Test report

Stormøllen A/S

Fish (*Poecilia reticulata*), acute toxicity test

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Sample material

On request by 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 9 - 25 July 2003.

Purpose

The purpose of the test was to detect a possible acute toxicity effect of Stalosan F on fish. As test organism was used *Poecilia reticulata* (guppy).

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

Test performance

Principle of the test

A pretest was performed in order to determine the concentration range for toxicity. However, the pretest indicated no toxicity. Therefore the final test was performed as a limit test using only two test concentrations.

Observations of the test systems were made at regular intervals during the test period of 96 hours in order to assess the effect concentrations. The test was made as a static test according to OECD Guideline 203 /1/.

Validity of the test

To fulfill the validity criteria of the test the following should be satisfied:

⇒ The mortality in the control(s) should not exceed 10% at the end of the test
⇒ The dissolved oxygen concentration must not be less than 60% throughout the test

Test system

The experiment was conducted under standard laboratory conditions in a temperature controlled room with continuous dimmed light and a temperature of 20 ± 2°C. The test was made as a static test with Guppy as test organism. Nine fish were used per treatment and a control group, giving a total of 27 fish. As water was used reconstituted water according to OECD 203.

Prior to the test the fish were acclimatized in the laboratory for 12 days followed by a 48 hours settling-in period. Two fish died during the settling-in period corresponding to 0.5% of the population leading to an acceptance of the batch. The fish were fed during the acclimatization period until 24 hours before test start and not fed during the test.

Test procedures

Study design

30 litre aquariums (all glass) with 20 litres of reconstituted water were used. Each test concentration of Stalosan F was dissolved as much as possible before adding it to the vessels. The test substance did not dissolve completely in the aquariums; part of the test substance was deposited at the bottom and part of it was stirred up by the aeration. The test medium had a pH of 7.8.

The test concentrations were nominal, meaning they were not verified by chemical analysis.

Exposure

Groups of 9 fish each were exposed to two test concentrations of Stalosan F:

0 mg/l (control); 100 mg/l and 1000 mg/l

The vessels containing the fish were aerated with atmospheric air. The fish were not fed during the 96-hour exposure period.

Observations

Mortality and clinical signs were recorded after 24, 48, 72 and 96 hours. The average weight and length of the fish after the termination of the test were determined to 0.58 g (load of 0.26 g/l of test medium) and 3.8 cm, respectively. The dissolved oxygen and pH were measured daily.

Calculations

The percent of dead fish was calculated for t = 24 hours, t = 48 hours, t = 72 hours and t = 96 hours.

Since the test was performed as a limit test, no effect concentrations can be calculated.

Results

pH, oxygen concentrations and temperature

The pH for all concentrations ranged from 6.8 to 8.3 during the whole study period. The oxygen concentrations were between 92 and 100% of the air saturation value.

The results are presented in Tables 1 and 2.

Table 1. *The pH in the test containers after 24, 48, 72 and 96 hours of exposure.*

Test concentration	pH				
(mg/l)	0 hours	24 hours	48 hours	72 hours	96 hours
0	7.8	7.8	7.8	8.1	8.3
100	7.8	7.8	7.7	7.6	7.9
1000	7.8	7.4	6.8	6.8	6.5

Table 2. *The O_2 in the test containers after 24, 48, 72 and 96 hours of exposure.*

Test concentration	O_2				
(mg/l)	0 hours	24 hours	48 hours	72 hours	96 hours
0	98	98	96	98	100
100	98	98	93	92	100
1000	98	98	98	95	100

Observations

Mortality

The mortality data are presented in Table 3.

Table 3. *The number and percent of dead fish after 24, 48, 72 and 96 hours of exposure.*

Test concentration	24 hours		48 hours		72 hours		96 hours	
(mg/l)	Number	%	Number	%	Number	%	Number	%
0	0	0	0	0	0	0	2	22
100	0	0	1	11	0	0	0	0
1000	0	0	1	11	0	0	1*	11

*: At the end of the test a dead fish was observed at the bottom of the aquarium. This was not observed earlier due to the opacity of the test solution.

Table 4. *The cumulative percent of dead fish after 24, 48, 72 and 96 hours of exposure.*

Test concentration	24 hours		48 hours		72 hours		96 hours	
(mg/l)	Number	%	Number	%	Number	%	Number	%
0	0	0	0	0	0	0	2	22
100	0	0	1	11	1	11	1	11
1000	0	0	1	11	1	11	2	22

Clinical observations

No clinical signs were observed in the control group of fish and in the group of the two test concentrations.

Validity of test

A mortality of 22% was observed in the control group normally leading to a rejection of the test. However the test is accepted, since no mortality was observed above 22% in the test concentrations and LC20-96h was assessed accordingly.

Conclusion

The acute test with the fish (*Poecilia reticulata*) 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:

LC20-96h	≈	1000 mg/l
LC50-96h	>	1000 mg/l

The assessment of LC20 is based on the 22% mortality in the highest test concentration of 1000 mg/l (1 g/l). However, since a mortality of 22% was also seen in the control group, the assessment of LC20-96h ≈ 1000 mg/l must be considered a conservative judgment of the data.

The test was accepted despite the mortality of the control group due to this conservative judgment of the data.