Toxicity of 3 water samples tested with the Bacteria luminescence inhibition test using *Vibrio fischeri* (Microtox)

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Wageningen University &
Research report C082/19

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Test report

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Wageningen Marine Research
Den Helder, August 2019

CONFIDENTIAL NO

Wageningen Marine Research report C082/19

WAGENINGEN
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Keywords: run-off, ecotoxicity, *Vibrio fischeri*, acute,

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This report can be downloaded for free from https://doi.org/10.18174/499253
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1 Introduction

A risk assessment concerning the potential environmental risk of highway run-off water is performed by WMR, under the assignment of TNO. To support the risk assessment, the potential toxicity of three samplesfrom run-off and surface water from different highways in Europe, is tested by WMR. Fresh water WET-tests are performed for species from multiple tropic levels, namely bacteria (*Vibrio fischeri*), algae (*Raphidocelis subcapitata*) and crustacean (*Daphnia magna*).

This report describes the results of the luminescence inhibition tests with the bacteria *Vibrio fischeri*.

Reported are the 30 minutes EC_{50} and NOEC as indication for acute effects

2 Materials and Methods

2.1 Abbreviations

EC_{xx}	Calculated concentration causing xx% (growth inhibition) effect
NOEC	No Observed effect concentration; i.e. the highest test concentration without significant negative effects

2.2 Test material

All 3 samples were tested in duplicate for more reliable results.

Sample 1a	52019067-015 (E18) replicate 1
Sample 1b	52019067-015 (E18) replicate 2
Sample 2a	52019067-001 (A61) replicate 1
Sample 2b	52019067-001 (A61) replicate 2
Sample 3a	52019067-007 (A2) replicate 1
Sample 3b	52019067-007 (A2) replicate 2

Sample transport: The samples were delivered at WMR's laboratory in Den Helder on August 6th and 15th, 2019. At arrival the samples were stored at 7°C and gradually brought back to room temperature before used in the test.

2.3 Test method

The test was carried out according to the procedure detailed in SOP E_4_183 'Microtox Basic test met *Vibrio fischeri*', which is summarised below. The test procedure is based upon ISO 11348-3 'Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) - Part 3: Method using freeze dried bacteria'.

Species	*Vibrio fischeri*
Batch	19A4002

Test vessels	Glass Microtox cuvettes
Dilution water	Diluent (2% NaCl dilution)
pH test water	6.0-8.5
Oxygen test water	>40%
Salinity	>10.0‰
Water treatment	Prior to the test, the test water was homogenised 24 hours before the test to enable particles to settle out before using the water in the test.
Test concentrations	0% (blank), 5.625%, 11.25%, 22.50%, 45.00%
Replicates	2
Temperature	15±0.5°C
Test duration	30 minutes
Endpoint	Luminescence inhibition
Observations	Luminescence (measured at 5, 15 and 30 minutes)
Water quality	In the test water pH, oxygen and salinity is measured and, if necessary, adjusted to the right values

Quality parameters

Reference toxicant	Several times per year, the reference toxicant phenol is tested. The results are added to a control chart.
Calculation of results	The EC_{XX} are calculated using a 'sigmoidal dose-response curve' with variable slope and is based on inhibition of luminescence in the test concentrations relative to the 0% blank. The calculated effect concentrations are not corrected for dilution by algal stock. The NOEC is derived from the data noting that the effect at the NOEC should not exceed 10% (ECB, 2003).

3 Results

3.1 Water quality

Table 1 shows that the pH and oxygen level of all 3 samples comply with the requirements of the test protocol. The salinity was adjusted to >10.00‰ using OAS (Osmotic Adjustment Salt), to avoid osmotic effects.

Table 1 pH of control (C0) and 100% (C5) at start and end of the test

Sample	pH	Oxygen (%)	Salinity (‰)
Sample 1	7.43	102.8	0.04
Sample 2	6.90	87.3	0.22
Sample 3	7.32	59.8	0.44

3.2 Luminescence inhibition

Table 2 to Table 7 show the luminescence intensity of the *Vibrio fischeri* in the test for all measurement times including the calculated inhibition. These tables show that for sample 1 and 2 there is a slight dose related inhibition, but the effects are minimal <12% effect. Sample 3 does not show luminescence inhibition.

Table 2 Luminescence intensity for sample 1a and % inhibition after 30 minutes

Concentration/minutes	0	5	15	30	Inhibition %
0.00	93.96	84.20	75.60	70.84	0
5.625	84.47	73.86	67.71	62.55	1.78
11.25	83.39	73.50	66.97	61.56	2.09
22.50	79.46	70.65	63.86	58.24	2.78
45.00	82.92	71.29	64.87	59.05	5.46

Table 3 Luminescence intensity for sample 1b and % inhibition after 30 minutes

Concentration/minutes	0	5	15	30	Inhibition %
0.00	93.99	84.30	76.76	71.57	0
5.625	82.34	72.73	66.63	61.26	2.30
11.25	81.27	74.48	67.52	60.95	1.51
22.50	82.91	72.29	67.21	59.16	6.29
45.00	82.00	70.28	63.33	57.04	8.65

Table 4 Luminescence intensity for sample 2a and % inhibition after 30 minutes

Concentration/minutes	0	5	15	30	Inhibition %
0.00	95.91	93.80	91.11	89.30	0
5.625	86.90	85.23	82.59	79.72	1.53
11.25	84.09	86.23	84.00	81.40	-3.97
22.50	81.95	83.25	81.24	78.80	-3.27
45.00	82.23	81.01	76.34	72.13	5.79

Table 5 Luminescence intensity for sample 2b and % inhibition after 30 minutes

Concentration/minutes	0	5	15	30	Inhibition %
0.00	92.83	94.26	91.53	89.80	0
5.625	88.05	88.81	87.70	83.47	2.00
11.25	82.94	86.69	82.68	80.33	-0.12
22.50	83.49	83.44	80.92	78.61	2.68
45.00	84.58	81.32	77.63	72.35	11.57

Table 6 Luminescence intensity for sample 3a and % inhibition after 30 minutes

Concentration/minutes	0	5	15	30	Inhibition %
0.00	89.77	93.51	92.42	92.74	0
5.625	90.59	90.81	89.74	89.31	4.57
11.25	86.84	92.92	91.45	91.25	-1.71
22.50	81.97	91.74	91.94	90.14	-6.45
45.00	68.22	82.86	81.82	79.58	-12.92

Table 7 Luminescence intensity for sample 3b and % inhibition after 30 minutes

Concentration/minutes	0	5	15	30	Inhibition %
0.00	104.56	111.86	117.72	117.89	0
5.625	95.21	110.36	113.17	111.88	-4.22
11.25	102.08	110.28	112.33	112.31	2.42
22.50	91.71	110.31	112.56	114.84	-11.06
45.00	98.51	116.66	118.97	122.74	-10.51

4 Conclusion

Table 8 shows the endpoints of the acute luminescence inhibition test with the bacteria *Vibrio fischeri*. No significant inhibition was observed in any of the 3 samples. However, a slight inhibition was noted in sample 1 and 2 at the highest concentration tested. Due to the need to suspend the bacteria in culture medium, 45% was the highest concentration that could be tested. Effects at higher sample concentrations can, therefore, not be assessed using this procedure. Sample 3 did not show any inhibition at any of the concentrations tested.

Table 8 Endpoints for all 3 samples. n.a.: not applicable.

30 minutes	EC_{50}	Effect	NOEC
Sample 1	>45%	7.06% effect at highest concentration	n.a.
Sample 2	>45%	8.68% effect at highest concentration	n.a.
Sample 3	>45%	n.a.	n.a.

5 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. This certificate is valid until 15 December 2021. The organisation has been certified since 27 February 2001. The certification was issued by DNV GL.

Justification

Report C082/19
Project Number: 431.51001.27

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

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