration were adjusted in the tests in order to comply with the test guideline criteria.

Each test included two control series, one with scrubber inlet water and one laboratory control. The laboratory control was pure laboratory medium.

The effect of scrubber discharge water is reported relative to the laboratory control by use of the endpoints: effect concentration (EC) or lethal concentration (LC), lowest observed effect concentration (LOEC), and no observed effect concentration (NOEC), where possible. The effect concentrations (EC) causing 10%, 25% and 50% effect in comparison with the laboratory control are referred to as EC10, EC25 and EC50, respectively. The lethal concentrations (LC) causing 10%, 25% and 50% effect in comparison with the laboratory control are referred to as LC10, LC25 and LC50, respectively.

Table 4.1 Whole effluent toxicity tests.

Organism	Test concentrations (mL/L)	Endpoints
Alga growth (*Skeletonema* sp.). ISO 10253, considered both acute and chronic /5/	Scrubber discharge water: 0; 31.25; 62.5; 125; 250; 500, 625 and 910 Inlet water: 910	NOEC/LOEC, EC10, EC25 and EC50
Crustacean acute (*Acartia tonsa*). ISO 14669 /3/	Scrubber discharge water: 31.25; 62.5; 125; 250; 500, 625 and 1,000 Inlet water: 1,000	LC10, LC25 and LC50
Fish, acute toxicity, European sea bass, limit test (renewal at 48h) OECD 203 /2/	Scrubber discharge water 910 Inlet water: 910	-
Crustacean chronic (*Acartia tonsa*). ISO 16778 Early life stage development test /4/	Scrubber discharge water: 0; 50; 100; 200; 400 and 800 Inlet water: 800	NOEC/LOEC, EC10, EC25 and EC50

4.1 Algal growth inhibition test with *Skeletonema* sp.

The toxicity of the samples to the growth rate of the marine alga *Skeletonema* sp. (clone: NIVA-BAC 1) was determined according to the ISO International Standard 10253 “Water quality – Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum*”.

The test concentrations (Table 4.1) were prepared in algal growth medium and algae were added to each of the test mixtures as described in the ISO 10253 standard.

The test conditions are summarized in Table 4.2.

Table 4.2 Test conditions for the growth inhibition test with *Skeletonema* sp.

Standard	**ISO 10253**
Test organism	*Skeletonema* sp. (clone: NIVA-BAC 1)
Test organism source and acclimatisation	NIVA, Norway, cultured at DHI. Cultured at a salinity of 28 PSU.
Test organism life stage	The alga is kept in log phase growth for at least 7 days before testing
Test duration	72 ± 2 hours
Test container	250-mL conical glass flask with air permeable lid, containing 100 mL test solution.
Initial algal concentration	*Skeletonema* sp.: 0.3 - 2.0 x 10^3 cells/mL
Replicates	• 6 × laboratory control • 6 × scrubber inlet • 3 × test concentration • 1 × blank control for each concentration
Method	Determination of specific growth rate by fluorescence measurements as a surrogate for biomass. Fluorescence is measured at the beginning of the test and after 24, 48 and 72 ± 2 hours of incubation in all replicates.
Endpoint	Growth rate (NOEC, EC10, EC25, EC50)
Laboratory control medium	Seawater (salinity 32 PSU) filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm), adjusted to a salinity of 28 PSU with Milli-Q water and heated to 73 °C.
Nutrient medium	Medium as described in ISO 10253
Photoperiod	Constant fluorescent light
Light intensity	60-120 µmol×m^{-2}×sec^{-1}
Shaking	120-140 rpm
Temperature	19 ± 1 °C

Standard	ISO 10253
Initial pH	In the laboratory control medium and samples: 8.0 ± 0.2
Validity criteria	• Average growth rate in laboratory controls ≥ 0.9.d^{-1} • pH increase in laboratory control ≤1.0 • Control variation coefficient: ≤ 7%
Reference test	Verification of the algae sensitivity with 3,5-DCP

4.2 Acute toxicity test with the crustacean *Acartia tonsa*

The toxicity of the samples to the marine copepod *Acartia tonsa* was determined according to the ISO International Standard 14669, 1999 “Water Quality - Determination of acute lethal toxicity to marine copepods (*Copepoda crustacea*)”.

The test concentrations described in Table 4.1 were prepared. The test conditions are summarized in Table 4.3.

Table 4.3 Test conditions for the acute test with *Acartia tonsa.*

Standard	ISO 14669
Test organism	*Acartia tonsa*
Test organism source	DHI Denmark (collected in the North Sea by the Danish Institute for Fisheries Research and has been cultured at DHI since 1987). Cultured at a salinity of 32 PSU.
Test organism life stage	Adults and copepodites
Test duration	48 hours
Test container	50-mL glass beaker with 25 mL test solution
Replicates	• 6 × laboratory control • 6 × scrubber inlet • 4 × test concentration
Test organisms/test container	5
Endpoint	Mortality (LC10, LC25, LC50)
Laboratory control medium	Seawater (salinity 32 PSU) filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm).
Food regime	No food supplied during testing
Photoperiod	16:8 hours
Temperature	20 °C ± 2 °C
Initial pH	In the laboratory control medium: 8.0 ± 0.3 and the samples

Standard	ISO 14669
Validity criteria	• Mortality in the laboratory control ≤10% • Dissolved oxygen concentration ≥70% throughout the test • Reference substance, 3,5-dichlorophenol, LC50 (48h) in the range 0.5-1.5 mg/L

4.3 Fish, Acute Toxicity test with European sea bass (*Dicentrarchus labrax*)

The toxicity of the samples to juveniles of European sea bass was determined in accordance with the OECD Guideline for the Testing of Chemicals No. 203 “Fish, Acute Toxicity Test” /2/.

Due to animal welfare regulations, the acute toxicity test with fish were performed according to the following threshold approach as described in OECD guidance document No. 126 /6/:

1. Derivation of the threshold concentration: The lowest E(L)C50 value of existing and reliable algae or acute invertebrate toxicity data is set as threshold concentration (TC).
2. As the TC was >910 mL/L, the test concentration should be 910 ML/L in the limit test. If sublethal effects are observed, these should be recorded. The test should be terminated when one or more fish from the test group die, since this finding requires a full study.
3. As no mortality was observed, the LC50 is above 910 mL/L and no further testing is required.

Test concentrations were prepared at the initiation of the test and renewed after 48 hours by removing approx. 90% of the test mixture and adding fresh test mixture into the aquaria.

The test conditions are summarized in Table 4.4

Table 4.4 Test conditions for the acute toxicity test on juvenile fish with the European sea bass.

Guideline	OECD 203
Test organism	European sea bass *(Dicentrarchus labrax)*
Test organism source and acclimatisation	Écloserie Marine de Gravelines, France. The fish were acclimatised to 20 °C ± 2 °C for at least 12 days before test start at a salinity of approx. 32 PSU.
Test organism life stage	Juvenile European sea bass (4-8 cm in length)
Test duration	96 hours (water renewal at 48 hours)
Test container	17-L aquaria with min. 15 L of test mixture (max. 0.8 g fish per L)
Replicates	• 1 × laboratory control • 1 × scrubber inlet • 1 × test concentration

Guideline	OECD 203
Test organisms/test container	7
Endpoint	Mortality (LC10, LC25 and LC50 if a full test must be performed)
Observations	Abnormal appearance and behaviour
Laboratory control medium	Seawater (salinity 32 PSU) filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm).
Food regime	Fish fed until 24 hours before test start. No food supplied during testing
Photoperiod	12:12 hours
Temperature	20°C ± 2 °C
Initial pH	In the laboratory control medium and samples: 8.0 ± 0.2
Validity criteria	• Mortality in the laboratory control ≤ 10% • Dissolved oxygen concentration ≥60%
Reference test	Verification of organism sensitivity with 3,5-dichlorophenol (3,5-DCP)

4.4 Early-life stage test with *Acartia tonsa (chronic toxicity test).*

The chronic toxicity of the samples to the marine copepod *Acartia tonsa* was determined according to the ISO International Standard 16778 (2015) “Water quality - Calanoid copepod early-life stage test with *Acartia tonsa”*.

The test concentrations described in Table 4.1 were prepared. The test conditions are summarized in Table 4.5.

Table 4.5 Test conditions for the early-life stage test with *Acartia tonsa (chronic toxicity test).*

Test guideline	**ISO 16778**
Test organism source	DHI Denmark (collected in the North Sea by the Danish Institute for Fisheries Research, cultured at DHI since 1987). Cultured at a salinity of approx. 32 PSU
Test organism life stage	Eggs collected from the *Acartia tonsa* culture
Test duration	5-7 days, until the copepodite ratio in laboratory control reach 60% (± 20%) of the total organisms.
Test container	250-mL glass beakers with 40/80 mL test solution
Replicates	• 12 × laboratory control (+ extra to determine the termination time) • 6 × scrubber inlet • 6 × test concentration
Eggs/ test container	60-90 eggs
Endpoints	• Early Life Stage Mortality (ELM) • Hatching Success (HS) • Larval Development Ratio (LDR)
Laboratory control medium	Seawater (salinity 32 PSU) filtered through Millipore filters (10; 5.0; 0.5 and 0.22 µm).
Food regime	*Rhodomonas salina* 50,000 cells/mL twice during the test
Photoperiod	16:8 hours light:dark at 5-10 µmol × m^{-2} × sec^{-1}
Temperature	20 °C ± 1 °C
Initial pH	In the laboratory control medium and samples: 8.0 ± 0.3

Validity criteria	• Early life stage mortality in the laboratory control (ELM): ≤ 30% • Hatching Success in the laboratory control (HS): ≥ 75% • Larval development ratio in the laboratory control (LDR): 60% ± 20% • Dissolved oxygen concentration: ≥ 70% throughout test • Laboratory control pH must not vary more than 1.0 from the initial pH • Temperature must not vary more than ± 1 °C • Salinity must not vary more than 10 % from the laboratory control start value • EC50 of the reference substance (3,5-DCP) within 500 µg/L ± 300 µg/L (20 °C and 20 PSU)

4.5 Chemical analyses

Chemical analyses of PAH, BTEX and selected metals normally found in scrubber discharge water were analysed in each collected sample as well as in the composite samples. The limit of detection as well as the used methods are presented in Annex B, together with the results of the analyses.

4.6 Statistical analysis

The results obtained in the WET tests with algae and the chronic *Acartia tonsa* test were used to derive the no observed effect concentration (NOEC), lowest observed effect concentration (LOEC) and the effect concentrations (EC) causing 10%, 25% and 50% effect in comparison with the laboratory control (i.e., EC10, EC25 and EC50). The results obtained in the acute WET tests with crustaceans and fish were used to derive the lethal concentrations (LC) causing 10%, 25% and 50% effect in comparison with the laboratory control (i.e., LC10, LC25 and LC50).

All the analyses were run using the free software R /7/ (R version 3.5.3 (2019-03-11) - "Great Truth").

The LOEC/NOEC values were determined by use of the Dunnett's test /8/. The NOEC values ($p>0.05$) were determined as the highest tested concentration, at which no significant negative effect was observed compared with the control. The LOEC ($t<0$ and $p<0.05$) is the concentration just above the NOEC.

To comply with the conditions of the Dunnett's test, the normality of the data was assessed but only by a visual description using a boxplot /9/. The variance was analysed with a Levene's test /10/.

When a significant effect was observed associated with a dose response, a Probit analysis was conducted to estimate the EC/LC10, EC/LC25 and EC/LC50.

The EC/LC values were calculated to attain the closest possible curve fit /11/.

5 Results

5.1 Ecotoxicity results

The results obtained in the WET tests with algae and the chronic *Acartia tonsa* test were used to derive the NOEC, LOEC, and EC10, EC25 and EC50. The results obtained in the WET tests with crustaceans and fish were used to derive the LC10, LC25 and LC50.

A summary of the results of the toxicity tests with scrubber discharge water is presented in Table 5.1. Negative effects of the scrubber inlet water were not significant when compared to the laboratory control. All data from the toxicity tests are presented in Appendix A.

Table 5.1 Results of aquatic toxicity tests in millilitres of scrubber water per litre of test medium. Numbers in parentheses are the 95% confidence intervals.

Test organism	Endpoint	NOEC (mL/L)	LOEC (mL/L)	EC/LC10 (mL/L)	EC/LC25 (mL/L)	EC/LC50 (mL/L)
Alga (*Skeletonema sp.*) ISO 10253	Growth	500	625	740 (480->910)	>910	>910
Crustacean (*Acartia tonsa*), acute ISO 14669	Mortality	-	-	720 (580-870)	>1000	>1,000
Fish (*Dicentrarchus labrax*) OECD 203	Mortality and behaviour	-	-	>910	>910	>910
Crustacean (*Acartia tonsa*), chronic ISO 16778	Hatching success	800	>800	>800	>800	>800
	Mortality	800	>800	>800	>800	>800
	Larval development ratio (LDR)	200	400	200 (110-280)	340 (250-430)	540 (470-610)

5.2 Results of chemical analyses

The results of the chemical analyses of all the individual samples as well as the composite samples are found in Appendix B. The individual samples from each ship were collected directly in the sampling bottles that were used for the chemical analyses, and, hence, volatile substances in the samples were preserved to the extent possible. The composite samples were prepared by mixing large volumes of sample water in the DHI laboratory, and this potentially led to loss of volatile substances, which implies that the concentrations of volatile substances were expected to be lower in the composite samples compared to the samples collected on board the ships.

Figures 1 to 3 below present the net positive concentration of substances for which an increase was observed from inlet to discharge in the composite samples.

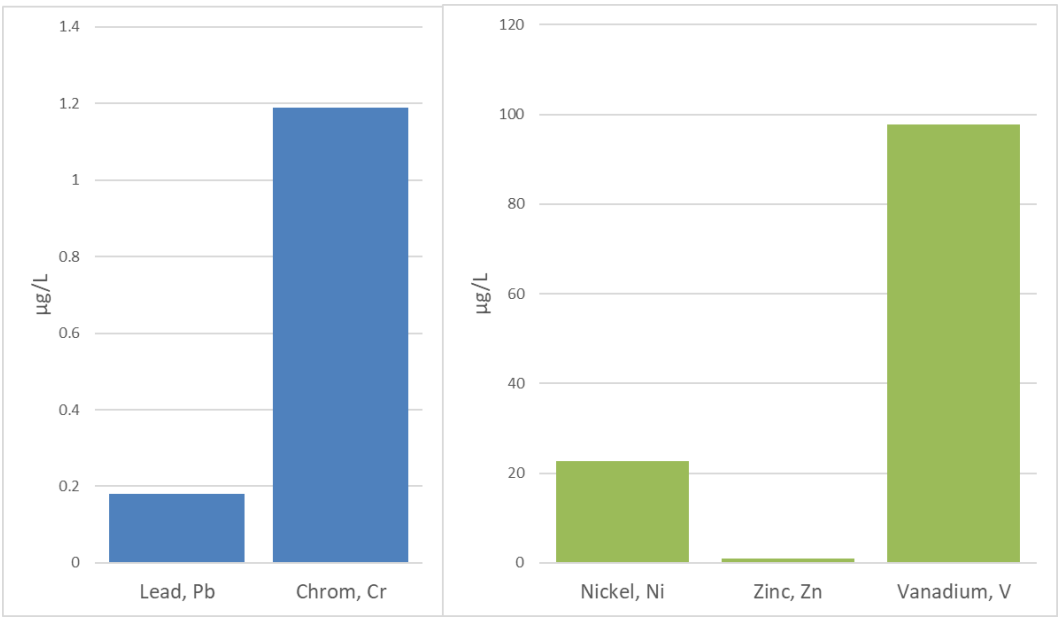


Figure 1 Increase in concentration of metals, calculated as the difference between the concentration in the composite inlet and composite discharge sample.

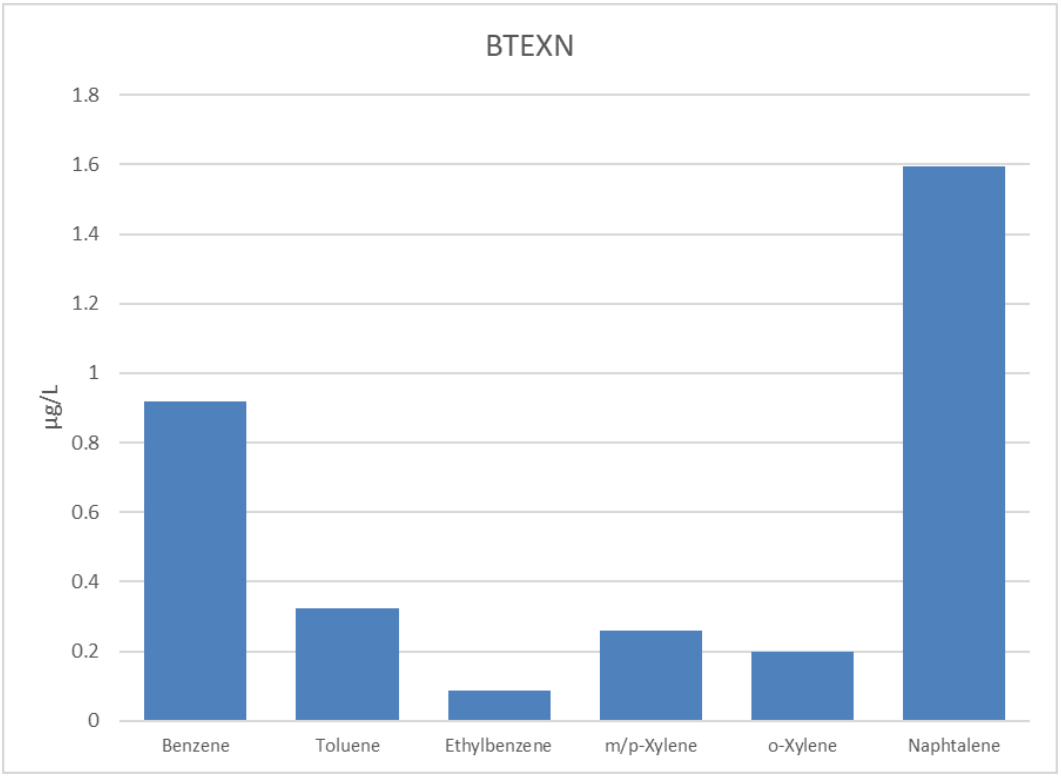


Figure 2 Increase in concentration of substances, calculated as the difference between the concentration in the composite inlet and composite discharge sample.

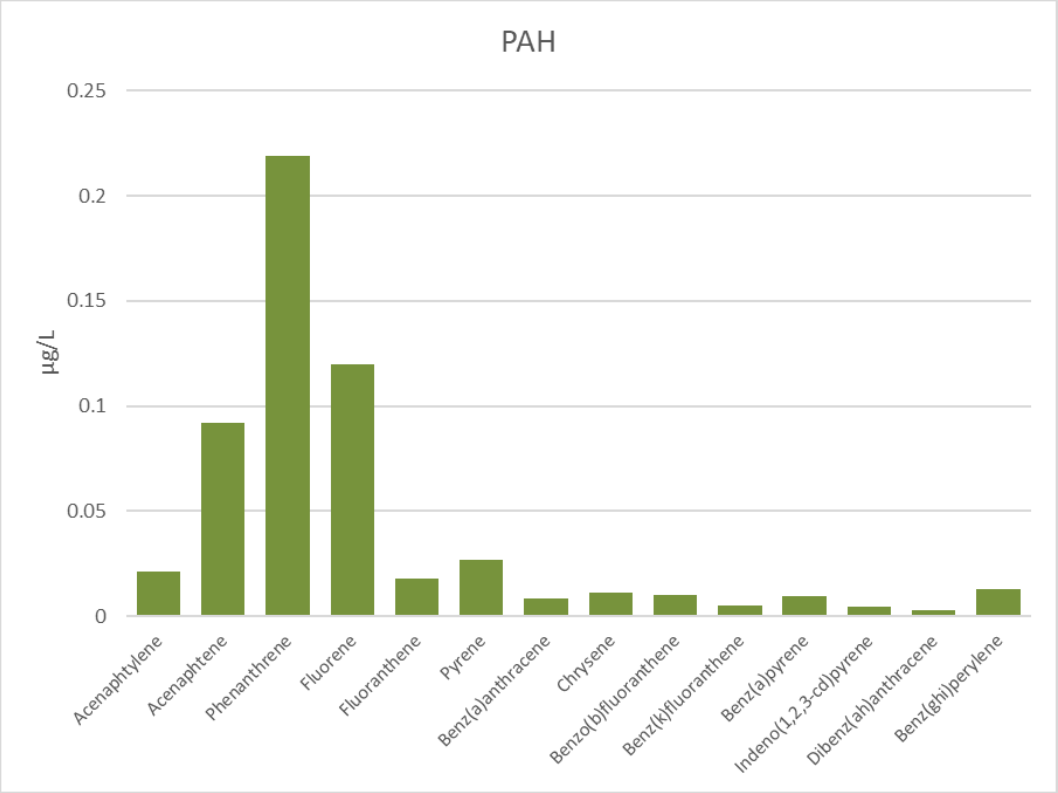


Figure 3 Increase in concentration of substances, calculated as the difference between the concentration in the composite inlet and composite discharge sample.

6 Environmental risk assessment

6.1 Environmental dilution of the scrubber discharge water

6.1.1 Environment scenarios

The marine antifoulant model MAMPEC, version 3.1.0.5 (https://www.deltares.nl/en/software/mampec/), was used to calculate the predicted environmental concentrations (PEC) of scrubber discharge water. The model integrates hydrodynamics and chemical fate and was originally developed for antifoulants in harbours, rivers, estuaries and open water. MAMPEC is now also being used to predict environmental concentrations for the exposure assessment of chemicals discharged via ballast water /12/.

MAMPEC calculates the PEC of chemicals in a defined scenario. The ballast water version contains a model harbour and an emission scenario, and the resulting PEC is used for the environmental risk assessment in connection with type approval of ballast water management systems.

Three scenarios were assumed for the release of the scrubber discharge water: The GESAMP-BWWG Model Harbour, the OECD-EU Commercial Harbour, and the OECD-EU Shipping Lane. The harbour models are equal in water volume, but the GESAMP harbour is further from the sea (x1 in Figure 6.1) and has a smaller mouth width (x3 in Figure 6.1) creating a scenario with less tidal water exchange compared to the OECD-EU

harbour. The harbours also represent a slight pH difference, 8.0 in the GESAMP harbour and 7.5 in the OECD-EU harbour. Flow velocity F in Figure 6.1 is 1 m/s in all scenarios.

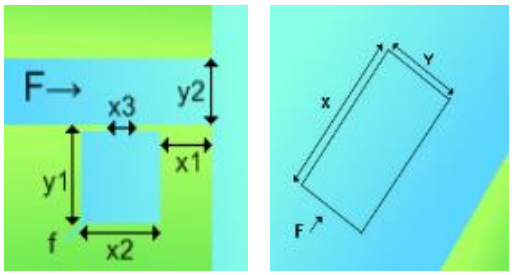


Figure 6.1 Model harbour scenario to the left and model shipping lane to the right. Details of the scenarios can be found in Appendix C.

The prediction of the emission of chemicals in the scenarios requires an input of release of chemical in g/d. When the model is used for ballast water, the release of chemical is calculated from the chemical concentration in the discharge multiplied by a daily discharge volume of 100,000 m^3. This volume is deemed to be too high for any scrubber discharge water emission scenario. The discharge of scrubber water is dependent on the engine load and typically also a minimum volume needed to keep the system running.

It is noted that the ecotoxicological studies are conducted with scrubber water sampled during high main engine load conditions for three of the four test vessels. In the emission scenarios the WET results are then transferred to harbour emission scenarios, where only auxiliary engines and boiler systems would be active meaning a lower fuel consumption, and therefore also lower concentrations in the exhaust gas to be handled by the scrubber.

6.1.2 Emission scenarios

For sampling of scrubber wash water, the water flow rate at the sample point and the power of the combustion unit(s) being scrubbed vary between vessels and likely also between sampling campaigns /13/. A sample taken at a lower specific water flow rate will have a relatively higher concentration of analytes compared with a similar sample taken at a higher specific water flow rate. The chemical content in scrubber water is thus often normalized to a flow rate through the scrubber system of 45 m^3/MWh to be able to compare different samples. A flow rate of 45 m^3/MWh was regarded as typical during development of the wash water guidelines in the IMO /14/.

For the present study in which whole effluent toxicity was evaluated instead of single chemicals, the normalization was omitted. The four discharge water samples were thus used as they are, i.e., four different discharges produced under four different circumstances and released to a common receiving environment. However, an estimate of normal daily discharge rate is needed for the MAMPEC model.

The reported flow rates for the four test vessels were 43, 44, 47 and 161 m^3/MWh giving an average of 74 m^3/MWh. This shows that the flow rate of 45 m^3/MWh is not always applicable for scrubber systems in operation. The same observation was recently reported in the literature /15/ as 90 m^3/MWh was found more suitable for the Baltic region, when data from the EGCSA and Euroshore report /13/ were evaluated. The value of 90 m^3/MWh is supported by a calculated average wash water discharge of 87 ± 50 m^3/MWh based on 44 vessels calling the port of Antwerp /16/. The authors /17/ also report that wash water is discharged in the surrounding surface water at a typical flow rate of 200–500 L/s for a vessel operating at 15 MW, i.e., there is large variation over a range of flow rates of 17,000-43,000 m^3/day for a sailing vessel.

During port stays most merchant vessels use auxiliary engines and boilers to generate power for domestic purposes such as heating/cooling of accommodation and cargo, and electricity for lighting and machinery. Therefore, a vessel at berth generally uses much less energy, and thus also less scrubber water compared to that when the main engine is in normal load condition during voyage. A study conducted by CE Delft identified a continuous port load of 8 MW, based on the consideration of the port sizes, ships' auxiliary load at berth, and shipping operations in the North Sea and Baltic Sea regions /17/:

> *"Some theoretical combinations or combinations thereof of what this loading could represent are included here:*
>
> - *10 Ferry – ro-pax (generic) at berth for 12 h/day 365 days/yr; or*
> - *7 Cruise ships (20,000–59,999 GT) at berth for 8 h/day 365 days/yr; or*
> - *4 Cruise ships (60,000–99,999 GT) at berth for 8 h/day 365 days/yr, or*
> - *24 General cargo ships (5,000–9,999 dwt) at berth for 24 h/day 365 days/yr; or*
> - *28 Bulk carriers (10,000-34,999 dwt) at berth for 24 h/day 365 days/yr; or*
> - *16 Container ships (8,000-11,999 TEU) at berth for 12 h/day 365 days/yr."*

Thus, using the default normalization flow rate of 45 m^3/MWh which according to EGCSA members is applied by most scrubber systems in normal operation, the average open loop scrubber wash water discharge rate for a standard port could be

$$45\frac{\mathrm{m}^3}{\mathrm{MWh}} \times 8\ \mathrm{MW} \times 24\frac{\mathrm{h}}{\mathrm{day}} = 8{,}640\frac{\mathrm{m}^3}{\mathrm{day}}$$

Using the higher average flow rate of 90 m^3/MWh, the average open loop scrubber wash water discharge rate for a standard port could be

$$90\frac{\mathrm{m}^3}{\mathrm{MWh}} \times 8\ \mathrm{MW} \times 24\frac{\mathrm{h}}{\mathrm{day}} = 17{,}280\frac{\mathrm{m}^3}{\mathrm{day}}$$

A study conducted for the Danish Environmental Protection Agency established a wash water discharge rate of approx. 50 m^3/MWh with an average power consumption of 1 MW for a ship at berth. The study assumed that a larger Danish port contains 10 ships at berth with an average discharge rate 12,000 m^3/day and a tanker with an inert gas generator, also applying wet scrubbing technology, adding a discharge of 5,000 m^3, i.e. a discharge water total of 17,000 m^3/day /18/.

Gross estimates of 10,000 and 20,000 m^3/day were applied in the harbour scenario calculations.

Discharge volumes in the shipping lane scenario were based on the Strait of Dover, being the busiest shipping route in the world with the passage of around 400 vessels per day /19/. Note that the 400 vessels per day sail further apart in Strait of Dover compared to the area applied in the MAMPEC shipping lane, and that the number includes all merchant and non-merchant ships in the area, not only the approx. 4% of the world fleet with an open or closed scrubber /20/.

$$400 \times 90\frac{\mathrm{m}^3}{\mathrm{MWh}} \times 15\ \mathrm{MW} \times 24\frac{\mathrm{h}}{\mathrm{day}} = 12{,}960{,}000\frac{\mathrm{m}^3}{\mathrm{day}}$$

For the present study, the discharge flow rate of the vessels was on average 74 m^3/MWh for an average main engine load of 5 MW:

$$400 \times 74\frac{\mathrm{m}^3}{\mathrm{MWh}} \times 5\ \mathrm{MW} \times 24\frac{\mathrm{h}}{\mathrm{day}} = 3{,}501{,}840\frac{\mathrm{m}^3}{\mathrm{day}}$$

Using the higher average flow rate of 90 m^3/MWh and larger main engine outputs /16/ the average open loop scrubber wash water discharge rate for the Strait of Dover would be

$$400 \times 90 \frac{\mathrm{m}^3}{\mathrm{MWh}} \times 15\ \mathrm{MW} \times 24 \frac{\mathrm{h}}{\mathrm{day}} = 12{,}960{,}000 \frac{\mathrm{m}^3}{\mathrm{day}}$$

Gross estimates of 3,500,000 and 13,000,000 m^3/day were applied in the shipping lane scenario calculations.

6.1.3 Predicted environmental concentration (PEC)

The predicted environmental concentration (PEC) in the aquatic environment was calculated by use of the marine antifoulant model to predict environmental concentrations (MAMPEC). To calculate the dilution of an input in the MAMPEC model's water phase, an inert chemical was created with properties given in Appendix C.4. For an assessment of ecotoxicological effects the use of an inert substance that remain dissolved in the water phase is a conservative approach. Both metals and organic substances will to some extent adhere to particles, and, hence, they will partition into the sediment which means that the presence of the substances in the water column will be short compared to the assumption applied in the modelling. Furthermore, no degradation was assumed for the inert chemical.

The dilution factors in Table 6.1 were calculated and applied to a sample of scrubber water. The minimum dilution factor was calculated from the maximum water concentration found in any part of the harbour, and the average dilution factor was calculated from the overall harbour concentration.

Table 6.1 Dilution factors and predicted environmental concentrations (PEC) of scrubber wash water in the three MAMPEC environment scenarios. The discharge volumes per day represent case-specific estimates and worst-case estimates.

Environment scenario	Discharge volume (m^3/day)	Min. dilution factor	Average dilution factor	Max. PEC (mL/L)	Avg. PEC (mL/L)
GESAMP-BWWG Model Harbour	10,000	592	1,116	1.7	0.90
	20,000	296	558	3.4	1.8
OECD-EU Commercial Harbour	10,000	2,817	5,208	0.36	0.19
	20,000	1,408	2,604	0.71	0.38
Shipping lane	3,500,000	993	9,403	1.0	0.11
	13,000,000	267	2,532	3.7	0.40

6.2 Predicted no effect concentration (PNEC)

The predicted no effect concentration (PNEC) means the concentration of the scrubber discharge water in the aquatic environment below which unacceptable effects will most likely not occur. The PNEC is calculated from the lowest no observed effect concentration (NOEC) which is divided with an assessment factor (AF) that depends on the available data on ecotoxicological effects. For the effects of discharge water on aquatic organisms, IMO has a history of risk assessment of discharged ballast water, and it was considered appropriate to follow this guideline /21/. An assessment factor of 50 can be applied to the lowest chronic NOEC from two marine species representing two trophic levels. NOECs in the current series of WET tests included 500 mL/L for algae (endpoint growth) and 200 mL/L for crustaceans (endpoint larval development ratio).

The lowest NOEC was observed in the early-life stage test with *Acartia tonsa* for the endpoint larval development ratio at a concentration of 200 mL of scrubber water per litre of test medium.

$$\mathrm{PNEC} = \frac{\mathrm{NOEC}}{\mathrm{AF}} = \frac{200\ \mathrm{mL/L}}{50} = 4\frac{\mathrm{mL}}{\mathrm{L}}$$

Thus, a PNEC of 4 mL of scrubber water per litre of sea water is applied.

6.3 Risk characterization ratio (RCR)

The environmental risk is estimated with a risk characterization ratio (RCR), where the PEC is divided with the PNEC. If the PEC is lower than the PNEC, and thus RCR is below 1, the risk to the aquatic environment is considered acceptable.

$$\text{Risk characterization ratio (RCR)} = \frac{\text{Predicted environmental concentration (PEC)}}{\text{Predicted no effect concentration (PNEC)}}$$

The RCRs for a variation of scenarios are presented in Table 6.2.

Table 6.2 Risk characterization ratios (RCR) based on a predicted no effect concentration (PNEC) of 4 mL/L and the predicted environmental concentration (PEC) from Table 6.1. The discharge volumes per day represent case-specific estimates and worst-case estimates.

Environment scenario	Discharge volume (m^3/day)	Max. RCR	Avg. RCR
GESAMP-BWWG Model Harbour	10,000	0.42	0.22
	20,000	0.85	0.45
OECD-EU Commercial Harbour	10,000	0.089	0.048
	20,000	0.18	0.10
Shipping lane	3,500,000	0.25	0.027
	13,000,000	0.94	0.43

The RCRs of the scenarios in Table 6.2 are below 1 indicating that no further assessment of direct toxic effects to the aquatic environment is necessary. The highest RCRs are generated by the peak concentrations of scrubber wash water discharged into the harbour with the lowest exchange of water and into the shipping lane with an assumed concentrated traffic consisting of all merchant vessels with an average main engine load of 15 MW and all using open loop scrubbers.

The MAMPEC modelling simulated an inert, fully water-soluble, non-degradable substance, and the derived PNEC was based on the most sensitive aquatic species (and endpoint) among the species examined in the present evaluation. Therefore, the RCRs are strictly an expression of the hydrodynamic conditions for the different emission scenarios used.

7 Conclusion

Ecotoxicological tests with aquatic species were performed for evaluating the effects of scrubber discharge water on the marine aquatic environment. The tests were conducted according to WET testing recommendations from the GESAMP task team. No toxicity on fish was observed, and in the short-term test with algae and the acute test with crustaceans effects were only observed at high concentrations. The most sensitive endpoint was the larval development ratio (LDR) in the chronic crustacean test.

The risk of the scrubber discharge water to the aquatic environment is characterized by use of:

- The predicted environmental concentration (PEC) in the aquatic environment which was calculated by use of MAMPEC
- The predicted no effect concentration (PNEC), i.e., the concentration of the scrubber discharge water in the aquatic environment below which unacceptable effects will most likely not occur

The risk of the scrubber discharge water to the aquatic environment is expressed by use of the risk characterization ratio (RCR) which is the ratio between PEC and PNEC. Thus, the RCR = PEC/PNEC, and unacceptable effects to the environment are unlikely, when RCR is below 1.

The PNEC was calculated on the basis of the lowest NOEC observed in the early-life stage test with *Acartia tonsa* at a concentration of 200 mL of scrubber water per litre of test medium. With an applied assessment factor (AF) of 50, a PNEC of 4 mL of scrubber water per litre of sea water was used in the risk assessment.

In the present risk assessment of the scrubber discharge water the RCR was below 1, and this means that the risk to the aquatic environment can be considered acceptable.

8 References

/1/ ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.

/2/ OECD Guidelines for Testing of Chemicals No. 203, ”Fish, Acute Toxicity Test”. Adopted 2019.06.18.

/3/ DS/ISO 14669 (1999): “Water quality - Determination of acute lethal toxicity to marine copepods (*Copepoda crustacea*)”. 1999.08.16.

/4/ ISO 16778 (2015): “Water quality - Calanoid copepod early-life stage test with *Acartia tonsa*”. Adopted 2015.06.15.

/5/ DS/EN ISO 10253 (2016): “Water quality - Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum*”. Third edition. 2016.11.15.

/6/ OECD guidance document No. 126: “Short guidance on the threshold approach for acute fish toxicity”. 2010.05.31.

/7/ R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

/8/ Frank Bretz, Torsten Hothorn and Peter Westfall (2010) (Dunnett’s test), Multiple Comparisons Using R, CRC Press, Boca Raton.

/9/ Fox, J. (2005) The R Commander: A Basic Statistics Graphical User Interface to R. Journal of Statistical Software, 14(9): 1–42

/10/ Fox, J. and Weisberg, S. (2011) An R Companion to Applied Regression, Second Edition, Sage.

/11/ Ritz, C (2010). Towards a unified approach to dose-response modelling in ecotoxicology. Environmental Toxicology & Chemistry, 29, 220–229.

/12/ B. van Hattum, J. van Gils, A. Markus, H. Elzinga, F. Kleisse, and A. Baart (2015). User Manual – Quick Guide, MAMPEC 3.1, MAMPEC-BW 3, Version 3.1.0.5.

/13/ EGCSA and Euroshore (2018) Study report on analyses of water samples from exhaust gas cleaning systems. MEPC 73/INF.5.

/14/ IMO (2008). Report of the Marine Environment Protection Committee on its Fifty-Eighth Session. MEPC 58/23

/15/ Ytreberg E., Åström S., Fridell E. (2021) Valuating environmental impacts from ship emissions – The marine perspective. Journal of Environmental Management, Volume 282, https://doi.org/10.1016/j.jenvman.2021.111958.

/16/ Teuchies J., Cox T.J.S., Van Itterbeeck K., Meysman F.J.R., and Blust R. (2020) The impact of scrubber discharge on the water quality in estuaries and ports. Environ Sci Eur 32, 103. https://doi.org/10.1186/s12302-020-00380-z

/17/ Faber J., Nelissen D., Huigen T., Shanti H., van Hattum B., and Kleissen F. (2019) The impacts of EGCS washwater discharges on port water and sediment. CE Delft Publication code: 19.4I09.141.

/18/ Kjølholt, J., Aakre, S., Jürgensen, C., Lauridsen, J. (2012). Assessment of possible impacts of scrubber water discharges on the marine environment. Danish Environmental Protection Agency. Environmental Project No. 1431, 2012.

/19/ Shamseer Mambra (2021). The Strait Of Dover – The Busiest Shipping Route In The World. Marine Navigation. https://www.marineinsight.com/marine-navigation/the-strait-of-dover-the-busiest-shipping-route-in-the-world/. Accessed on March 31st, 2021.

/20/ Chambers, S. (2021) Number of installed scrubbers has doubled since last year's global sulphur cap introduction. Number of installed scrubbers has doubled since last year's global sulphur cap introduction - Splash247. Accessed on April 20th, 2021.

/21/ IMO (2017) Methodology for information gathering and conduct of work of the GESAMP*-BWWG. BWM.2/Circ.13/Rev.4.

APPENDIX A – Toxicity Tests

A Toxicity Tests

A.1 Algal growth inhibition test with *Skeletonema* sp. with scrubber discharge and inlet water

A.1.1 Primary data on fluorescence

Table A. 1 Raw data of the fluorescence (RFU) generated in the algal growth inhibition test with scrubber discharge and inlet water. Test period from 2021-03-17 to 2021-03-20. Blanks were prepared as the dilution series but did not contain algae.

Flask	Scrubber discharge water (mL/L)	0 hour	22 hours	46 hours	71.5 hours
Laboratory control A	0	2.5	7.4	69.9	874
Laboratory control B	0	2.5	7.4	71.5	823
Laboratory control C	0	2.5	7.4	81.2	988
Laboratory control D	0	2.5	7.4	71.5	852
Laboratory control E	0	2.5	6.6	60.0	578
Laboratory control F	0	2.5	7.4	72.7	916
Blank	0	0.8	0.5	0.4	0.4
A	31.25	2.3	7.7	74.4	829
B	31.25	2.3	7.0	75.8	648
C	31.25	2.3	7.6	72.3	644
Blank	31.25	0.8	0.5	0.4	0.4
A	62.5	2.1	8.3	76.3	878
B	62.5	2.1	7.8	80.9	879
C	62.5	2.1	8.0	73.9	849
Blank	62.5	0.8	0.5	0.4	0.6
A	125	2.2	8.7	74.7	837
B	125	2.2	7.9	71.4	805
C	125	2.2	7.9	74.2	809
Blank	125	0.8	0.5	0.4	0.5
A	250	2.0	7.3	64.2	671
B	250	2.0	7.3	70.1	795
C	250	2.0	7.4	69.5	806
Blank	250	0.8	0.5	0.4	0.4
A	500	2.1	5.4	48.7	524
B	500	2.1	5.0	42.8	448

Flask	Scrubber discharge water (mL/L)	0 hour	22 hours	46 hours	71.5 hours
C	500	2.1	5.4	52.1	610
Blank	500	0.9	0.5	0.4	0.4
A	625	2.1	5.3	39.1	419
B	625	2.1	5.1	35.9	434
C	625	2.1	5.1	45.4	532
Blank	625	0.8	0.5	0.4	0.4
A	910	1.9	3.9	27.7	259
B	910	1.9	3.4	23.8	199
C	910	1.9	3.1	21.6	188
Blank	910	0.9	0.5	0.4	0.5
Scrubber inlet water A	910	1.6	6.9	74.8	1,038
Scrubber inlet water B	910	1.6	7.6	79.4	1,151
Scrubber inlet water C	910	1.6	6.6	74.7	997
Scrubber inlet water D	910	1.6	8.1	83.1	1,030
Scrubber inlet water E	910	1.6	7.4	80.7	1,018
Scrubber inlet water F	910	1.6	6.4	71.8	858
Blank	910	0.4	0.3	0.3	0.3

A.1.2 Physical parameters

Table A. 2 Measurements of pH and salinity during the algal growth inhibition test.

Date	2021-03-17 – Day 0		2021-03-20 – Day 3
Scrubber discharge water (mL/L)	pH	Salinity (PSU)	pH
Laboratory control	8.0	27.3	8.5
31.25	8.1	27.5	8.5
62.5	8.1	27.6	8.5
125	8.1	27.9	8.5
250	8.1	28.6	8.4
500	8.1	29.8	8.3
625	8.1	30.5	8.1
910	8.0	31.1	7.8
Scrubber inlet water 910 mL/L	8.1	31.2	8.5

A.1.3 Statistical analysis – Determination of effect concentrations after exposure to scrubber discharge and inlet water

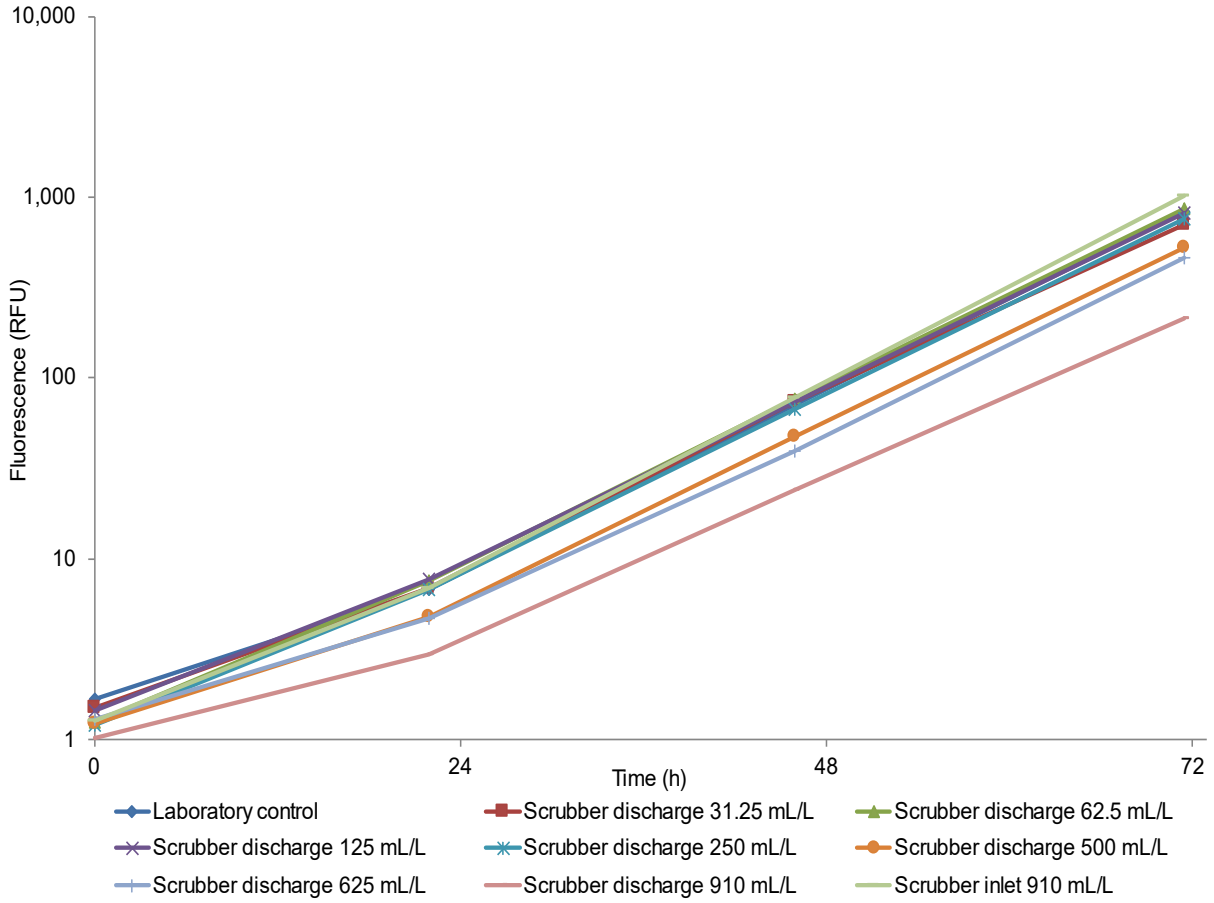


Figure A.1 Fluorescence versus time in the growth inhibition test with *Skeletonema* sp. exposed to different concentrations of scrubber discharge water and one concentration of scrubber inlet water.

A.1.3.1 Results of the statistical analysis for the growth rate (NOEC and LOEC) with scrubber discharge water and scrubber inlet water

Table A.3 Experimental data of the growth rate (growth per hour) in the laboratory control, scrubber discharge water and scrubber inlet water groups.

Replicate No.	Laboratory control	Scrubber discharge water concentrations							Scrubber inlet water 910 mL/L
		31.25 mL/L	62.5 mL/L	125 mL/L	250 mL/L	500 mL/L	625 mL/L	910 mL/L	
1	0.089	0.089	0.092	0.089	0.089	0.086	0.082	0.079	0.095
2	0.088	0.087	0.093	0.089	0.092	0.084	0.082	0.075	0.096
3	0.091	0.086	0.092	0.089	0.092	0.088	0.086	0.075	0.095
4	0.088	-	-	-	-	-	-	-	0.094
5	0.083	-	-	-	-	-	-	-	0.095
6	0.089	-	-	-	-	-	-	-	0.093
Count	6	3	3	3	3	3	3	3	6
mean	0.088	0.087	0.092	0.089	0.091	0.086	0.083	0.076	0.094

- Normality

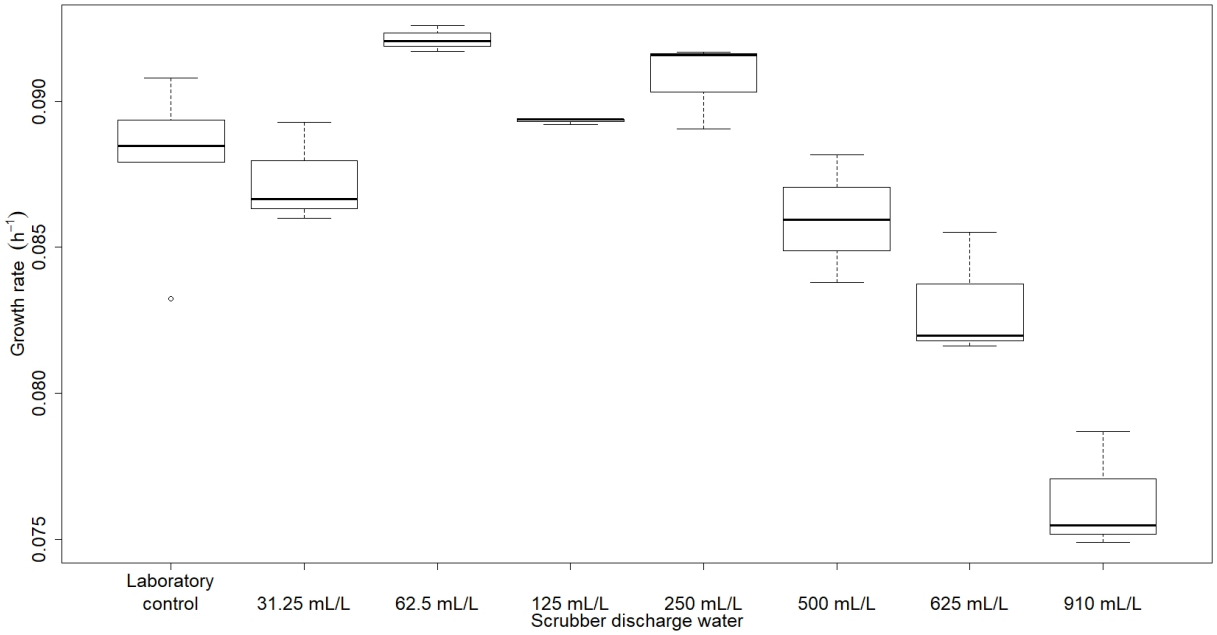


Figure A.2 Boxplots of growth rate for test groups with scrubber discharge water.

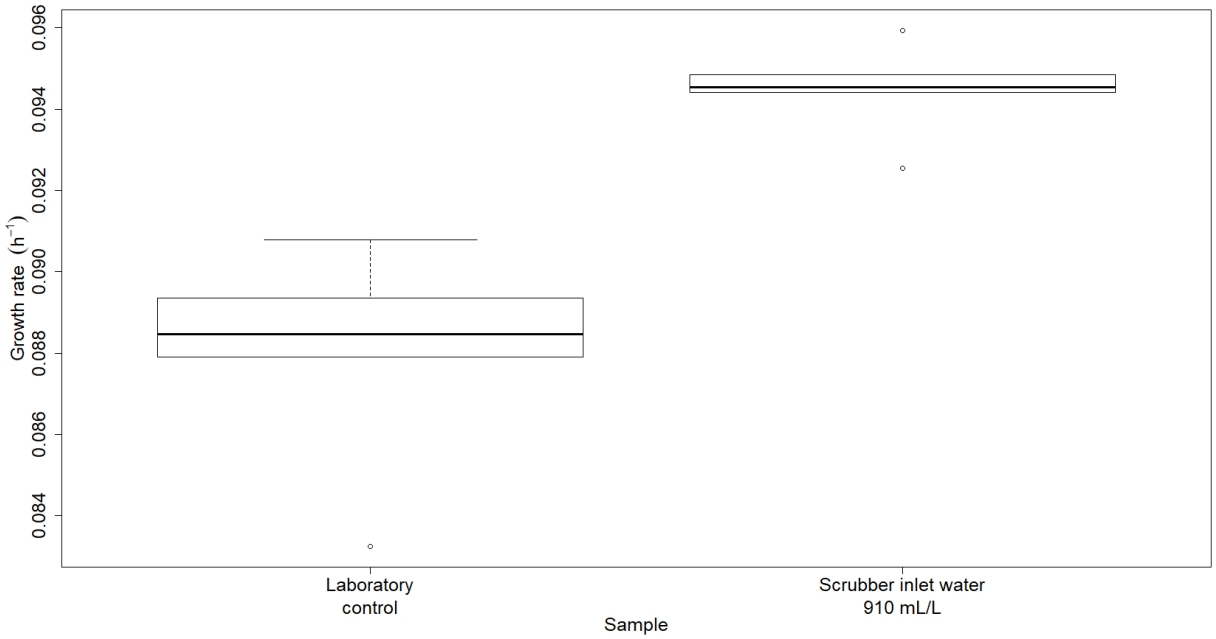


Figure A.3 Boxplots of growth rate for the test group with scrubber inlet water.

The boxplot patterns show the data distribution (Figure A.2 and Figure A.3). A normal distribution in each test group will be assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups are not different from the control group (Table A.4).

Table A.4 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(>|t|)	Significance[1)]
Scrubber discharge-31.25 - Lab control = 0	-0.001	0.001	-0.54	1.00	
Scrubber discharge-62.5 - Lab control = 0	0.004	0.001	2.99	0.04	*
Scrubber discharge-125 - Lab control = 0	0.001	0.001	0.95	0.91	
Scrubber discharge-250 - Lab control = 0	0.003	0.001	2.00	0.29	
Scrubber discharge-500 - Lab control = 0	-0.002	0.001	-1.51	0.58	
Scrubber discharge-625 - Lab control = 0	-0.005	0.001	-3.66	0.01	*
Scrubber discharge-910 - Lab control = 0	-0.012	0.001	-8.56	<0.001	***
Mean comparisons	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	**Significance[1)]**
Scrubber inlet-910 - Lab control = 0	0.006	0.001	5.66	<0.001	***

1) Signif. codes: 0 '***' 0.001'**' 0.01 '*' 0.05 '.'0.1 ' '1 (Adjusted p values reported - single-step method)

The hypothesis that the mean of any test groups with scrubber discharge water is significantly lower than the laboratory control was verified (t<0 and P<0.05) at 625 mL/L of scrubber discharge water and above. The scrubber discharge concentration 62.5 mL/L

was furthermore significantly higher than the laboratory control (t>0 and P<0.05) (Table A.4). The NOEC is therefore estimated to be 500 mL/L (Table A.5).

The scrubber inlet water was significantly higher than the laboratory control (t>0 and P<0.05) (Table A.4).

Table A.5 Growth rate endpoint, estimations of NOEC and LOEC, Dunnett's test.

Growth rate endpoint	NOEC (mL/L)	LOEC (mL/L)
Scrubber discharge water	500	625
Scrubber inlet water	910	>910

A.1.3.2 Inhibition of the growth rate of *Skeletonema* sp. with scrubber discharge and inlet water

Table A.6 Inhibition (in %) of the scrubber discharge and inlet water growth rates compared to the average laboratory control growth rate after 3 days of exposure.

Replicate No.	Laboratory control	Scrubber discharge water concentrations							Scrubber inlet water 910 mL/L
		31.25 mL/L	62.5 mL/L	125 mL/L	250 mL/L	500 mL/L	625 mL/L	910 mL/L	
1	-1%	-1%	-5%	-2%	-1%	2%	7%	11%	-8%
2	0%	2%	-5%	-1%	-4%	5%	7%	14%	-9%
3	-3%	2%	-4%	-2%	-4%	0%	3%	15%	-7%
4	0%	-	-	-	-	-	-	-	-7%
5	5%	-	-	-	-	-	-	-	-7%
6	-2%	-	-	-	-	-	-	-	-5%
mean	0%	1%	-5%	-1%	-3%	2%	6%	13%	-7%

A.1.3.3 Results of the Probit analysis with scrubber discharge water

Table A.7 ECx estimates, Probit analysis with R after 3 days.

ECx[1)]	Estimated scrubber discharge water concentration (mL/L)	Std. Error	Lower (mL/L)	Upper (mL/L)
EC05	600	55	487	714
EC10	741	123	486	996

1) The ECx were absolute values from the dose-response curve and the maximum effect observed was 13% (Figure A. 4), consequently, EC25 and EC50 were not calculated.

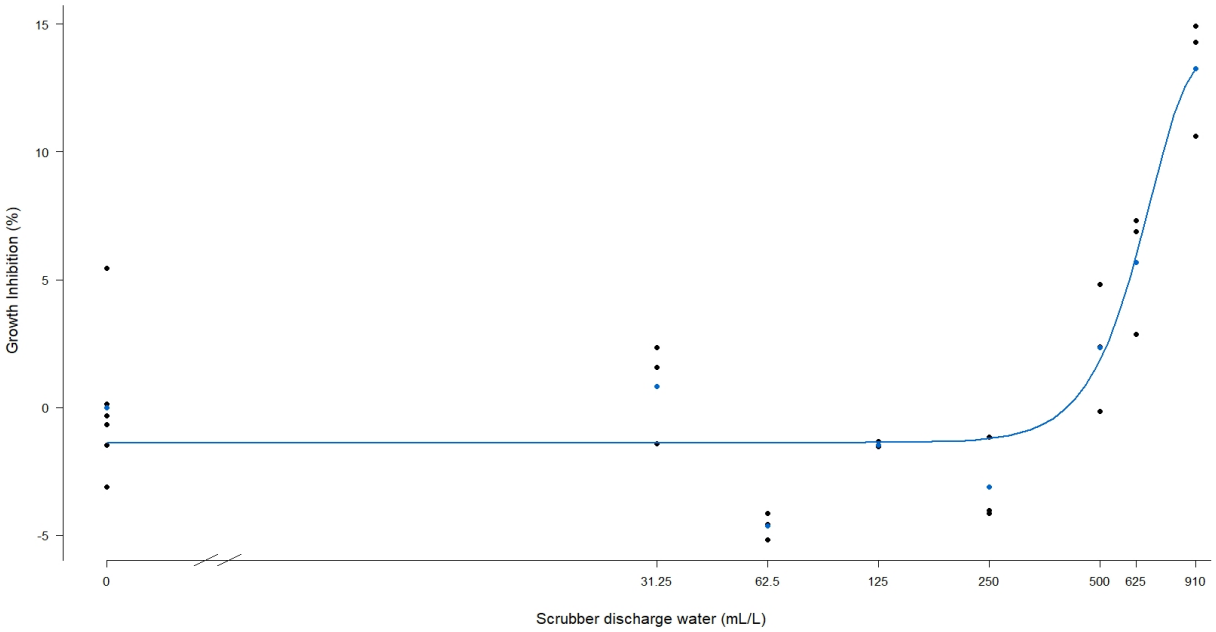


Figure A. 4 Effect of the scrubber discharge water on the growth rate after 3 days of exposure. In blue, the dose response curve established by use of the free software R, (W2.4 {drc} /21/ and /22/). The blue points correspond to the average inhibition per concentrations. The black points correspond to the inhibition per replicate.

Table A.8 Growth rate endpoint, estimations of EC10, EC25 and EC50, Probit analysis. In parenthesis the 95% confidence intervals.

Growth rate endpoint	EC10 (mL/L)	EC25 (mL/L)	EC50 (mL/L)
Scrubber discharge water	741 (486->910)	>910	>910

A.1.4 Calculation of the mean coefficient of variation for section-by-section specific growth rates

Table A. 9 Calculation of the mean coefficient of variation for section-by-section specific growth rates (between 0-1, 1-2 and 2-3 days) in the laboratory control cultures.

Replicate	Growth rate per section (growth per hour)			Average growth	Variation coefficient
	0-22 hours	22-46 hours	46-71.5 hours		
Control 1	0.064	0.096	0.099	0.087	23%
Control 2	0.064	0.097	0.096	0.086	22%
Control 3	0.064	0.103	0.098	0.088	24%
Control 4	0.065	0.097	0.097	0.086	22%
Control 5	0.058	0.095	0.089	0.081	24%
Control 6	0.064	0.098	0.100	0.087	23%
Mean value	0.063	0.098	0.097	0.086	23%

A.1.5 Validity criteria

Table A.10 Validity criteria for the growth rate inhibition test with *Skeletonema* sp. according to the ISO 10253 /5/.

Criteria in the control	Target value	Fulfilled
Control pH increase during test	< 1.0 (observed: 0.5)	Yes
Control specific growth rate	> 0.9 per day (observed: 2.1 per day)	Yes
Control variation coefficient	≤ 7% (observed: 2.9%)	Yes

A.2 Acute test with *Acartia tonsa* with scrubber discharge and inlet water

A.2.1 Primary data on mortality

Table A. 11 Daily observation of the total number of live and dead copepods in the acute test with scrubber discharge and inlet water.

Date		17 March 2021 (Start)											
Scrubber discharge water (mL/L)	Initial number of copepods	Number of live copepods						Number of dead copepods					
		A	B	C	D	E	F	A	B	C	D	E	F
Laboratory control	30	5	5	5	5	5	5	0	0	0	0	0	0
31.25	20	5	5	5	5	-	-	0	0	0	0	-	-
62.5	20	5	5	5	5	-	-	0	0	0	0	-	-
125	20	5	5	5	5	-	-	0	0	0	0	-	-
250	20	5	5	5	5	-	-	0	0	0	0	-	-
500	20	5	5	5	5	-	-	0	0	0	0	-	-
625	20	5	5	5	5	-	-	0	0	0	0	-	-
1,000	20	5	5	5	5	-	-	0	0	0	0	-	-
Scrubber inlet water 1,000 mL/L	30	5	5	5	5	5	5	0	0	0	0	0	0

Date		18 March 2021 (24 hours)											
Scrubber discharge water (mL/L)	Initial number of copepods	Number of live copepods						Number of dead copepods					
		A	B	C	D	E	F	A	B	C	D	E	F
Laboratory control	30	5	5	4	5	5	5	0	0	1	0	0	0
31.25	20	5	5	5	5	-	-	0	0	0	0	-	-
62.5	20	5	4	5	5	-	-	0	1	0	0	-	-
125	20	5	5	5	5	-	-	0	0	0	0	-	-
250	20	5	5	5	5	-	-	0	0	0	0	-	-
500	20	5	5	5	5	-	-	0	0	0	0	-	-
625	20	5	5	5	5	-	-	0	0	0	0	-	-
1,000	20	5	5	5	5	-	-	0	0	0	0	-	-
Scrubber inlet water 1,000 mL/L	30	5	5	5	5	5	5	0	0	0	0	0	0

Date		19 March 2021 (48 hours)											
Scrubber discharge water (mL/L)	Initial number of copepods	Number of live copepods						Number of dead copepods					
		A	B	C	D	E	F	A	B	C	D	E	F
Laboratory control	30	5	5	4	5	5	5	0	0	1	0	0	0
31.25	20	5	5	5	5	-	-	0	0	0	0	-	-
62.5	20	5	4	5	5	-	-	0	1	0	0	-	-
125	20	5	5	5	4	-	-	0	0	0	1	-	-
250	20	5	4	5	5	-	-	0	1	0	0	-	-
500	20	5	5	5	5	-	-	0	0	0	0	-	-
625	20	5	4	5	5	-	-	0	1	0	0	-	-
1,000	20	4	4	2	4	-	-	1	1	3	1	-	-
Scrubber inlet water 1,000 mL/L	30	5	5	5	5	5	5	0	0	0	0	0	0

A.2.2 Physical parameters

Table A. 12 Measurement of pH, salinity, and dissolved oxygen at the start and termination of the acute test.

Date	17 March 2021 (Start)		
Scrubber discharge water (mL/L)	Oxygen (% sat)	Salinity (PSU)	pH
Laboratory control	100	33	7.8
31.25	100	33	8.0
62.5	100	33	8.0
125	100	33	8.0
250	100	33	8.0
500	100	33	7.9
625	100	33	7.9
1,000	99	34	7.8
Scrubber inlet water 1,000 mL/L	100	34	8.0

Date	19 March 2021 (48 hours)	
Scrubber discharge water (mL/L)	**Oxygen (% sat)**	**pH**
Laboratory control	99	8.2
31.25	99	8.2
62.5	100	8.2
125	100	8.1
250	99	8.1
500	99	8.1
625	99	8.0
1,000	99	7.7
Scrubber inlet water 1,000 mL/L	99	8.2

Table A. 13 Physical parameters – temperature monitoring in the test period.

Mean (°C)	Minimum (°C)	Maximum (°C)
20.1	20.0	20.2

A.2.3 Statistical analysis – Determination of lethal concentrations after exposure to scrubber discharge and inlet water

A.2.3.1 Determination of the effect on mortality with scrubber discharge water

Table A. 14 Experimental data of the mortality (number of dead copepods and percentage) in the laboratory control, scrubber discharge water concentrations, and the scrubber inlet water.

Scrubber discharge water (mL/L)	No. of copepods exposed	No. of dead copepods 24h	No. of dead copepods 48h	Mortality 24h (%)	Mortality 48h (%)
Laboratory control	30	1	1	3	3
31.25	20	0	0	0	0
62.5	20	1	1	5	5
125	20	0	1	0	5
250	20	0	1	0	5
500	20	0	0	0	0
625	20	0	1	0	5
1,000	20	0	6	0	30
Scrubber inlet water 1,000 mL/L	30	0	0	0	0

A.2.3.2 Results of the Probit analysis

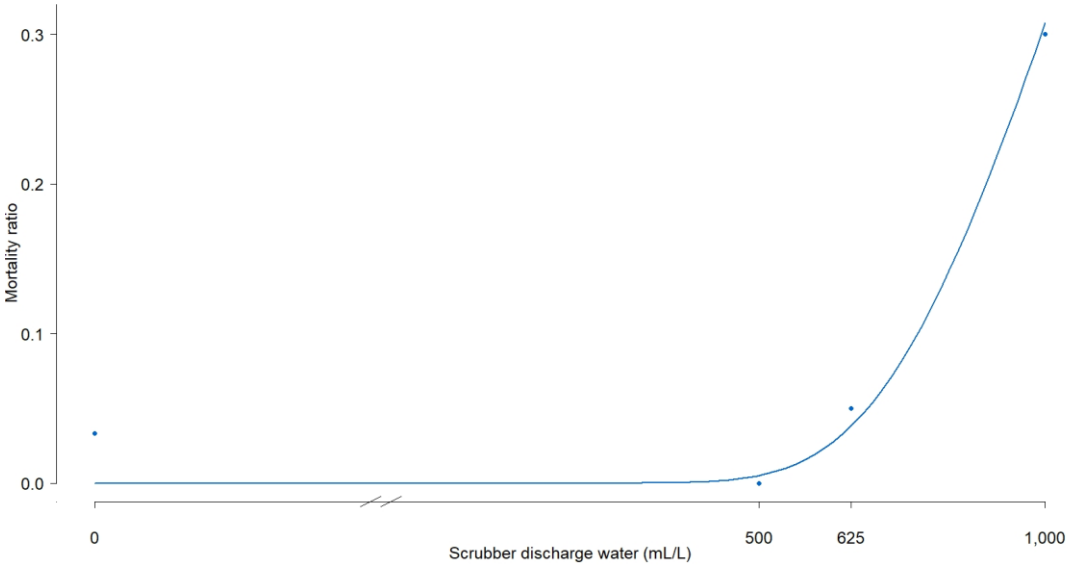


Figure A. 5 Effect of scrubber discharge water on the mortality after 48 hours exposure. In blue, the dose response curve established by use of the free software R. The blue points correspond to the average inhibition per concentration.

The mortality found in test concentrations below 500 mL/L were not included in the estimation of the effect concentrations, as no clear dose-response relationship was observed.

The test results can be seen in Table A.15.

Table A.15 Mortality endpoint, estimations of LC10, LC25 and LC50 with R. In parenthesis the 95% confidence intervals.

Mortality endpoint	LC10 (mL/L)	LC25 (mL/L)	LC50 (mL/L)
Scrubber discharge water	734 (594-874)	928 (721->1,000)	>1,000

A.2.4 Validity criteria

Table A.16 Validity criteria of the test according to the ISO 14669 /3/ in the acute test with *Acartia tonsa.*

Criteria	Target value	Fulfilled
Control mortality rate	≤ 10% (observed: 3%)	Yes
Dissolved oxygen	≥70% (observed: ≥99%)	Yes
Reference substance, 3,5-DCP, $LC50_{48\ h}$	0.5-1.5 mg/L (observed: 0.53 (0.44-0.61) mg/L) (2021.02.24)	Yes

A.3 Early-life stage test with *Acartia tonsa* with scrubber discharge and inlet water

A.3.1 Primary data on the early-life stage test

Table A. 17 Raw data of the number and larvae stage of organisms generated in the early-life stage test with scrubber discharge and inlet water. Test period from 2021-03-24 to 2021-03-30.

Concentration	Replicate	Number at the initiation of the test:			Number at the end of exposure at Day 6:		
		Eggs	Nauplii	Total	Eggs unhatched	Nauplii	Copepodites
Laboratory control	A	61	0	61	0	17	34
	B	68	0	68	0	22	33
	C	74	0	74	4	26	26
	D	78	0	78	0	39	34
	E	75	0	75	3	30	31
	F	74	0	74	1	32	29
	G	78	0	78	1	20	33
	H	69	0	69	0	25	43
	I[1)]	62	0	62	-	-	-
	J	61	1	62	2	17	34
	K	76	1	77	0	19	36
	L	76	0	76	1	26	39
Scrubber discharge water 50 mL/L	A	84	0	84	1	33	37
	B	89	0	89	2	25	33
	C	74	1	75	1	20	49
	D	77	2	79	1	27	40
	E	72	0	72	1	14	40
	F	68	0	68	1	25	30

Concentration	Replicate	Number at the initiation of the test:			Number at the end of exposure at Day 6:		
		Eggs	Nauplii	Total	Eggs unhatched	Nauplii	Copepodites
Scrubber discharge water 100 mL/L	A	68	0	68	1	21	23
	B	80	0	80	1	30	31
	C	73	0	73	1	29	28
	D	65	0	65	1	31	24
	E	69	0	69	0	27	26
	F	74	0	74	1	25	27
Scrubber discharge water 200 mL/L	A	78	0	78	1	24	31
	B	83	0	83	0	29	32
	C	72	0	72	2	32	17
	D	72	0	72	1	26	30
	E	68	0	68	0	33	27
	F	76	0	76	1	33	28
Scrubber discharge water 400 mL/L	A	80	0	80	4	32	30
	B	78	0	78	2	32	31
	C	71	0	71	2	47	5
	D	86	0	86	3	37	25
	E	67	1	68	3	21	23
	F	90	0	90	2	34	32
Scrubber discharge water 800 mL/L	A	63	0	63	1	39	7
	B	81	0	81	4	43	4
	C	77	0	77	1	49	7
	D	76	0	76	1	50	10
	E	69	0	69	2	21	1
	F	78	0	78	3	62	10
Scrubber inlet water 800 mL/L	A	60	1	61	1	23	23
	B	78	0	78	2	24	26
	C[1)]	61	0	61	-	-	-
	D	79	0	79	1	24	33
	E	76	0	76	1	33	37
	F	88	0	88	2	35	29

1) The replicate was lost

A.3.2 Physical parameters

Table A. 18 Physical parameters – start of the test, Day 0: 2021-03-24.

Scrubber discharge water (mL/L)	Dissolved oxygen (%)	Salinity (PSU)	pH
Laboratory control	100	32.5	8.0
50	100	32.8	7.9
100	100	32.8	7.9
200	100	33.0	7.9
400	100	33.2	7.9
800	100	33.4	7.9
Scrubber inlet water 800 mL/L	100	33.6	8.0

Table A. 19 Physical parameters – before addition of test solution, Day 2: 2021-03-26.

Scrubber discharge water (mL/L)	Dissolved oxygen (%)	Salinity (PSU)	pH
Laboratory control	100	32.5	8.4
50	100	32.9	8.5
100	100	33.2	8.4
200	100	33.5	8.4
400	100	33.4	8.4
800	100	33.9	8.2
Scrubber inlet water 800 mL/L	100	33.9	8.4

Table A. 20 Physical parameters – after addition of test solution, Day 2: 2021-03-26.

Scrubber discharge water (mL/L)	Dissolved oxygen (%)	Salinity (PSU)	pH
Laboratory control	100	32.7	8.2
50	100	32.9	8.2
100	100	33.0	8.1
200	100	33.2	8.1
400	100	33.3	8.1
800	100	33.6	8.0
Scrubber inlet water 800 mL/L	100	33.7	8.2

Table A. 21 Physical parameters – end of the test, Day 6: 2021-03-30.

Scrubber discharge water (mL/L)	Dissolved oxygen (%)	Salinity (PSU)	pH
Laboratory control	98	32.7	8.3
50	98	32.9	8.3
100	97	33.9	8.3
200	98	33.4	8.2
400	98	33.5	8.2
800	97	34.0	8.0
Scrubber inlet water 800 mL/L	98	34.2	8.3

Table A. 22 Physical parameters – temperature monitoring from 2021-03-24 to 2021-03-30.

Mean	Minimum	Maximum
20.0	19.8	20.5

A.3.3 Statistical analysis – Determination of effect concentrations after exposure to scrubber discharge water

A.3.3.1 Determination of NOEC and LOEC for hatching success after exposure to scrubber discharge and inlet water

Table A.23 Experimental data of hatching success (%) in the laboratory control, scrubber discharge water and scrubber inlet water groups.

Replicate No.	Laboratory control	Scrubber discharge water 50 mL/L	Scrubber discharge water 100 mL/L	Scrubber discharge water 200 mL/L	Scrubber discharge water 400 mL/L	Scrubber discharge water 800 mL/L	Scrubber inlet water 800 mL/L
1	100%	99%	99%	99%	95%	98%	98%
2	100%	98%	99%	100%	97%	95%	97%
3	95%	99%	99%	97%	97%	99%	1)
4	100%	99%	98%	99%	97%	99%	99%
5	96%	99%	100%	100%	96%	97%	99%
6	99%	99%	99%	99%	98%	96%	98%
7	99%	-	-	-	-	-	-
8	100%	-	-	-	-	-	-
9	1)	-	-	-	-	-	-
10	97%	-	-	-	-	-	-
11	100%	-	-	-	-	-	-
12	99%	-	-	-	-	-	-
Count	11	6	6	6	6	6	5
Mean	98%	99%	99%	99%	97%	97%	98%

1) The replicate was lost

- Normality

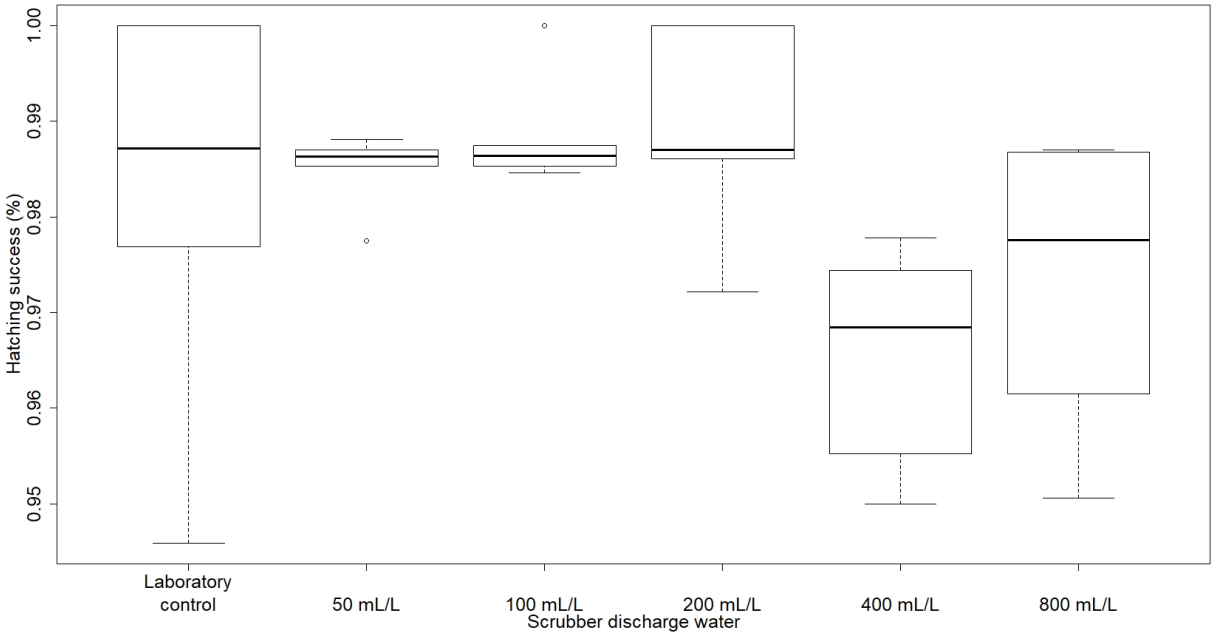


Figure A. 6 Boxplots of the hatching success for test groups with scrubber discharge water.

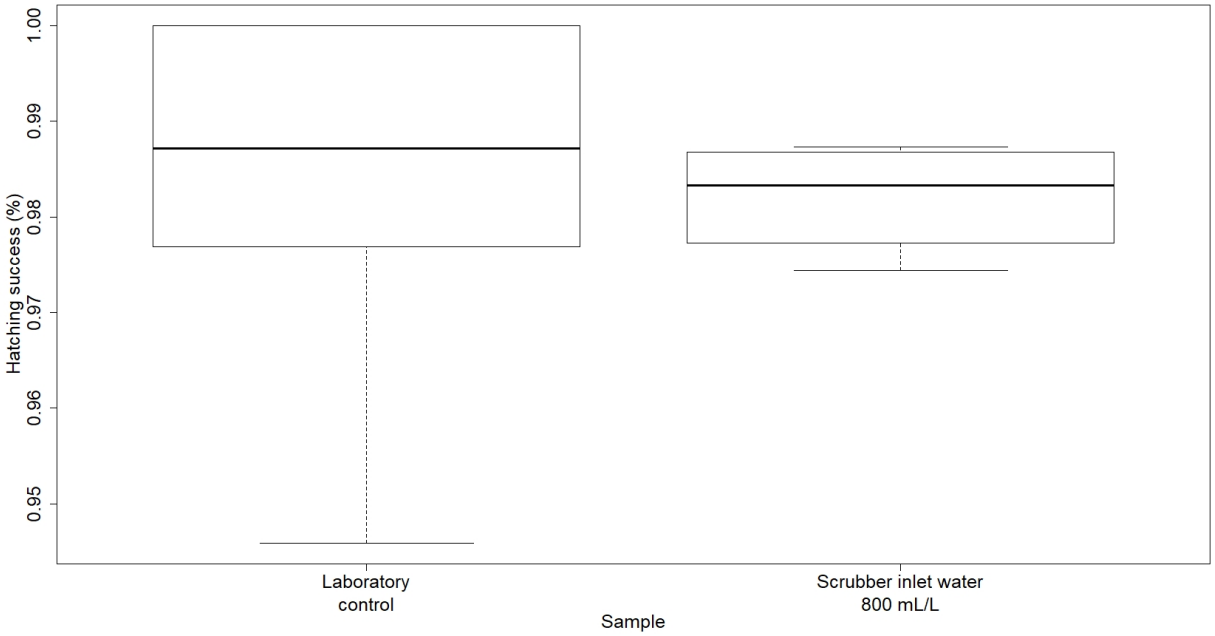


Figure A. 7 Boxplots of the hatching success for test group with scrubber inlet water.

The boxplot patterns show the data distribution (Figure A. 6 and Figure A. 7). A normal distribution in each test group will be assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups are not different from the control group (Table A.24).

Table A.24 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(>|t|)	Significance[1)]
Scrubber discharge-50 - Lab control = 0	0.00	0.0	0.03	1.00	
Scrubber discharge-100 - Lab control = 0	0.00	0.0	0.52	0.98	
Scrubber discharge-200 - Lab control = 0	0.00	0.0	0.57	0.98	
Scrubber discharge-400 - Lab control = 0	-0.02	0.0	-2.8	0.03	*
Scrubber discharge-800 - Lab control = 0	-0.01	0.0	-1.7	0.36	
Mean comparisons	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	**Significance[1)]**
Scrubber inlet-800 - Lab control = 0	0.00	0.0	-0.3	0.73	

1) Signif. codes: 0 '***' 0.001'**' 0.01 '*' 0.05 '.'0.1 ''1 (Adjusted p values reported - single-step method)

The hypothesis that the mean of any test group with scrubber discharge water is significantly different from the laboratory control water was rejected (t>0 and P>0.05), except for 400 mL/L (t<0 and P< 0.05) (Table A.24). Therefore, the NOEC value was estimated to 800 mL/L (Table A.25).

The scrubber inlet water was not significantly different from the laboratory control (t<0 and P>0.05) (Table A.24

Table A.25 Hatching success (HS) endpoint, estimations of NOEC and LOEC, Dunnett's test.

Hatching success endpoint	NOEC (mL/L)	LOEC (mL/L)
Scrubber discharge water	800	>800

A.3.3.2 Results of the Probit analysis with scrubber discharge water

As no negative effect was observed among the test groups, the data to estimate the ECX were not computed.

Table A.26 Hatching success endpoint, estimations of EC10, EC25 and EC50, Probit analysis.

Hatching success endpoint	EC10 (mL/L)	EC25 (mL/L)	EC50 (mL/L)
Scrubber discharge water	>800	>800	>800

A.3.3.3 Determination of NOEC and LOEC for the early-life stage mortality with scrubber discharge water

Table A.27 Experimental data of early-life stage mortality (%) in the laboratory control, scrubber discharge water and scrubber inlet water groups.

Replicate No.	Laboratory control	Scrubber discharge water 50 mL/L	Scrubber discharge water 100 mL/L	Scrubber discharge water 200 mL/L	Scrubber discharge water 400 mL/L	Scrubber discharge water 800 mL/L	Scrubber inlet water 800 mL/L
1	16%	16%	34%	29%	18%	26%	23%
2	19%	33%	23%	27%	17%	39%	34%
3	26%	7%	21%	30%	25%	26%	1)
4	6%	14%	14%	21%	25%	20%	27%
5	15%	24%	23%	12%	32%	67%	7%
6	16%	18%	29%	19%	25%	4%	26%
7	31%	-	-	-	-	-	-
8	1%	-	-	-	-	-	-
9	1)	-	-	-	-	-	-
10	15%	-	-	-	-	-	-
11	29%	-	-	-	-	-	-
12	13%	-	-	-	-	-	-
Count	11	6	6	6	6	6	5
mean	17%	19%	24%	23%	24%	30%	23%

1) The replicate was lost

- Normality

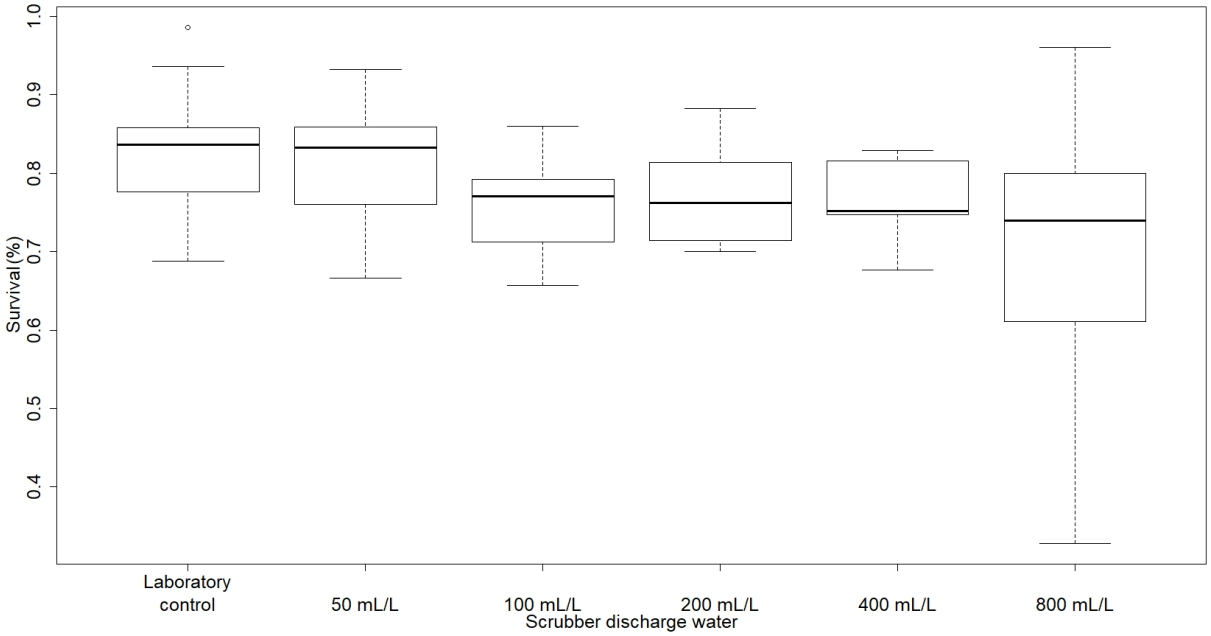


Figure A. 8 Boxplots of the early-life stage mortality, represented as survival, for test groups with scrubber discharge water.

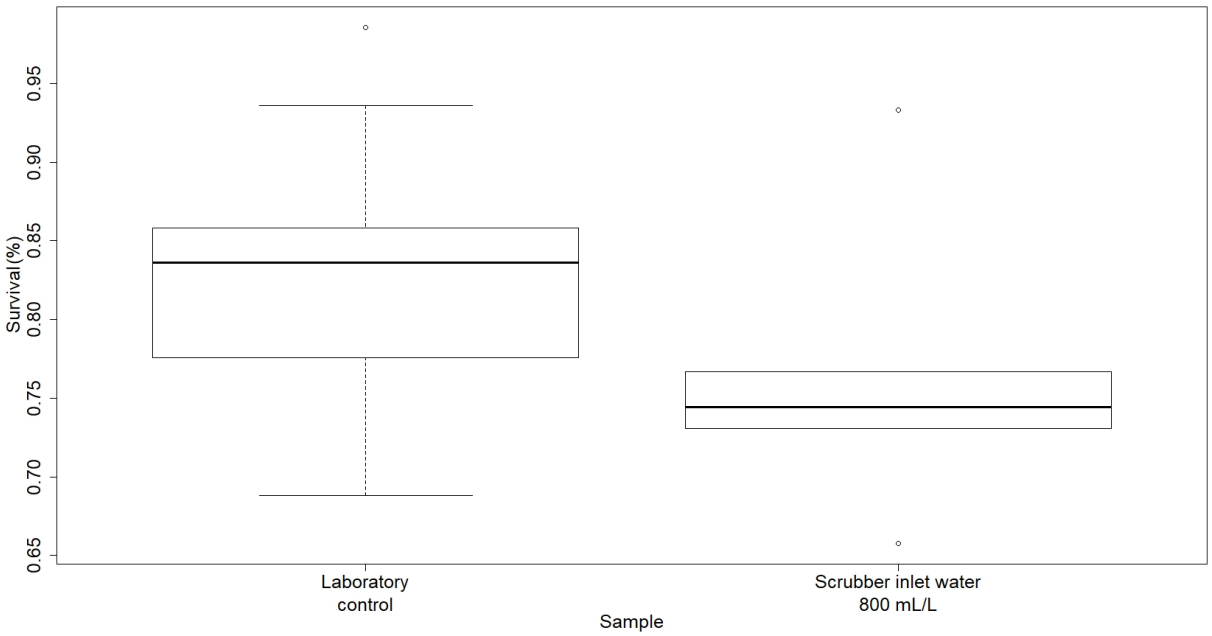


Figure A. 9 Boxplots of the early-life stage mortality, represented as survival, for test group with scrubber inlet water.

The boxplot patterns show the data distribution (Figure A. 8 and Figure A. 9). A normal distribution in each test group will be assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups are not different from the control group (Table A.28).

Table A.28 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(>|t|)	Significance[1)]
Scrubber discharge-50 - Lab control = 0	-0.01	0.05	-0.26	1.00	
Scrubber discharge-100 - Lab control = 0	-0.07	0.05	-1.24	0.65	
Scrubber discharge-200 - Lab control = 0	-0.06	0.05	-1.02	0.80	
Scrubber discharge-400 - Lab control = 0	-0.07	0.05	-1.21	0.68	
Scrubber discharge-800 - Lab control = 0	-0.13	0.05	-2.40	0.09	.
Mean comparisons	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	**Significance[1)]**
Scrubber inlet-800 - Lab control = 0	-0.06	0.05	-1.23	0.24	

1) Signif. codes: 0 '***' 0.001'**' 0.01 '*' 0.05 '.'0.1 ''1 (Adjusted p values reported - single-step method)

The hypothesis that the mean of any test group with scrubber discharge water is significantly different from the laboratory control water was rejected (t>0 and P>0.05) (Table A.28). Therefore, the NOEC value was estimated to 800 mL/L (Table A.29).

The scrubber inlet water was not significantly different from the laboratory control (t<0 and P>0.05) (Table A.28)

Table A.29 Early-life stage mortality endpoint, estimations of NOEC and LOEC, Dunnett's test.

Early-life stage mortality	NOEC (mL/L)	LOEC (mL/L)
Scrubber discharge water	800	>800
Scrubber inlet water	800	>800

A.3.3.4 Results of the Probit analysis with scrubber discharge water

As no negative effect was observed among the test groups, the data to estimate the ECX were not computed.

Table A.30 Early-life stage mortality endpoint, estimations of LC10, LC25 and LC50, Probit analysis.

Early life stage mortality	LC10 (mL/L)	LC25 (mL/L)	LC50 (mL/L)
Scrubber discharge water	>800	>800	>800

A.3.3.5 Determination of NOEC and LOEC for the larval development ratio with scrubber discharge water

Table A.31 Experimental data of larval development ratio (%) in the laboratory control, scrubber discharge water and scrubber inlet water groups.

Replicate No.	Laboratory control	Scrubber discharge water 50 mL/L	Scrubber discharge water 100 mL/L	Scrubber discharge water 200 mL/L	Scrubber discharge water 400 mL/L	Scrubber discharge water 800 mL/L	Scrubber inlet water 800 mL/L
1	67%	53%	52%	56%	48%	15%	50%
2	60%	57%	51%	52%	49%	9%	52%
3	50%	71%	49%	35%	10%	13%	1)
4	47%	60%	44%	54%	40%	17%	58%
5	51%	74%	49%	45%	52%	5%	53%
6	48%	55%	52%	46%	48%	14%	45%
7	62%	-	-	-	-	-	-
8	63%	-	-	-	-	-	-
9	1)	-	-	-	-	-	-
10	67%	-	-	-	-	-	-
11	65%	-	-	-	-	-	-
12	60%	-	-	-	-	-	-
Count	11	6	6	6	6	6	5
Mean	58%	62%	49%	48%	41%	12%	52%

1) The replicate was lost

- Normality

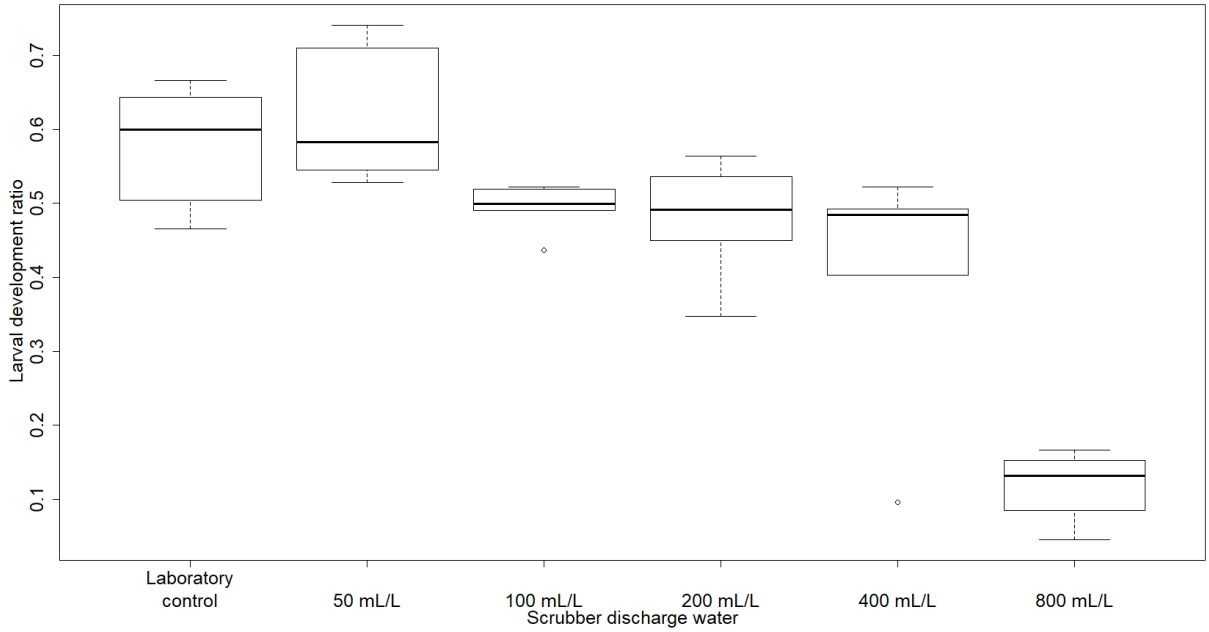


Figure A. 10 Boxplots of the larval development ratio for test groups with scrubber discharge water.

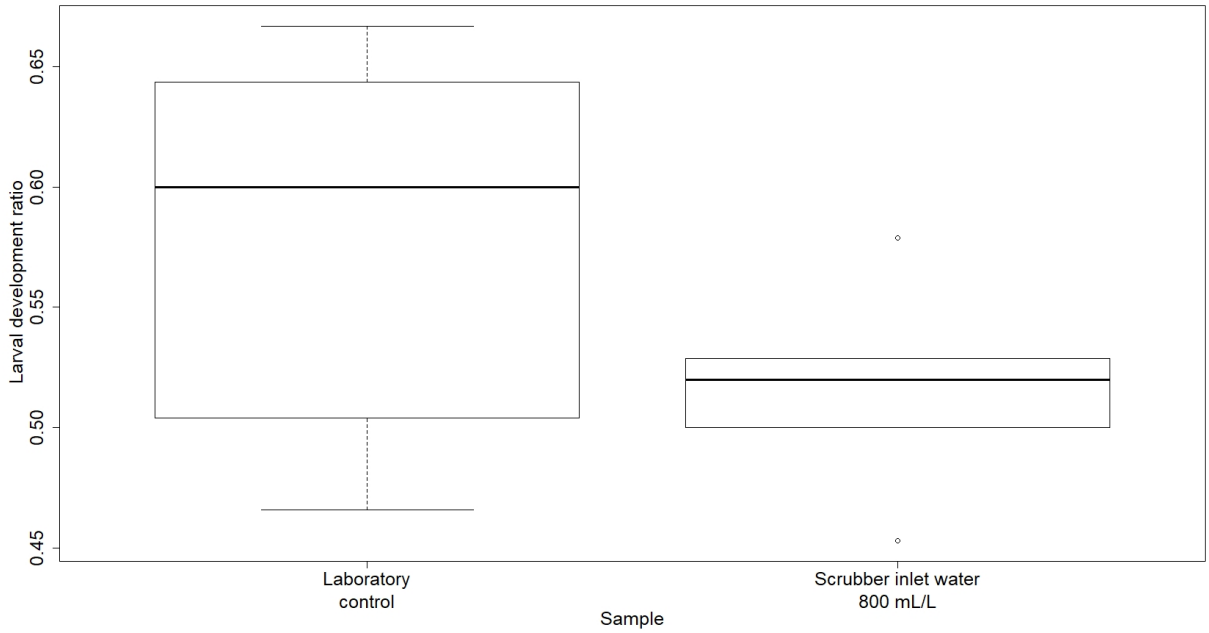


Figure A. 11 Boxplots of the larval development ratio for test group with scrubber inlet water.

The boxplot patterns show the data distribution (Figure A. 10 and Figure A. 11). A normal distribution in each test group will be assumed in the following study.

- Dunnett's test

Dunnett's test tests the null hypothesis that the averages of every test groups are not different from the control group (Table A.32).

Table A.32 Multiple comparisons of means: Dunnett's contrasts.

Mean comparisons	Estimate	Std. Error	t value	Pr(>|t|)	Significance[1)]
Scrubber discharge-50 - Lab control = 0	0.03	0.05	0.75	0.93	
Scrubber discharge-100 - Lab control = 0	-0.09	0.05	-1.92	0.25	
Scrubber discharge-200 - Lab control = 0	-0.10	0.05	-2.24	0.13	
Scrubber discharge-400 - Lab control = 0	-0.17	0.05	-3.71	0.003	**
Scrubber discharge-800 - Lab control = 0	-0.46	0.05	-10.2	< 0.001	***
Mean comparisons	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	**Significance[1)]**
Scrubber inlet-800 - Lab control = 0	-0.07	0.04	-1.71	0.11	

1) Signif. codes: 0 '***' 0.001'**' 0.01 '*' 0.05 '.'0.1 ''1 (Adjusted p values reported - single-step method)

The hypothesis that the mean of any test groups with scrubber discharge water is significantly lower than the laboratory control water was verified (t<0 and P< 0.05) at 400 mL/L of scrubber discharge water and above (Table A.32). Therefore, the NOEC value was estimated to 200 mL/L (Table A.33).

The scrubber inlet water was not significantly different from the laboratory control (t<0 and P>0.05) (Table A.32)

Table A.33 Larval development ratio endpoint, estimations of NOEC and LOEC, Dunnett's test.

Larval development ratio endpoint	NOEC (mL/L)	LOEC (mL/L)
Scrubber discharge water	200	400
Scrubber inlet water	800	>800

A.3.3.6 Results of the Probit analysis of the larval development ratio

Table A. 34 Experimental data – inhibition of the larval development ratio after 6 days of exposure.

Replicate No.	Laboratory control	Scrubber discharge water 50 mL/L	Scrubber discharge water 100 mL/L	Scrubber discharge water 200 mL/L	Scrubber discharge water 400 mL/L	Scrubber discharge water 800 mL/L	Scrubber inlet water 800 mL/L
1	-15%	9%	10%	3%	17%	74%	14%
2	-3%	2%	13%	10%	15%	85%	11%
3	14%	-22%	15%	40%	83%	78%	1)
4	20%	-3%	25%	8%	31%	71%	0%
5	13%	-27%	16%	23%	10%	92%	9%
6	18%	6%	11%	21%	17%	76%	22%
7	-7%	-	-	-	-	-	-
8	-9%	-	-	-	-	-	-

Replicate No.	Laboratory control	Scrubber discharge water 50 mL/L	Scrubber discharge water 100 mL/L	Scrubber discharge water 200 mL/L	Scrubber discharge water 400 mL/L	Scrubber discharge water 800 mL/L	Scrubber inlet water 800 mL/L
9	1)	-	-	-	-	-	-
10	-15%	-	-	-	-	-	-
11	-13%	-	-	-	-	-	-
12	-3%	-	-	-	-	-	-
Mean	0%	-6%	15%	17%	29%	80%	11%

1) The replicate was lost

Table A.35 ECx estimates, Probit analysis with R after 6 days.

ECx	Estimate scrubber discharge water concentration (mL/L)	Std. Error	Lower (mL/L)	Upper (mL/L)
EC05	135	38	60	211
EC10	199	44	113	284
EC25	340	45	252	428
EC50	543	36	474	613

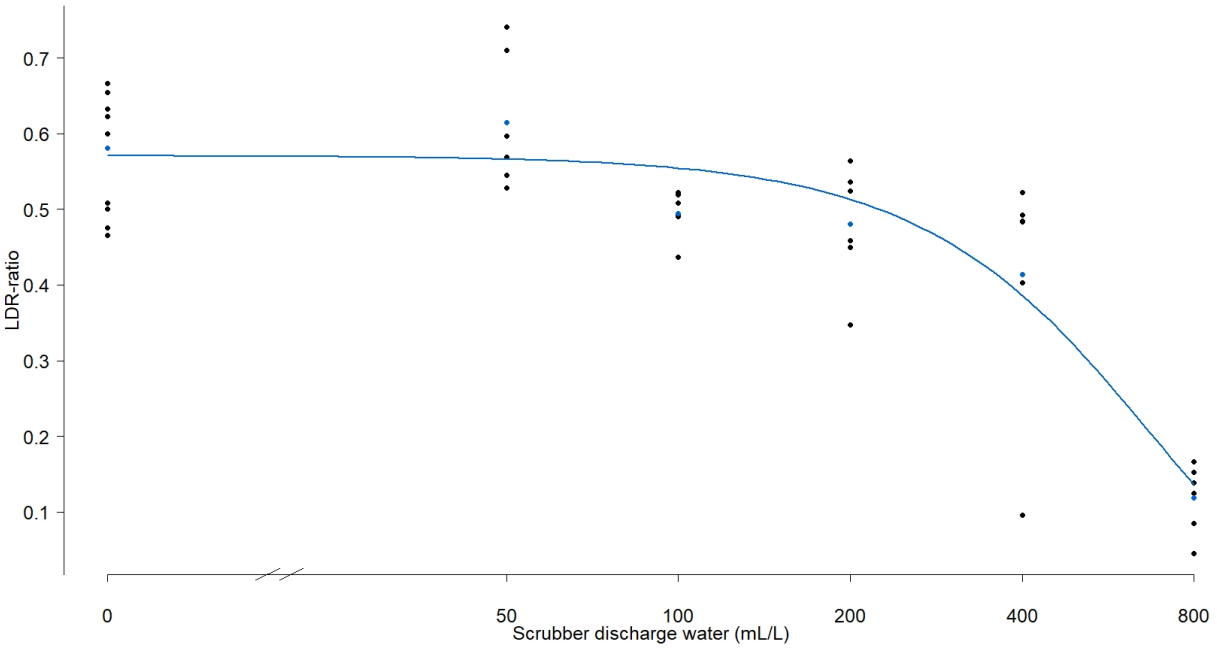


Figure A. 12 Effect of the scrubber discharge water on the larval development ratio (LDR) after 6 days of exposure. In blue, the dose response curve established by use of the free software R. The blue points correspond to the average inhibition per concentration. The black points correspond to the inhibition per replicate.

Table A. 36 Larval development ratio endpoint, estimations of EC10, EC25, and EC50, Probit analysis. In parenthesis the 95% confidence intervals.

Larval development ratio endpoint	EC10 (mL/L)	EC25 (mL/L)	EC50 (mL/L)
Scrubber discharge water	200 (110-280)	340 (250-430)	540 (470-610)

A.3.4 Validity criteria

Table A. 37 Validity criteria according to the ISO 16778 /4/ in the early-life stage test with *Acartia tonsa.*

Criteria	Target value	Fulfilled
Early-life stage mortality in the control	≤ 30% (observed value: 17%)	Yes
Hatching success in the control	≥ 75% (observed value: 98%)	Yes
Larval development ratio in the control	60% ± 20% (observed value: 58%)	Yes
Temperature variation	< ± 1 °C (observed value: 19.8-20.5 °C)	Yes
Control pH increase during test	< 1.0 (observed value: 0.4)	Yes
Dissolved oxygen concentration	> 70% throughout test (observed value: ≥ 97%)	Yes
Salinity variation from the control start (Si) value	Si‰ ± 10% (observed value: 33‰ ± 5%)	Yes
EC50 of the reference substance (3,5-DCP) within 500 µg/L ± 300 µg/L (20 °C and 20 ‰)	LDR EC50 for 3,5-DCP at 20 °C and 32‰ salinity: 460 (400 - 510) µg/L	Yes

A.4 Acute limit test with European sea bass *Dicentrarchus labrax* with scrubber discharge and inlet water

A.4.1 Primary data on mortality, visible abnormalities, and size

Table A. 38 Daily observation of the total number of live and dead fish in the acute limit test with the test item.

Date	22 March 2021 (Start, 0 hours)		
Sample	**Initial number of fish**	**Number of live fish**	**Number of dead fish**
Laboratory control	7	7	0
Scrubber discharge water, 910 mL/L	7	7	0
Scrubber inlet water, 910 mL/L	7	7	0

Date	22 March 2021 (Start, 3 hours)		
Sample	**Initial number of fish**	**Number of live fish**	**Number of dead fish**
Laboratory control	7	7	0
Scrubber discharge water, 910 mL/L	7	7	0
Scrubber inlet water, 910 mL/L	7	7	0

Date	23 March 2021 (Day 1)		
Sample	**Initial number of fish**	**Number of live fish**	**Number of dead fish**
Laboratory control	7	7	0
Scrubber discharge water, 910 mL/L	7	7	0
Scrubber inlet water, 910 mL/L	7	7	0

Date	24 March 2021 (Day 2)		
Sample	**Initial number of fish**	**Number of live fish**	**Number of dead fish**
Laboratory control	7	7	0
Scrubber discharge water, 910 mL/L	7	7	0
Scrubber inlet water, 910 mL/L	7	7	0

Date	25 March 2021 (Day 3)		
Sample	**Initial number of fish**	**Number of live fish**	**Number of dead fish**
Laboratory control	7	7	0
Scrubber discharge water, 910 mL/L	7	7	0
Scrubber inlet water, 910 mL/L	7	7	0

Date	26 March 2021 (Day 4)		
Sample	**Initial number of fish**	**Number of live fish**	**Number of dead fish**
Laboratory control	7	7	0
Scrubber discharge water, 910 mL/L	7	7	0
Scrubber inlet water, 910 mL/L	7	7	0

On days 1-3 of the test, all aquaria were inspected twice per day.

No visible abnormalities regarding equilibrium, appearance, ventilatory behaviour and swimming behaviour were observed during the test.

The average length of the laboratory control fish was 5.1 cm (4.4-5.6 cm) and the average weight 1.81 g (1.23-2.19 g).

A.4.2 Physical parameters

Table A. 39 Measurements of temperature, dissolved oxygen, salinity, and pH generated in the acute limit test with scrubber discharge and inlet water.

Date	22 March 2021 (Start, 0 hours)			
Sample	**Temperature (°C)**	**Oxygen (% sat)**	**Salinity (PSU)**	**pH**
Laboratory control	20.5	100	32.3	7.9
Scrubber discharge water, 910 mL/L	19.6	97	33.4	7.9
Scrubber inlet water, 910 mL/L	19.5	97	33.7	7.9

Date	22 March 2021 (2 hours)			
Sample	**Temperature (°C)**	**Oxygen (% sat)**	**Salinity (PSU)**	**pH**
Laboratory control	19.8	93	32.1	7.9
Scrubber discharge water, 910 mL/L	19.6	96	33.4	7.8
Scrubber inlet water, 910 mL/L	19.5	96	33.5	7.9

Date	23 March 2021 (Day 1)			
Sample	**Temperature (°C)**	**Oxygen (% sat)**	**Salinity (PSU)**	**pH**
Laboratory control	20.3	90	32.0	7.8
Scrubber discharge water, 910 mL/L	19.8	95	33.5	7.5
Scrubber inlet water, 910 mL/L	19.7	95	33.7	8.0

Date	24 March 2021 (Day 2, before and after renewal)							
Sample	Temperature (°C)		Oxygen (% sat.)		Salinity (PSU)		pH	
Laboratory control	20.2	20.4	93	96	32.4	32.1	7.8	7.9
Scrubber discharge water, 910 mL/L	20.0	20.1	94	96	33.5	33.4	7.4	8.0
Scrubber inlet water, 910 mL/L	19.8	20.1	95	98	33.9	33.5	8.1	8.0

Date	25 March 2021 (Day 3)			
Sample	Temperature (°C)	Oxygen (% sat)	Salinity (PSU)	pH
Laboratory control	20.4	90	32.0	7.8
Scrubber discharge water, 910 mL/L	20.3	92	33.4	7.1
Scrubber inlet water, 910 mL/L	20.0	95	33.7	8.0

Date	26 March 2021 (Day 4)			
Sample	Temperature (°C)	Oxygen (% sat)	Salinity (PSU)	pH
Laboratory control	20.4	92	31.8	7.8
Scrubber discharge water, 910 mL/L	20.4	91	33.4	7.3
Scrubber inlet water, 910 mL/L	20.3	91	33.8	7.9

A.4.3 Statistical analysis – Determination of lethal concentration after exposure to scrubber discharge and inlet water

A.4.3.1 Determination of the effect on mortality with scrubber discharge and inlet water

Table A. 40 Experimental data of the mortality rate (%) in the laboratory control, scrubber discharge and inlet water.

Endpoint	Laboratory control	Scrubber discharge water 910 mL/L	Scrubber inlet water 910 mL/L
Mortality	0%	0%	0%

A.4.3.2 Results of the Probit analysis

As no negative effect was observed, the data to estimate the LCx were not computed.

Table A. 41 Mortality endpoint, estimations of LC10, LC25 and LC50.

Mortality endpoint	LC10 (mL/L)	LC25 (mL/L)	LC50 (mL/L)
Scrubber discharge water	>910	>910	>910

A.4.4 Validity criteria

Table A. 42 Validity criteria of the test according to OECD 203 /2/ in the acute limit test with European sea bass *(Dicentrarchus labrax).*

Criteria	Target value	Fulfilled
Control mortality rate	≤14% (≤1 of 7 fish) (observed: 0%)	Yes
Dissolved oxygen	≥60% (minimum observed: 90%)	Yes

APPENDIX B – Chemical Analyses

B Chemical Analyses

B.1 Methods and limit of detection

Table B.1 Chemical analysis. Limit of detection may be higher due to matrix interference.

Chemical analysis	Limit of detection	Method
METALS		
Lead, Pb	0.025 µg/L	ISO 17294-2:2016
Cadmium, Cd	0.003 µg/L	
Chromium, Cr	0.01 µg/L	
Cobber, Cu	0.03 µg/L	
Nickel, Ni	0.03 µg/L	
Zink, Zn	0.3 µg/L	
Vanadium, V	0.05 µg/L	
Mercury, Hg	0.002 µg/L	SS EN ISO 17852:2008
BTEX		
Benzene	0.020 µg/L	ISO 10301:2000
Toluene	0.020 µg/L	
Ethylbenzene	0.020 µg/L	
Xylenes	0.020 µg/L	
PAHs		
Naphthalene	0.01 µg/L	DIN 38407-F39
Acenaphthylene	0.01 µg/L	
Acenaphthene	0.01 µg/L	
Phenanthrene	0.01 µg/L	
Anthracene	0.01 µg/L	
Fluorene	0.01 µg/L	
Fluoranthene	0.01 µg/L	
Pyrene	0.01 µg/L	
Benzo(a)anthracene	0.01 µg/L	
Chrysene	0.01 µg/L	
Benzo(b+j+k)fluoranthenes	0.01 µg/L	
Benz(a)pyrene	0.01 µg/L	

Chemical analysis	Limit of detection	Method
Indeno(1,2,3-cd)pyrene	0.01 µg/L	
Dibenzo(a,h)anthracene	0.01 µg/L	
Benzo(ghi)perylene	0.01 µg/L	
Benz(e)pyrene	0.01 µg/L	

B.2 Results of Chemical Analyses

Table B. 2 Overview of all chemical analyses. ND = Not detected, i.e. the concentration below the limit of quantification.

	Ship 1		Ship 2		Ship 3		Ship 4		Composite sample used for testing at DHI [1)]	
Substance (µg/L)	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge
Lead, Pb	<0.25	<0.25	<0.25	<0.25	0.83	1.3	1.9	3.4	0.74	0.92
Cadmium, Cd	<0.030	0.039	<0.030	0.043	<0.030	<0.030	0.077	0.073	<0.030	<0.030
Chrom, Cr	0.48	2.1	0.24	1.8	0.9	2.9	0.73	2.8	0.71	1.9
Copper, Cu	6.2	6.8	2.3	4.8	2.8	3.2	8.1	18	6.3	4.0
Mercury, Hg	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.00594	0.00261	<0.002	<0.002
Nickel, Ni	1.2	34	0.36	20	0.30	9.7	2.0	18	0.31	23
Zinc, Zn	320	170	16	12	850	14	26	1600	39	40
Vanadium, V	1.8	160	1.6	90	2.2	54	3.2	71	2.3	100
Benzene	<0.020	0.33	<0.020	0.83	<0.020	0.65	<0.020	1.8	<0.020	0.92
Toluene	0.022	0.17	<0.020	0.38	<0.020	0.60	<0.020	0.76	0.045	0.37
Ethylbenzene	0.021	0.040	0.026	0.1	<0.020	0.36	<0.020	0.13	<0.020	0.088
m/p-Xylene	0.075	0.14	0.14	0.33	<0.020	1.1	<0.020	0.37	<0.020	0.26
o-Xylen	0.045	0.082	0.13	0.21	<0.020	0.89	<0.020	0.21	0.021	0.22
Xylenes	0.12	0.22	0.27	0.54	<0.040	2.0	<0.040	0.58	<0.040	0.48

Substance (µg/L)	Ship 1		Ship 2		Ship 3		Ship 4		Composite sample used for testing at DHI [1)]	
	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge
Xylenes (o,-m-, p-xylene + ethylbenzene)	0.14	0.26	0.3	0.64	<0.060	2.4	<0.060	0.71	<0.060	0.57
Naphtalene	0.0034	4.9	ND	3.3	0.0086	6.4	0.0017	5.2	0.005	1.6
Acenaphtylene	<0.0010	0.36	<0.0010	0.35	<0.0010	0.51	<0.0010	0.068	<0.0010	0.021
Acenaphtene	<0.0010	1.1	<0.0010	1.1	0.0022	1.1	<0.0010	0.27	<0.0010	0.092
Phenanthrene	<0.0010	3.3	<0.0010	4.0	0.011	10	0.0017	1.6	0.0012	0.22
Anthracene	<0.0010	0.089	<0.0010	0.034	<0.0010	0.19	<0.0010	<0.0010	<0.0010	<0.0010
Fluorene	<0.0010	1.8	<0.0010	1.5	0.0046	2.2	<0.0010	0.44	<0.0010	0.12
Fluoranthene	<0.0010	0.082	<0.0010	0.27	0.0015	0.36	0.0033	0.12	<0.0010	0.018
Pyrene	<0.0010	0.032	<0.0010	0.75	0.0019	0.94	0.0026	0.11	<0.0010	0.027
Benz(a)anthracene	<0.0010	0.0013	<0.0010	0.089	<0.0010	0.022	0.001	0.0056	<0.0010	0.0083
Chrysene	<0.0010	0.0054	<0.0010	0.10	<0.0010	0.049	<0.0010	0.025	<0.0010	0.011
Benzo(b)fluoranthene	<0.0010	<0.0010	<0.0010	0.028	<0.0010	0.0066	0.0013	0.019	<0.0010	0.0098
Benz(k)fluoranthene	<0.0010	<0.0010	<0.0010	0.012	<0.0010	0.0034	0.0010	0.011	<0.0010	0.005
Benz(a)pyrene	<0.0010	<0.0010	<0.0010	0.056	<0.0010	0.0048	0.0014	0.019	<0.0010	0.0095
Indeno(1,2,3-cd)pyrene	<0.0010	<0.0010	<0.0010	0.014	<0.0010	0.010	0.0025	0.0079	<0.0010	0.0044

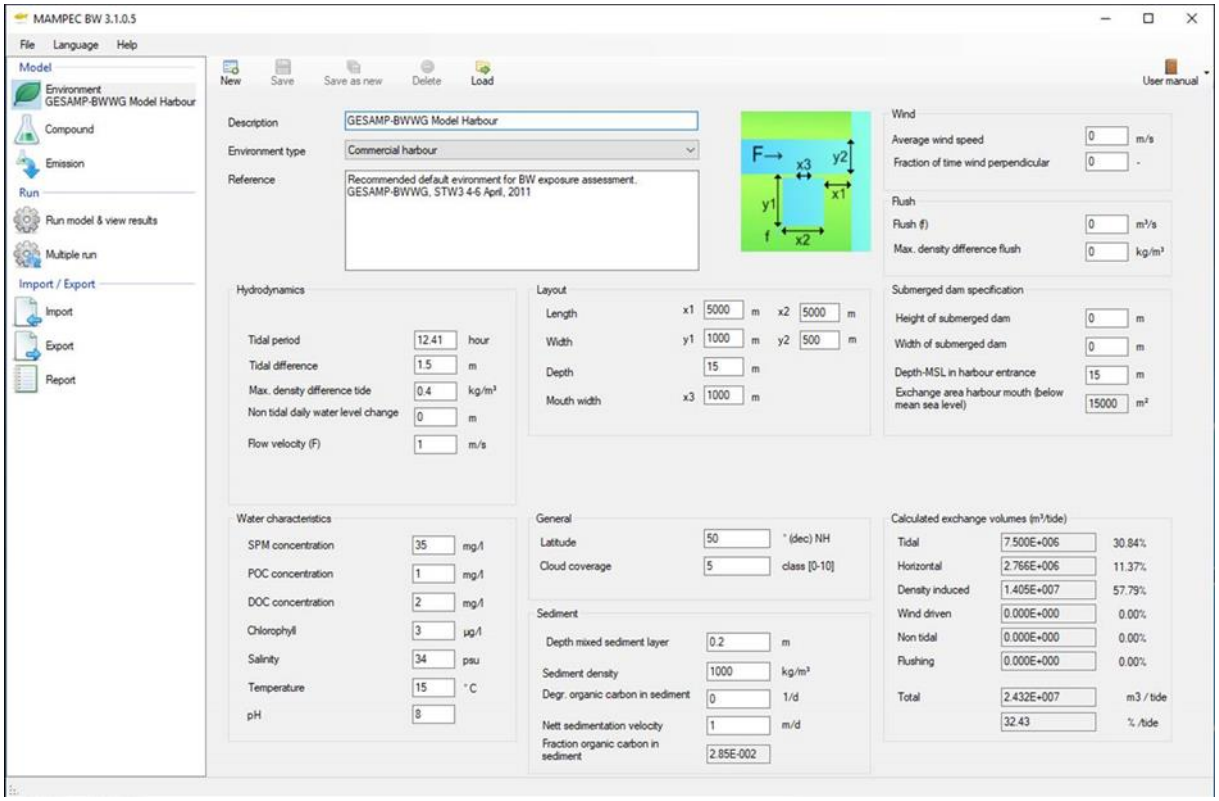
Substance (µg/L)	Ship 1		Ship 2		Ship 3		Ship 4		Composite sample used for testing at DHI [1)]	
	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge	Inlet	Discharge
Dibenz(ah)anthracene	<0.0010	<0.0010	<0.0010	0.015	<0.0010	0.0026	<0.0010	0.0054	<0.0010	0.0027
Benz(ghi)perylene	<0.0010	<0.0010	<0.0010	0.068	<0.0010	0.0089	0.0024	0.020	<0.0010	0.013
Sum of PAH'es, 16 EPA substances	0.0034	12	ND	12	0.03	22	0.019	7.9	0.0062	2.2

1) The composite sample was prepared by mixing large volumes of water sampled for Whole effluent toxicity and has therefore been handled several times. The samples from each ship were added directly to the sampling bottles and sent for analyses. Hence, the concentrations of volatile substances in the mixed sample are expected to be lower.

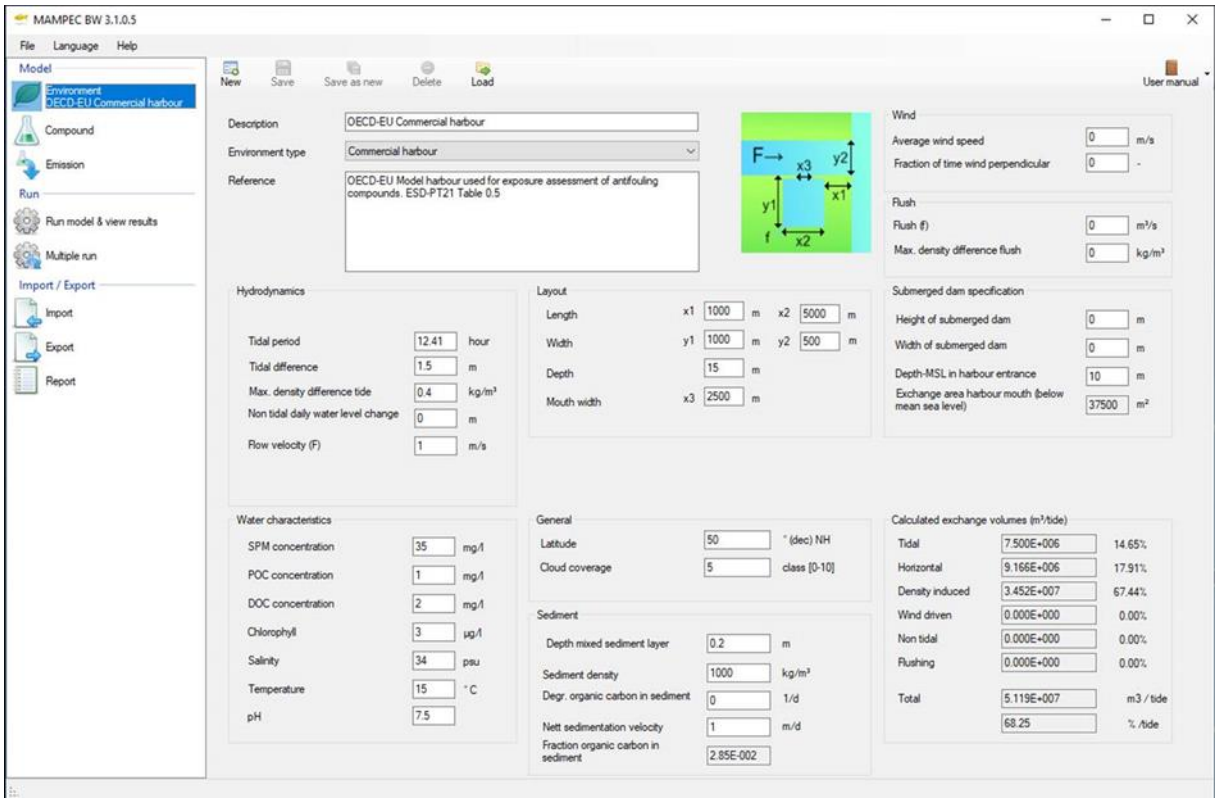
APPENDIX C – Environmental model scenarios in MAMPEC

C MAMPEC input

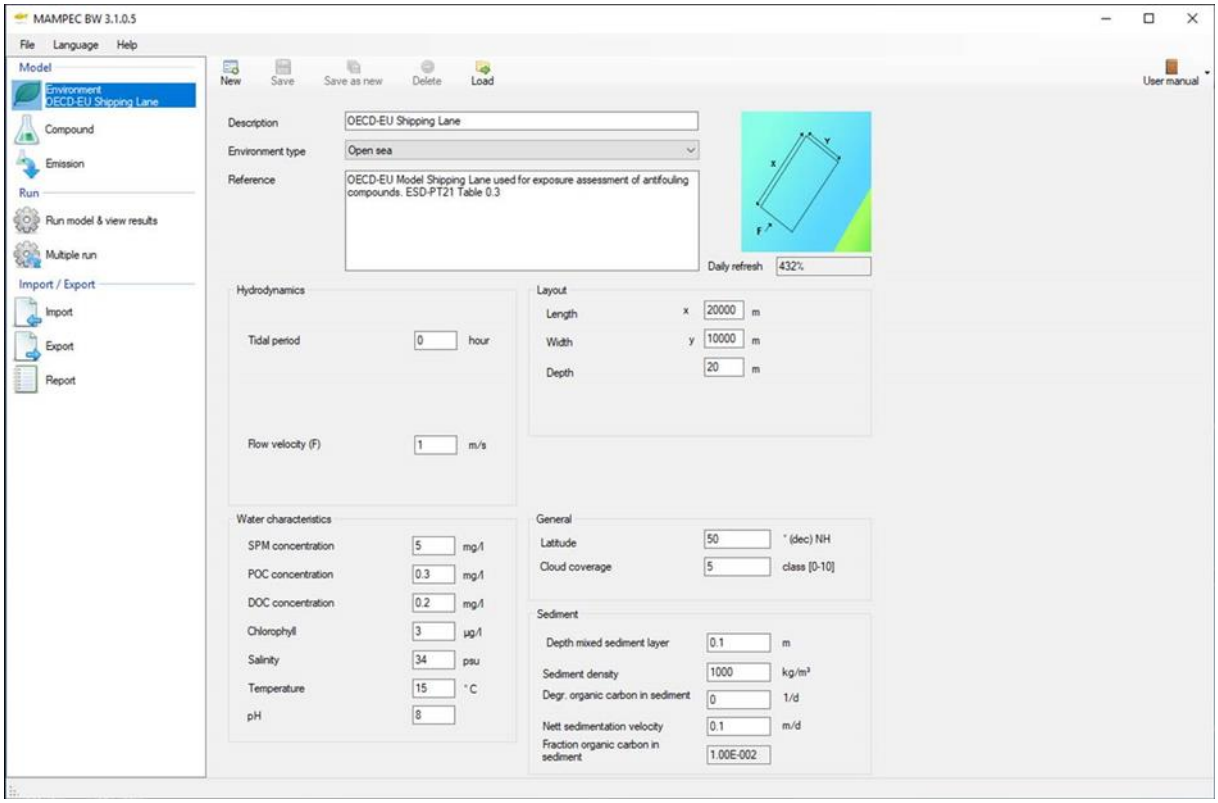
C.1 GESAMP-BWWG Model Harbour



C.2 OECD-EU Commercial Harbour



C.3 OECD-EU Shipping Lane



C.4 Inert substance