

Section A7.4.1.1**Acute toxicity to fish****Annex Point IIA7.1***96-h LC₅₀, *Salmo gairdneri**

3.4.3	Test system	For details see table A7_4_1_1-4
3.4.4	Test conditions	For details see table A7_4_1_1-5
3.4.5	Duration of the test	96 h
3.4.6	Test parameter	Mortality
3.4.7	Sampling	All concentrations were observed once every 24 hours for mortality and abnormal effects such as surfacing, loss of equilibrium and dark discoloration.
3.4.8	Monitoring of TS concentration	No
3.4.9	Statistics	A computerized LC ₅₀ program developed by Stephan et al. (1975) was used to calculate the LC ₅₀ and its 95% confidence limits using the binomial, the moving average and the probit tests. The method which gave the narrowest confidence limits for the LC ₅₀ was reported in the report.

4 RESULTS

4.1	Limit Test	A preliminary test was performed.
4.1.1	Concentration	1.0, 10, and 100 mg/L
4.1.2	Number/ percentage of animals showing adverse effects	Not reported, based on the results, a logarithmic series from 32 to 320 mg/L was chosen for the actual test.
4.1.3	Nature of adverse effects	Not reported
4.2	Results test substance	
4.2.1	Initial concentrations of test substance	Nominal test concentrations were: 0 (control), 32, 56, 100, 180, and 320 mg/L
4.2.2	Actual concentrations of test substance	Not measured
4.2.3	Effect data (Mortality)	For details see table A7_4_1_1-6 and table A7_4_1_1-7.
4.2.4	Concentration / response curve	Not reported
4.2.5	Other effects	Abnormal effects of mortality, surfacing and/or loss of equilibrium was observed.
4.3	Results of controls	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects observed in control animals

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4.3.2	Nature of adverse effects	Not applicable	
4.4	Test with reference substance	Challenge with Antimycin A	
4.4.1	Concentrations	1.4×10 ⁻⁵ , 2.4×10 ⁻⁵ , 4.2×10 ⁻⁵ , 7.5×10 ⁻⁵ , and 14×10 ⁻⁵ mg/L	
4.4.2	Results	96 h LC ₅₀ was 4.8 ×10 ⁻⁵ mg/L (95% c.i. 2.4×10 ⁻⁵ -7.5×10 ⁻⁵ mg/L), which is within the 95% confidence intervals reported in the literature.	
5 APPLICANT'S SUMMARY AND CONCLUSION			
5.1	Materials and methods	The acute toxicity of SY-83 to rainbow trout (<i>Salmo gairdneri</i>) was assessed by exposing fish to 0, 32, 56, 100, 180, and 320 mg/L (ten fish/concentration). As a reference, fish were exposed to Atimycin A. Mortality and abnormal effects such as surfacing, loss of equilibrium and dark discolorisation was evaluated.	x
5.2	Results and discussion	The 96 h LC ₅₀ for SY-83 is 130 mg/L (100-180 mg/L). The NOEC was 56 mg/L, based on observations of mortality, surfacing and/or loss of equilibrium in concentrations of 100, 180, and 320 mg/L.	
5.2.1	NOEC	56 mg/L	
5.2.2	LC ₀	100 mg/L	
5.2.3	LC ₅₀	130 mg/L	
5.2.4	LC ₁₀₀	Not reported	
5.3	Conclusion	The 96 h LC ₅₀ for SY-83 is 130 mg/L (100-180 mg/L). The pH values were very low in the highest doses (≥100 mg/L), it is more than likely that the low pH value affected the survival of the fishes.	x
5.3.1	Other Conclusions		
5.3.2	Reliability	2	x
5.3.3	Deficiencies	No	x

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE**Date**

2012/05/07

Materials and Methods

Applicant's version is acceptable with the following comments

To point 3.1 and 5.1: Test material: The test substance is not exactly specified.

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Results and discussion	Applicant's version can be adopted with the following remarks: To point 4.2.2: In the study the actual concentration of test substance was not measured. Therefore one important validity criterion could not be estimated. To point 5.3.: As the applicant stated pH values were very low in the highest concentrations (≥ 100 mg/L), so it is more than likely that the low pH value affected the survival of the fishes.
Conclusion	Applicant's version can be adopted.
Reliability	3
Acceptability	Not acceptable, because the pH values were out of the range of 6-8.5 during the test. It could not be excluded, that the toxicity is caused by the lowering of pH value during the test.
Remarks	This study could not be used for the environmental effect assessment because of invalidity. But for supportive information the results are useable.
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Soft reconstituted water
Alkalinity	30-35 mg/L as CaCO ₃
Hardness	40-45 mg/L as CaCO ₃
pH	7.2-7.6 (initial)
Oxygen content	Dissolved oxygen 9.2 mg/L
Conductance	Not reported
Holding water different from dilution water	Not reported

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	<i>Salmo gairdneri</i> (rainbow trout)
Source	Trout Lodge (McMillan, Washington)
Wild caught	No
Age/size	Mean weight: 1.09 (\pm 0.28) g Mean standard length: 42 (\pm 3.4) mm
Kind of food	Standard commercial fish food (Rangen's) until 48 h prior to testing, feeding was discontinued from that point
Amount of food	Not applicable, fishes were not fed during the test
Feeding frequency	Daily up till 48 h before test, fishes were not fed during the test
Pretreatment	Observations for at least 14 days prior to testing
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	No
Volume of test vessels	Five gallon glass vessels containing 15 L water
Volume/animal	150 mL/fish
Number of animals/vessel	10 fish/vessel
Number of vessels/ concentration	1 vessel/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	12 \pm 1°C
Dissolved oxygen	9.2 mg/L
pH	7.2
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	Not reported
Photoperiod	16 h daylight photoperiod

Table A7_4_1_1-6: Mortality data

Test-Substance Concentration (nominal) [mg/l]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	10	10		10	0	0		0
32	10	10		10	0	0		0
56	10	10		10	0	0		0
100	10	10		10	0	0		0
180	2	0		0	80	100		100
320	0	0		0	100	100		100
Temperature [°C]	13	12		12				
pH								
0	7.2	7.0		7.3				
32	7.1	6.9		7.2				
100		4.5		4.6				
320	3.5							
Oxygen [mg/l]	9.2	7.8-8.2		6.1-7.1				

Table A7_4_1_1-7: Effect data

	48 h [mg/l]	95 % c.l.	96 h [mg/l]	95 % c.l.
LC ₀	Not reported		100	Not reported
LC ₅₀			130	100-180
LC ₁₀₀			Not reported	

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fulfilled
Mortality of control animals <10%	x	
Concentration of dissolved oxygen in all test vessels > 60% saturation	x	
Concentration of test substance ≥80% of initial concentration during test		x (not measured)

Criteria for poorly soluble test substances	n.a.	