Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *48-h EC_{50}, Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Hooftman, R.N., Kauffman-van Bommel, J.A., Van Drongelen-Sevenhuijsen, D., 1992. The acute toxicity of L(+) lactic acid to *Daphnia magna* (OECD Guideline no. 202, 48 h). TNO, report nr. IMW-91-0076-01. GLP, Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Purac Biochem	
1.2.2	Companies with letter of access	No	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 202	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch no.: ZO 3456	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	79.5-80.5%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	Enzymatic analysis with a Boehringer Mannheim test kit (cat. no. 1 112 821).	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Ground water (for details, see table A7_4_1_2-2)	
3.4.2	Test organisms	*Daphnia magna* (for details see table A7_4_1_2-3)	
3.4.3	Test system	For details see table A7_4_1_2-4	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *48-h EC_{50}, Daphnia magna*

3.4.4	Test conditions	For details see table A7_4_1_2-5	
3.4.5	Duration of the test	48 h	
3.4.6	Test parameter	Immobility	x
3.4.7	Sampling	Immobile animals were counted after 24 h and at the end of the test (according to OECD 202). At the same time the condition of the animals was visually compared with that of the control animals (swimming behaviour, colour or other visual observable morphological or behavioural criteria).	
3.4.8	Monitoring of TS concentration	Yes	
3.4.9	Statistics	The maximum likelihood estimates of the EC50 values were calculated assuming a log-logistic dose-effect relation, and likelihood-ratio confidence intervals were derived from the confidence intervals according to van der Hoeven (1991, "LC50 estimates and their confidence intervals derived for tests with only one concentration with partial effect, Water Reseaerch, 25, p. 401-408)..	

4 RESULTS

4.1	**Limit Test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**	Non-entry field	
4.2.1	Initial concentrations of test substance	Concentrations of L(+) lactic acid at the start of the test:	

Nominal test substance	Nominal lactic acid	Actual lactic acid	
0	0	<5	mg/L
32	26	15	mg/L
180	144	60	mg/L
560	448	340	mg/L

4.2.2	Actual concentrations of test substance	Concentrations of L(+) lactic acid at the end of the test (after 48 h):	x

Nominal test substance	Nominal lactic acid	Actual lactic acid	
0	0	<5	mg/L
32	26	15	mg/L
180	144	110	mg/L
560	448	350	mg/L

4.2.3	Effect data (Immobilisation)	For details see table A7_4_1_2-6 and A7_4_1_2-7 (please note that the EC50 value was recalculated, see 5.2 Results and discussion)	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *48-h EC_{50}, Daphnia magna*

4.2.4	Concentration / response curve	Not presented	
4.2.5	Other effects	No other effects observed	
4.3	**Results of controls**	Data included in table A7_4_1_2-6 and A7_4_1_2-7	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test performed according to OECD 202.	
5.2	**Results and discussion**	According to the study report, the EC_{50} was calculated by assuming a log-logistic dose-effect relation, and likelihood-ratio confidence intervals were derived from the confidence intervals. However, as the mortality changes from 0 to 100% in two consecutive concentrations, the EC_{50} cannot be calculated with this method. Therefore, the EC50 was recalculated as the mean of the two consecutive concentrations.	
5.2.1	EC_0	180	
5.2.2	EC_{50}	250 mg/L (180-320)	x
5.2.3	EC_{100}	320	
5.3	**Conclusion**	Not all validity criteria were fulfilled, the concentration of the test substance was 65% of the initial concentration. The pH values were very low in the highest doses (3.6-4.1), it is more than likely that the low pH value affected the survival of the fishes.	x
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

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	Applicants version can be adopted with the following remark: 3.4.6: According to the test protocol daphnids were dead in the two highest test concentrations (320 mg/L and 560 mg/L) after 24 h of exposition. Therefore the test parameter is mortality instead of immobility.

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *48-h EC_{50}, Daphnia magna*

Results and discussion	Applicants version can be adopted with the following remarks: 4.2.2. and 5.2.2.: Effect values in the study are related to nominal concentrations although the measured values for three concentration levels show a decrease during the exposure period. Based on the geometric mean of the test substance concentration from start to the end of the test a mean recovery rate of 65 % was calculated by RMS. By applying this mean recovery rate to the nominal effect concentration (240 mg/L) the following effect value was determined: **EC_{50} (48 h) = 156 mg a.s./L** In the original study an EC_{50} value of 240 mg/L instead of 250 mg/L is stated. Therefore this value was used for recalculation. 5.3.: There is a typo. It should be „…survival of daphnia, not fishes".
Conclusion	Applicant's version can be adopted with the following remark: 5.3.: We are in line with the explanation of the applicant that the low pH values more than likely affected the mortality of the daphnids. In this study the pH was not adjusted and it is possible that effects on the animals are based on low pH values in the test solution rather than to the toxicity of the test substance.
Reliability	3
Acceptability	Not acceptable, because the pH values in the highest test concentrations were much lower than the recommended pH values in the guideline; furthermore, the pH values were not stable during the test. Therefore it has to be assumed that toxic effects are more likely linked to the low pH values than to the toxicity of the test substance.
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	*Give date of comments submitted*
Materials and Methods	*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*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Groundwater from a location near Linschoten (the Netherlands)
Alkalinity	Not reported
Hardness	220 mg/L, expressed as $CaCO_3$
pH	8.0-8.2
Ca / Mg ratio	1.86
Na / K ratio	5.95
Oxygen content	Not reported
Conductance	Not reported
Holding water different from dilution water	Not reported

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	Cultured in the laboratorium
Age	Less than 24 h old at the beginning of the test
Breeding method	In the laboratorium under standard conditions, according to the principles of NPR 6503 (ref. 3)
Kind of food	Not applicable, daphnia were not fed during the test
Amount of food	Not applicable, daphnia were not fed during the test
Feeding frequency	Not applicable, daphnia were not fed during the test
Pretreatment	Not reported
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	No renewal of test solution
Volume of test vessels	150 mL all-glass beakers, all containing 100 mL of test solution or control medium
Volume/animal	100 mL/5 animals
Number of animals/vessel	5 animals/vessel
Number of vessels/ concentration	4 vessels/concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	20 ± 1°C
Dissolved oxygen	7.9-9.0 mg/L
pH	3.6-8.2
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	Not reported
Photoperiod	16 h light – 8 h dark regime with transition periods of 30 minutes

Table A7_4_1_2-6: Immobilisation data

Test-Substance Concentration (nominal) [mg/l]	**Immobile *Daphnia***						
	Number		**Percentage**		**Oxygen [mg/l]**	**pH**	**Tempera-ture [°C]**
	24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
0	20	20	0	0	8.3	8.0	
32	20	20	0	0	8.6	8.0	
56	20	20	0	0	8.9	7.9	
100	20	20	0	0	8.9	7.8	
180	20	20	0	0	8.8	7.4	
320	0	0	100	100	8.8 (24 h)	4.1	
560	0	0	100	100	8.1 (23 h)	3.7	

Table A7_4_1_2-7: Effect data

	EC_{50} (nominal)	**95 % c l.**	**EC_0 (nominal)**	**EC_{100} (nominal)**
24 h [mg/l]	250	180-320	180	320
48 h [mg/l]	250	180-320	180	320

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	**Not fullfilled**
Immobilisation of control animals <10%	**x**	
Control animals not staying at the surface	**x**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**x**	
Concentration of test substance ≥80% of initial concentration during test		**x**

Criteria for poorly soluble test substances ~~ergänzen~~	**n.a.**	
