



Toxicity of 3 water samples tested with the Acute fresh crustacean test using *Daphnia magna*

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Test report

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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 samples from run-off and surface water from different highways in Europe, is tested by WMR. Fresh water WET-tests are performed for species from multiple trophic levels, namely bacteria (*Vibrio fischeri*), algae (*Raphidocelis subcapitata*) and crustacean (*Daphnia magna*).

This report describes the results of the acute immobilization tests with the freshwater crustacean *Daphnia magna*.

Reported are the 24hour and 48hour EC₂₅ and EC₅₀, NOEC and toxic unit.

2 Materials and Methods

2.1 Abbreviations

SFW	Standard freshwater, following ISO guidelines
EC _{xx}	Calculated concentration causing XX% (immobilization) effect
Toxic unit (TU _c)	Chronic Toxicity Units (100/EC ₂₅)
NOEC	No Observed Effect Concentration; i.e. the highest test concentration without significant negative effects

2.2 Test material

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

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 being used in the test.

2.3 Test method

The test was carried out according to the procedure detailed in SOP E_4_054 '*Daphnia magna* immobiliteitstest' which is summarised below. The test procedure is based upon the standard acute test 'Daphtoxkit F' by MicroBioTests™ and ISO 6341:2012 'Determination of the inhibition of the immobility of *Daphnia magna* Straus (Cladocera, Crustacea) - Acute toxicity test'.

Species	<i>Daphnia magna</i>
Stage	Neonates
Batch	DM131218, MicroBioTests
Hatching time	72-80 hours
Test vessels	24 well-multiwell plates
Dilution water	Standard freshwater (SFW ISO medium)
pH dilution and test water	6-9
CaCO ₃ dilution water	140-275 mg/l CaCO ₃

Water treatment	Prior to the test, the dilution water was aerated to ensure dissolved oxygen was >90%. The samples were homogenised 24 hours before the test to enable particles to settle out before using the water in the test.
Test concentrations	0% (blank), 31.6%, 42.2%, 56.2%, 75.0% and 100%
Organisms per well	5 neonates
Test volume	10 ml
Replicates	4
Temperature	20±2 °C
Photoperiod	dark
Test duration	48 hours
Endpoint	Immobilization
Observations	Number of (not)immobilized individuals after 24 and 48 hours

Quality parameters

Control wells	<10% immobilization
Reference toxicant	Several times per year, the reference K ₂ Cr ₂ O ₇ is tested. The results are added to a control chart.

Calculation of results

The EC_{xx} and its confidence intervals were calculated by using a "sigmodal dose response curve with variable slope (ISO, 2006) built into the software program GraphPad Prism (version 7.05, Aug 2018). This is based on a maximum effect of 100% and a minimum effect between 0 and 10% (blank condition).

The NOEC is derived from the data noting that the effect at the NOEC should not exceed 10% when used in risk assessment (ECB, 2003).

The TU_c is derived from the data by calculating 100/EC₂₅.

3 Results

3.1 Quality parameters

Table 1 shows that the mean immobilization of the control wells complies with the requirements of the test protocol, being <10%.

Table 1 Mean immobilization (% of total organisms) in control wells for sample 1 and 2 after 24 and 48 hours

Sample	Sample 1	Sample 2	Sample 3
Mean immobilization (24h)	0	0	0
Mean immobilization (48h)	5	5	0

To check if there is any pH and/or oxygen effect present during the test the pH and oxygen (%) are measured at the start of the test and after 48 hours. Table 2 shows that the pH has an average change of 0.43 units comparing the starting pH with the end pH and is still within the range of 6-9. Table 2 also shows that the oxygen level has an average change of 7.4% comparing the start and the end percentages and is still >60%. This indicates that, if there is any effect present, this is not caused by pH or oxygen level changes.

Table 2 pH and oxygen (%) of control (C0) and 100% (C5) at start and end of the test

Sample	pH start	pH end	Oxygen (%) start	Oxygen (%) end
Sample 1 (C0)	8.06	7.71	95.4	104.7
Sample 1 (C5)	7.75	8.19	112.1	101.9
Sample 2 (C0)	8.06	7.61	95.4	101.3
Sample 2 (C5)	7.14	7.92	106.5	102.2
Sample 3 (C0)	8.06	7.65	95.4	103.6
Sample 3 (C5)	7.55	7.69	107.0	100.7

3.2 Immobilization

Table 3, Table 4 and Table 5 show the mean number of alive/ not-immobilized neonates and the % of immobilization for the 24 and 48 hours measurements. These tables show that there is no doses-response effect present for any of the 3 samples.

Table 3 Mean not-immobilized neonates and % immobilization for Sample 1

Concentration (%)	Mean number (24h)	% immobilization (24h)	Mean number (48h)	% immobilization (48h)
0.00	20	0.0	19	5.0
31.6	20	0.0	19	5.0
42.2	20	0.0	20	0.0
56.2	20	0.0	20	0.0
75.0	20	0.0	20	0.0
100	19	5.0	18	10.0

Table 4 Mean not-immobilized neonates and % immobilization for Sample 2

Concentration (%)	Mean number (24h)	% immobilization (24h)	Mean number (48h)	% immobilization (48h)
0.00	20	0.0	19	5.0
31.6	20	0.0	20	0.0
42.2	20	0.0	20	0.0
56.2	18	10.0	18	10.0
75.0	20	0.0	20	0.0
100	20	0.0	20	0.0

Table 5 Mean not-immobilized neonates and % immobilization for Sample 3

Concentration (%)	Mean number (24h)	% immobilization (24h)	Mean number (48h)	% immobilization (48h)
0.00	20	0.0	20	0.0
31.6	19	5.0	19	5.0
42.2	20	0.0	20	0.0
56.2	20	0.0	20	0.0
75.0	20	0.0	20	0.0
100	20	0.0	18	10.0

4 Conclusion

Table 6 and Table 7 show the endpoints of the acute immobilization test with the freshwater crustacean *Daphnia magna*. In none of the three samples effects of the samples were observed. The highest effect seen was 10%, which was within the normal range of variation for this test. The EC₂₅- and EC₅₀-values, therefore, are >100% and the TUC-values <1, indicating absence of significant toxicity to the freshwater crustacean *Daphnia magna*.

Table 6 endpoints for all 3 samples for 24h data. n.a.: not applicable.

24h	EC ₅₀	Effects	NOEC	EC ₂₅	TU _c
Sample 1	>100%	5% effect in highest concentration	n.a.	>100%	<1
Sample 2	>100%	0% effect in highest concentration	n.a.	>100%	<1
Sample 3	>100%	0% effect in highest concentration	n.a.	>100%	<1

Table 7 endpoints for all 3 samples for 48h data. n.a.: not applicable.

48h	EC ₅₀	Effects	NOEC	EC ₂₅	TU _c
Sample 1	>100%	10% effect in highest concentration	n.a.	>100%	<1
Sample 2	>100%	0% effect in highest concentration	n.a.	>100%	<1
Sample 3	>100%	10% effect in highest concentration	n.a.	>100%	<1

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

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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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