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Norway Five Toxicity Tests

The Norwegian Institute for Water Research, performed 5 toxicity tests on OSE II, for the Norwegian EPA to understand, the non-toxic characteristics associated with the use of OSE II.

The OSEI Corporation distributor Premium Green Technologies of Norway, had 5 toxicity tests performed in order to show the Norwegian government that OSE II was safe, and non toxic to the particular species they require toxicity testing for Algae Skeletonema Pseudocstatum.

The 72 hour EC50 toxicity test showed n.d. or non detect, the EC50 95/%-CL lower test showed n.d., or non detect, the EC50 95/%-CL upper showed an n.d., or non detect, the LOEC was >100, or greater than 100, and the NOEC was _>100, or greater than or equal to 100.

These test are extremely conclusive in proving OSE II is much less toxic than the required limit of greater than >10, the tests stopped at >100 the upper testing limit for these toxicity tests. These tests are great toxicity tests, once again showing that OSE II is safe and non toxic, and in particular for use, in Norwegian waters.

The toxicity test performed was the Determination of the 72 hour toxicity of OSE II to the marine algae Skeletonema pseudocostatum. The results showed that OSE II has no detection (ND) for the three EC 50 tests showing no toxicity, while the LOEC was greater than 100 ul/l and the NOEC showed great results as well.

The full report follows this page.

Steven Pedigo

CEO/Chairman OSEI Corporation

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TEST REPORT

Determination of the 72 hour toxicity of OSE II to the marine algae *Skeletonema pseudocostatum*

NIVA Report number: 190167

April 2020

PREFACE

SPONSOR

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TESTING FACILITY

Norwegian Institute for Water Research (NIVA)
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PERSONNEL INVOLVED IN THE STUDY

Name and Company	Title	Function
Ana Almeida, NIVA, Gaustadalléen 21, 0349 Oslo, Norway	Research scientist	Study Director and Test personnel
Tânia Gomes, NIVA	Research scientist	Test personnel
Adam Lillicrap, NIVA	Research manager	Test Facility Manager

TIME SCHEDULE

Study initiation date: 25th February 2020
Start of test: 25th February 2020
Completion of test: 28th February 2020

REPORT APPROVED BY GLP MANAGER

GLP Manager: _____ Date: 24.04.2020

Adam Lillicrap
Research Manager
Norwegian Institute for Water Research

SUMMARY

The inhibitory effects of OSE II on the growth of the marine microalgae *Skeletonema pseudocostatum*, strain NIVA BAC 1, was investigated. The test was performed according to ISO 10253:2016, Water quality – Marine algal growth inhibition test with *Skeletonema* sp. and *Phaeodactylum tricornutum*. ISO/TC 147/SC 5 Biological methods, ICS:13.060.70, 19p (1).

A series of test solutions were prepared by dissolving different concentrations of the test substance OSE II in ISO media 10253 (1 µL/L, 3.2 µL/L, 10 µL/L, 32 µL/L and 100 µL/L), plus a control.

The test solutions were inoculated with approximately 5×10^3 cells/mL of an exponentially growing culture of *Skeletonema pseudocostatum*. Three replicates of each concentration were incubated in 25 mL glass flasks with 15 mL test volume in an incubator with orbital shaking, set to 20± 2 °C and under continuous light. Six replicate cultures in growth medium were used as controls. Growth was monitored using a coulter counter at 24, 48 and 72 hours.

The test substance showed no effects in *Skeletonema pseudocostatum* growth. The results of the study are summarised as follows:

Endpoint	Test substance OSE II (µL/L)
	72 h
EC_{50}	n.d.
EC_{50} 95%-CL lower	n.d.
EC_{50} 95%-CL upper	n.d.
LOEC	>100
NOEC	≥100

Where:

EC_{50} – effective concentration for 50% reduction
95% – CL 95 – confidence limits
LOEC – lowest observed effect concentration
NOEC – no observed effect concentration
n.d. - not determined