

Test report

Stormøllen A/S

Daphnia magna, acute toxicity test

February 2004

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Sample material

On a request from the client, the laboratory bought 25 kg of "Stalosan F" at a local pet shop on 21 May 2003. Stalosan F is used as a stable bedding material.

The sample was stored dark at room temperature until test start.

The test was carried out during the period 17 - 21 June 2003.

Purpose

The purpose of the test is to detect a possible acute toxicity effect of Stalosan F on the freshwater crustacean *Daphnia magna*.

Test methods

Stalosan F: Physical/chemical properties

Description:	Powder, light brown
pH:	3.6 (according to MSDS)
Batch:	Not given
Expiry date:	Not given
Storage:	In original container in dimmed light at room temperature
Solubility:	Slightly soluble in water at 25°C
Stability of test concentrations:	The stability was not verified by chemical analysis

Test performance

Source and culturing of *Daphnia magna*

Daphnia magna neonates at an age of < 24 hours were used for the test. The parent animals were cultured in aged lake water from the mesotrophic lake "Hald Sø" at 20°C ± 2°C and a light/dark cycle of 16/8 hour with a light intensity < 50 µmol m⁻² s⁻¹. The parent animals were fed with *Chlorella vulgaris*.

Preparation of the test material

The test concentrations were all prepared directly by adding the test product to the medium. The mixtures were stirred but not filtrated though not all of the test products were soluble.

Test procedure

The study was carried out as a 48-hour static test. To each 20 ml test container (glass) were added 10 ml of the medium or a mixture of the medium and test material. To each test container were added 5 neonates. 6 controls and 4 replicates of each test concentration were used for this study, resulting in a total of 30 individuals of the control and 20 individuals of each test concentration. To avoid evaporation, the test containers were covered with Parafilm®.

The test containers were placed in a temperature-controlled cabinet at a temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and a light/dark cycle of 16/8 hours ($< 50 \mu\text{mol m}^{-2} \text{s}^{-1}$). The animals were not fed during the test.

After 24 and 48 hours the immobilization in each test container was registered. After 48 hours, the pH and O_2 were measured.

Limit test

The test was performed as a limit test since the pretest did not give any indication of toxicity. The test concentrations chosen for the test were 100 mg/l and 1000 mg/l.

Calculations

The percent of immobile animals was calculated for $t = 24$ hours and $t = 48$ hours. Since the test was performed as a limit test, no effect concentrations can be calculated.

Results

The results of the acute test after 24 hours of exposure are given in Table 1 and after 48 hours in Table 2. No animals were immobile in the control group. The raw data are given in Appendix 2.

Table 1. *The number and percent of immobile animals after 24 hours of exposure.*

Test concentration (mg/l)	Number of test animals	Number of immobile after 24 hours	Percent of immobile after 24 hours
0	31	0	0
100	19	0	0
1000	19	0	0

Table 2. *The number and percent of immobile animals after 48 hours of exposure.*

Test concentration (mg/l)	Number of test animals	Number of immobile after 48 hours	Percent of immobile after 48 hours
0	31	0	0
100	19	0	0
1000	19	1	5

The pH and oxygen contents in the test containers after 48 hours of exposure are listed in Table 3.

Table 3. *The pH and O₂ in the test containers after 48 hours of exposure.*

Test concentration (mg/l)	pH after 48 hours of exposure	O ₂ after 48 hours of exposure (%)
0	8.0	> 90
100	7.9	> 90
1000	8.0	> 90

Conclusion

The acute test with the *Daphnia magna* was performed with two concentrations of Stalosan F. The test was performed as a limit test and effect concentrations can therefore not be determined using statistical calculation. However, the effect concentrations can be assessed to be the following:

EC20-24h > 1000 mg/l

EC50-24h > 1000 mg/l

EC20-48h > 1000 mg/l

EC50-48h > 1000 mg/l

Appendix – Raw data

Data for acute test											
Testorganism:		<i>Daphnia magna</i>				Project/study no.:					
Technician:		GS				Reg.no.:		207285-71-184			
Method :						Study director:		JP			
Preliminary:						Testsubstance :		Stalosan F			
Definitive :		x				Static :		x			
Date of teststart :		2003.06.18.				Temperature:		20			
Testduration :		48h				Salinity:		0			
		pH				O2-content (% saturation)					
		Start		End		Start		End			
Control						8.0		>90			
Highest conc. value						8.0		>90			
Concentration (mg/l)		0		0.1		1.0					
Total number of animals per conc.		31		19		19					
Animals per testvessel	A B	5	5	5	5	4	5				
	C D	6	5	5	4	5	5				
	E F	5	5								
Date & initials		Number of dead animals, 1. day									
2003.06.19 GS	A B	0	0	0	0	0	0				
	C D	0	0	0	0	0	0				
	E F	0	0								
	Total	0	0	0							
Date & initials		Number of dead animals, 2. day									
2003.06.20 GS	A B	0	0	0	0	0	0				
	C D	0	0	0	0	1	0				
	E F	0	0								
	Total	0	0	1							
Date & initials		Number of dead animals, 3. day									
	A B										
	C D										
	E F										
	Total										
Date & initials		Number of dead animals, 4. day									
	A B										
	C D										
	E F										
	Total										
Comments											
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The test results relate only to the items tested.

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