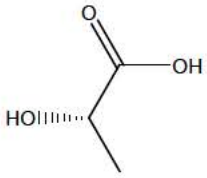
Section A7 Annex Point A7.1.1.1.1	Hydrolysis	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	From the structural formula of lactic acid (Figure 1) it is clear that only one hydrolysable group is present: the acid group. For the hydrolysis of the acid group, the dissociation constant (pK) of 3.8 should be taken into account. As no further hydrolysable groups are available, no further data on hydrolysis is necessary.  (S)-2-hydroxypropanoic acid Figure 1: Structural formula of lactic acid.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009/03/03	
Evaluation of applicant's justification	Applicant's justification is acceptable.	
Conclusion	Applicant's justification can be adopted.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7 Annex Point A7.1.1.1.2	Phototransformation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	In the OECD Guidance Document (No.7, 1997) it is mentioned that no direct photoreaction is possible without absorption of light quanta. Only quanta of UV/visible light are energetic enough to break bonds between atoms in a molecule and only the wavelength range 290-800 nm is relevant for photolysis in the water compartment. As a consequence, chemicals that absorb light significantly only in the UV region below 290 nm, and in the IR region above 800 nm, can not undergo direct photolysis in the water compartment. The UV-spectrum of pure lactic acid shows that it is absorbed in the region of 210 to 250 nm. In a aqueous solution, lactoyllactic acid and dilactide may be present. When lactoyllactic acid is present, a shoulder at 275 nm is seen, and the UV-spectrum of lactic acid containing dilactide shows a shoulder at 270-290 nm. The UV-spectrum of lactic acid shows that no absorbance in the wavelength range of 290-800 nm occurs; therefore no direct phototransformation is expected.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009/03/03	
Evaluation of applicant's justification	Applicant's justification is accepted.	
Conclusion	Applicant's justification can be adopted.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Determination of BOD and COD

			Official use only
		1 REFERENCE	
1.1	**Reference**	Hanstveit, A.O., Pullens, M.A.H.L., 1993 BOD and COD of the product L(+) lactic acid according to EC Test Guidelines C.8 and C.9. TNO, report nr. IMW-91-0076-03. 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, Dutch guidelines NEN 6634 and NEN 6633, similar to EC test guidelines C.8 and C.9	x
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%	x
3.1.4	Further relevant properties	Not applicable	x
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	Not reported	x
3.1.7	Specific chemical analysis	Not performed	
3.2	**Reference substance**	No	x
3.2.1	Initial concentration of reference substance	Not applicable	
3.3	**Test ing procedure**		
3.3.1	Inoculum / test species	Activated sludge from an oxidation ditch. For details, see table A7_1_1_2-2	
3.3.2	Test system	For details see table A7_1_1_2-3	
3.3.3	Test conditions	For details see table A7_1_1_2-4	

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Determination of BOD and COD

3.3.4	Method of preparation of test solution	Not applicable	
3.3.5	Initial TS concentration	Nominal test concentrations 2.0 and 4.0 mg/L, no chemical analysis performed	x
3.3.6	Duration of test	20 days	
3.3.7	Analytical parameter	Biological and theoretical oxygen demand (BOD and ThOD, mentioned as COD in the study report).	
3.3.8	Sampling	O_2 concentrations were measured after 0, 5, and 20 days.	
3.3.9	Intermediates/ degradation products	Not identified	
3.3.10	Nitrate/nitrite measurement	Yes, nitrification control was included by adding 2.5 mg/L allylthiourea to bottles containing 2.0 mg/L lactic acid.	
3.3.11	Controls	BOD: quadruplicate BOD bottles without lactic acid	x
		Toxicity: glucose and glutamic acid were added to control bottles and bottles containing 4 mg/L lactic acid.	
		Nitrification: allylthiourea was added to bottles containing 2 mg/L lactic acid.	
3.3.12	Statistics	The percentage degradation was calculated as (BOD/ThOD)×100	x
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	Not presented	
4.1.2	Degradation	After 5 days: 50%	x
		After 20 days: 67%	
4.1.3	Other observations	BOD values in bottles containing glucose and glutamic acid revealed that lactic acid did not inhibit the activity of the inoculum.	x
		Addition of allylthiourea resulted in some nitrification.	
4.1.4	Degradation of TS in abiotic control	COD (ThOD): theoretical oxygen demand was 0.85 mg O_2	x
4.1.5	Degradation of reference substance	Not applicable	x
4.1.6	Intermediates/ degradation products	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	BOD and COD were determined according to the Dutch Guidelines "Water-determination of biological oxygen demand after n days (BODn)" (NEN 6634) and "Water-determination of chemical oxygen demand after n days (CODn)" NEN 6633, similar to EC test guidelines C.8 and C.9. An activated sludge inoculum was used. A control test	x

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Determination of BOD and COD

		with glucose and glumatic acid as substrate was included in order to assess possible toxic effects of lactic acid on microbiological activity. Furthermore, nitrification control was included by adding allylthiourea.	
5.2	**Results and discussion**	The degradation after 5 days was 50%, after 20 days 67%. No toxic effects were found. Based on these results, lactic acid can be considered readily biodegradable.	x
5.3	**Conclusion**	See pass levels in tables A7_1_1_2-5.	
5.3.1	Reliability	2	
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	2009/10/14
Materials and Methods	Applicant's version is acceptable apart from the following amendments: 2.1: To be correct, it has to be stated that the mentioned Dutch guidelines NEN 6634 and NEN 6633 are similar to EC test guidelines C.5 and C.6 instead of C.8 and C.9. 3.1.3: The purity of the test substance is 79.5-80% (water is the other constituent of the test substance?). All measured values refer to the purity 79.5-80%. 3.1.4: The measured COD of the test substance is 0.902 mg O_2 mg^{-1} and the theoretical oxygen demand was calculated to be 0.85 mg O_2 mg^{-1}. 3.1.6: In a study according to OECD 209 (cf. Doc III A7.4.1.4_01), an EC_{50} >100 mg L^{-1} was observed for the test substance. 3.2: Following the Dutch guideline, reference substances used in this test are glucose and glutamic acid (aniline is used as reference substance according to OECD 301D). 3.2.1.: Initial concentrations of reference substances in the procedure control are 3 mg L^{-1} glucose and 3 mg L^{-1} glutamic acid. 3.3.3: Composition of the medium was comparable to OECD 301D with two exceptions: (a) instead of $Na_2HPO_4.2H_2O$, $Na_2HPO_4.\underline{7H_2O}$ was used and (b) instead of 0.50 g NH_4Cl, <u>1.7 g</u> NH_4Cl was used for the Phosphate buffer solution. 3.3.5: Concentrations refer to the test substance (purity 79.5-80 %.) 3.3.11: Inoculum blank (4 bottles), procedure control (4 bottles), toxicity control (4 bottles, containing 3 mg L^{-1} glucose, 3 mg L^{-1} glutamic acid and 4 mg L^{-1} test substance), nitrification control (4 bottles, containing 2.5 mg L^{-1} allylthiourea and 2 mg L^{-1} test substance). 3.3.12: COD value of test substance was used for the calculation. 5.1: Refer to comment number 2.1 and 3.3.11.

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Determination of BOD and COD

Results and discussion	Applicant's version is acceptable apart from the following amendments: 4.1.2: Degradation after 5 days: 48% (concentration 2 mg L^{-1}), 50 % (concentration 4 mg L-1). Degradation after 20 days: 60% (concentration 2 mg L^{-1}), 67 % (concentration 4 mg L^{-1}). The concentrations are based on test substance (80 % purity). The difference of degradation of the two test concentrations is less than 20 %. 4.1.3: In the toxicity test the degradation was 51 % (day 5) and 75 % (day 20). The test substance can be assumed not to be inhibitory. Nitrification control revealed that nitrification took place in the medium. However, this applies to blanks as well as to test substance batches. Therefore the study result is not affected. Oxygen depletion in the inoculum blank was 1.74 mg dissolved oxygen L^{-1} after 20 days. According to OECD 301D, oxygen depletion should not exceed 1.5 mg O_2 L^{-1} after 28 days. The reason for the higher value in the present study presumably is the higher nitrification since more ammonium is added to the mineral medium compared to the OECD mineral medium. The residual concentration of oxygen in the test bottles did not fall below 0.5 mg L^{-1} at any time (except toxicity control after 20 days). 4.1.4: Refer to comment number 3.1.4. 4.1.5: Degradation of the reference substances: 49% (day 5) and 90% (day 20). Even if degradation of the reference substances were not measured after 14 days, it is expected that the validity criterion of OECD 301D (degradation of reference compound reaches pass level after 14 days) is fulfilled. 5.2: Refer to comment number 4.1.2, 4.1.3, and 4.1.5.
Conclusion	Applicant's version is acceptable apart from the following amendments: 5.3: The Dutch guideline NEN 6634 used in the present study is basically comparable to OECD 301D. Deviations are: (a) more ammonium in mineral medium, (b) only 3 sampling points, (c) duration 20 days, and (d) a different reference substance. Because degradation of test substance was above the pass level after 20 days, the short study duration is not a problem. Due to the deviations, the validity criteria of guideline OECD 301D can only be roughly controlled. Nevertheless, the test is regarded as valid (refer to comment number 4.1.2, 4.1.3, and 4.1.5) and acceptable. Lactic acid is readily biodegradable, however the 10-days window criterion is not fulfilled/cannot be assessed.
Reliability	2
Acceptability	Acceptable
Remarks	

	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*

Section A7.1.1.2.1 Biodegradability (ready)

Annex Point IIA7.6.1.1
Annex Point IIA7.6.1.2

Determination of BOD and COD

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_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	Not specified
Strain	Not specified
Source	Oxidation ditch, used to treat domestic sewage
Sampling site	TNO, Delft, the Netherlands
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The original sludge (containing 3.5-4.0 g of solid substance/L) was allowed to settle for 4-8 minutes. 2 mL of the supernatant was used to inoculate.
Pretreatment	Vigorous aeration
Initial cell concentration	Not reported

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	BOD bottles
Number of culture flasks/concentration	4 bottles/concentration
Aeration device	Not reported
Measuring equipment	Oxygen electrode
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	BOD dilution water was prepared from concentrated stock solutions in Milli-Q water, according to the Dutch Guideline "Water-determination of biochemical oxygen demand after n days (BODn)" (NEN 6634)
Additional substrate	Yes, glucose and glutamic acid were added to check the activity of the inoculum and the possible toxicity of the test substance
Test temperature	20 °C
pH	Start: 7.0-7.1 End: 6.6-6.9 End (bottles with glucose): 6.1-6.3
Aeration of dilution water	Yes, dilution water was aerated vigorously before use
Suspended solids concentration	Not reported
Other relevant citeria	Nitrification control was included by adding 2.5 mg/L allythiourea to bottles containing 2 mg/L lactic acid

Table A7_1_1_2-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	**x**	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		**x**
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	**x**	
Percentage of removal of reference substance reaches pass level by day 14		**x**

5.3.2.1 Criteria for poorly soluble test substances	5.3.2.2	5.3.2.3
5.3.2.4	**5.3.2.5**	**5.3.2.6**
5.3.2.7	**5.3.2.8**	**5.3.2.9**

Section A7 Annex Point A7.1.2	Rate and route of degradation in aquatic systems	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	According to the 'Technical Guidance Document on data requirements', subjects under 7.1.2 are additional data requirements. As lactic acid will be used in products that are to be used in-house only, information on the rate and route of degradation in aquatic systems is not required.	x
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009/10/21	
Evaluation of applicant's justification	Applicant's justification is acceptable. Lactic acid is readily biodegradable (10-days window criterion not fulfilled/cannot be assessed). No further biodegradation studies are required.	
Conclusion	Applicant's justification is acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

Official use only

1 REFERENCE

1.1	**Reference**	Baltussen, E. (2008). Estimation of the adsorption coefficient (K_{OC}) of lactic acid 93% aq on soil and on sewage sludge using high performance liquid chromatography (HPLC). Notox Document 489046 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 121
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

3.1	**Test material**	Lactic acid 93% aqueous solution
3.1.1	Lot/Batch number	0712002519
3.1.2	Specification	As given in section 2
3.1.3	Purity	99.8% (solution was 92.8% lactic acid in water vs. nominal concentration of 93% lactic acid in water).
3.1.4	Further relevant properties	Substance is a strong acid; explicit instructions in the guideline apply.
3.1.5	Method of analysis	Not relevant. OECD is a direct screening test based on chromatography of the active substance; no analysis of samples from an experimental test is needed.
3.2	**Degradation products**	Degradation products tested: Not relevant; in the HPLC screening test no metabolites/degradates are formed. OECD 121 only tests the acsorption/desorption behaviour of the parent compound.
3.2.1	Method of analysis for degradation products	Not applicable
3.3	**Reference substance**	Yes; phenol
3.3.1	Method of analysis for reference substance	Not relevant. OECD is a direct screening test based on chromatography of the active substance; no analysis of samples from an experimental test is needed.
3.4	**Soil types**	Not relevant. OECD is a direct screening test based on chromatography of the active substance; no analysis of samples from an experimental test is needed.
3.5	**Testing procedure**	
3.5.1	Test system	Direct injection of solution of test substance onto HPLC column.
3.5.2	Test solution and Test conditions	Test at neutral pH Standard: Stock solution: 1 g/L solution of phenol in methanol. Test solution: 0.05 mL stock solution in 5 mL mobile phase. Test substance: Stock solution: 1.744 g/L solution of test substance in methanol. Test solution: 0.6 mL stock solution and 1.4 mL water in 5 mL mobile phase; final concentration test solution: 0.209 g/L. Test at pH 2.0 Standard: Stock solution: 1 g/L solution of phenol in methanol. Test solution: 0.05 mL stock solution in 5 mL mobile phase. Test substance: Stock solution: 1.128 g/L solution of test substance in methanol. Test solution: 0.6 mL stock solution and 1.4 mL phosphate buffer pH 2.0 in 5 mL mobile phase; final concentration test solution: 0.135 g/L.
3.6	**Test performance**	
3.6.1	Preliminary test	Not applicable
3.6.2	Screening test:	Not applicable

	Adsorption	
3.6.3	Screening test: Desorption	Not applicable
3.6.4	HPLC-method	Yes. HPLC: Alliance 2695 with UV detector 2487, Waters Column: Hypersil BDS-CN cyanopropyl mixed phase column, dp = 5 µm, Thermo Eluent: 30/70 v/v methanol/water (neutral pH); 30/70 v/v methanol/0.05 M phosphate buffer pH 2.0 (pH 2.0) Flow: 1 mL/min Injection volume: 10 µL Detection: 210 nm Data capture and calculations: Cary WinUV 3.1, Varian; Empower 5.00, Waters
3.6.5	Other test	

4 RESULTS

4.1	**Preliminary test**	see table A7_1 _3-2
4.2	**Screening test: Adsorption**	pKa of lactic acid (Perrin method) is 3.08. Therefore, HPLC test is done at neutral pH and at pH 2.0. R_t phenol is 2.98 minutes at neutral pH and 2.785 minutes at pH 2.0. R_t test substance is shorter than R_t phenol at neutral pH and at pH 2.0. K_{OC} test substance therefore < 20.9 (log K_{OC} < 1.32)
4.3	**Screening test: Desorption**	N.A.
	Calculations	
4.3.1	Ka , Kd	N.A.
4.3.2	Ka_{oc} , Kd_{oc}	N.A.
	Degradation product(s)	N.A.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	HPLC method is suitable for estimating the overall adsorption/desorption behaviour of organic acids, especially at low pH. Lactic acid is retained less by a cyanopropyl column than the reference substance phenol. Test is therefore valid.
5.2	**Results and discussion**	Lactic acid is retained less by a cyanopropyl column than the reference substance phenol. Peak shapes are not quite ideal but acceptable. Two major components are evident at R_t 1.21 minutes and 1.55 minutes at neutral pH; at pH 2.0 three major peaks are evident at R_t 2.08 minutes, R_t 2.20 minutes, and R_t 2.41 minutes. Individual peaks correspond to lactic acid and major oligomers. All peaks have shorter retention times than phenol. Log K_{OC} estimates are therefore all < 1.32.
5.2.1	Adsorbed a.s. [%]	N.A.
5.2.2	K_a	N.A.
5.2.3	K_d	N.A.
5.2.4	Ka_{oc}	N.A.
5.2.5	Ka/Kd	N.A.
5.2.6	Degradation products (% of a.s.)	N.A.
5.3	**Conclusion**	Results are valid within the constraints of the OECD 121 HPLC screening method for KOC. Applicability of OECD 121 may be subject to future validation investigations, but from the test results it appears that the test is relevant for lactic acid. Lactic acid is expected to be reasonably mobile in soil.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2009/05/18
Materials and Methods	A HPLC-screening test according to the OECD test guideline (TG) No. 121 is submitted. As the substance is expected to be ionized for at least 10% within pH 5.5 to 7.5, the pKa-value was calculated and the HPLC-analysis was performed with both the ionized (measured at neutral pH) and the non-ionized form (measured at pH=2). In the test report it is described, how a calibration graph has to be prepared, but it is not documented that a calibration graph is performed. Instead of using a calibration graph, the retention time of the a.s. is compared with the retention time of phenol, one of the reference substances of the method with a low log Koc (1.32 L/kg). Although this value alone is outside of the range for which the method is applicable (log Koc 1.5 to 5 L/kg, see OECD TG), this approach can be accepted under consideration of all circumstances.
Results and discussion	Under the chromatographic conditions of the method, the retention time of the active substance is lower than the retention time of the reference substance phenol with the known log Koc of1.32 L/kg. Therefore it was concluded, that the log Koc of lactic acid at neutral pH, as well as at pH=2 is < 1.32 L/kg (Koc<20.9 L/kg).
Conclusion	Applicant's version can be adopted with constraints.
Reliability	2
Acceptability	Considering the properties of lactic acid (e.g. high water solubility, low log Kow, biodegradability) as a naturally occurring substance with low risk potential, the test can be accepted. Formally a test according to OECD TG No. 106 would have to be required; but it is not expected, that such a requirement will lead to considerably other results. In a literature study, performed by RMS, Sansone, FJ et al. (Geochim Cosmochim Acta 51: 1889-96 (1987)) reported, for example, an experimental estimated K_{OC}-value on a clastic mud for 5.7 L/kg and of 0.08 L/kg for a lateralic muddy sand.
Remarks	Our conclusion to accept the test, due to the circumstances mentioned above, is shared by the Swedish CA in the frame of an inquiry to the electronic discussion group.
	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*

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

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_1 _3-1: Classification and physico-chemical properties of soils used as adsorbents

Not applicable

Table A7_1 _3-2: Results of preliminary test:

Test substance	Lactic acid 93%
Sample purity	99.8%
Weighed soil	N.A.
Volume of $CaCl_2$ solution	N.A.
Nominal concentration of a.s. final solution	1.744 g/L ; 1.128 g/L
Analytical concentration final of a.s. solution	N.A.
Concentration of the test solution (show calculation)	0.209 g/L ; 0.135 g/L
Details of the analytical method used:	N.A.; HPLC screening method
Method	
Recovery rate	
Detection limit	

Table A7_1 _3-3: Results of screening test - adsorption:

Not applicable

Table A7_1 _3-4: Results of screening test - desorption:

Not applicable

Section A7 Annex Point A7.2	Fate and behaviour in soil	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	According to the 'Technical Guidance Document on data requirements', subjects under 7.2 are additional data requirements. As lactic acid will be used in products that are to be used in-house only, information on the fate and behaviour in soil is not required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009/10/21	
Evaluation of applicant's justification	Applicant's justification is acceptable. Lactic acid is readily biodegradable (10-days window criterion not fulfilled/cannot be assessed). No further biodegradation studies are required.	
Conclusion	Applicant's justification is acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7 Annex Point A7.3	Fate and behaviour in air	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [x]** **Other justification [ ]**	
Detailed justification:	According to the 'Technical Guidance Document on data requirements', subjects under 7.3 are additional data requirements. As lactic acid will be used in products that are to be used in-house only, information on the fate and behaviour in air is not required. The atmospheric oxidation by hydroxyl radicals and ozone was calculated using the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN v. 4.01, in EPISUITE v 3.11). The estimation methods used by AOPWIN are based on the structure-activity relationship (SAR) methods developed by Atkinson. The calculated overall OH rate constant is 5.92 x 10^{-12} cm^3 mol^{-1} sec^{-1}. Assuming a 12-h day and an OH concentration of 1.5 x 106 cm-1 this gives a half-life of 1.8 days.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009/04/23	
Evaluation of applicant's justification	Applicant's justification is accepted.	
Conclusion	Applicant's justification can be adopted with minor restrictions regarding the applied input parameters for AOPWIN.	
Remarks	According to the TGD (Part II, Chapter 2.3.6.3) a 24 hours day in QSAR calculation of half life should be assumed. Considering the 24-h day and the corresponding OH–radical concentration of 5 x 10^5 molecules x cm^{-3}, the half life is 2.71 days.	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, Salmo gairdneri*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1984. Acute toxicity of SY-83 to rainbow trout (Salmo gairdneri). ABC Inc. Report nr. #32147 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**	Studies performed according to the "Methods of acute toxicity tests with fish, macroinvertebrates and amphibians" (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975, Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009) and "Standards methods for the examination of waste and wastewater" (American Public Health Organization, 1980, 15th ed.).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 (SY-83)	x
3.1.1	Lot/Batch number	Not available	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	100%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	Not analysed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Antimycin A	
3.3.1	Method of analysis for reference substance	Not reported	
3.4	**Testing procedure**		
3.4.1	Dilution water	Soft reconstituted water (for details see table A7_4_1_1-2)	
3.4.2	Test organisms	Rainbow trout (*Salmo gairdneri,* for details see table A7_4_1_1-3)	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, 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	x
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	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, Salmo gairdneri*

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 LC50 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 (*Salmo gairdneri*) 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/L0. 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/L0. 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.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, Salmo gairdneri*

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	*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_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	*Salmo gairdneri* (rainbow trout)
Source	Trout Lodge (McMillan, Washington)
Wild caught	No
Age/size	Mean weight: 1.09 (± 0.28) g Mean standard length: 42 (± 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 ± 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 fullfilled**
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.**	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, Lepomis macrochirus*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1984. Acute toxicity of SY-83 to bluegill sunfish (*Lepomis macrochirus*). ABC Inc. Report nr. #32146 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**	Studies performed according to the "Methods of acute toxicity tests with fish, macroinvertebrates and amphibians" (Committee on Methods for Toxicity Tests with Aquatic Organisms, 1975, Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009) and "Standards methods for the examination of waste and wastewater" (American Public Health Organization, 1980, 15th ed.).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 (SY-83)	x
3.1.1	Lot/Batch number	Not available	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	100%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	Not applicable	
3.1.6	Method of analysis	Not analysed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.3	**Reference substance**	Antimycin A	
3.3.1	Method of analysis for reference substance	Not reported	
3.4	**Testing procedure**		
3.4.1	Dilution water	Soft reconstituted water (for details see table A7_4_1_1-2)	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, Lepomis macrochirus*

3.4.2	Test organisms	Bluegill sunfish (*Lepomis macrochirus*, for details see table A7_4_1_1-3)	
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	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 56 to 560 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), 56, 100, 180, 320, and 560 mg/L	
4.2.2	Actual concentrations of test substance	Not measured	x
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.	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, Lepomis macrochirus*

4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects observed in control animals	
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 LC50 was 1.0×10^{-4} 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 (*Salmo gairdneri*) was assessed by exposing fish to 0, 56, 100, 180, 320, and 560 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}	180 mg/L	
5.3	**Conclusion**	The 96 h LC_{50} for SY-83 is 130 mg/L (100-180 mg/L0. The pH values were very low in the highest doses (3.1 at 560 mg/L at 0 h, 4.7 at 100 mg/L after 48 h), 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

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1 *96-h LC_{50}, Lepomis macrochirus*

Materials and Methods	Applicant's version is acceptable with the following remarks: To point 3.1 and 5.1: Test material: The test substance is not exactly specified. To point 5.1: There is a transcription error. It should be *Lepomis macrochirus* instead of *Salmo gairdneri* as stated by the applicant.
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	*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_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
Oxygen content	Dissolved oxygen 9.0 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	*Lepomis macrochirus* (bluegill sunfish)
Source	Osage Catfisheries (Osage beach, Missouri)
Wild caught	No
Age/size	Mean weight: 0.37 (± 0.15) g Mean standard length: 24 (± 2.3) 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	22 ± 1°C
Dissolved oxygen	9.0 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
56	10	10		10	0	0		0
100	10	10		9	0	0		10
180	1	0		0	90	100		100
320	0	0		0	100	100		100
560	0	0		0	100	100		100
Temperature [°C]		22		22				
pH								
0		7.0		6.9				
56		6.7		6.7				
100		4.9		4.9				
Oxygen [mg/l]		6.2-7.0		5.6-6.8				

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	**95 % c l.**	**96 h [mg/l][1]**	**95 % c.l.**
LC_0	100 (NOEC)		56 (NOEC)	
LC_{50}	130	100-180	130	100-180
LC_{100}	180		180	

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

	fulfilled	**Not fullfilled**
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.**	

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	**n.a.**	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

Official use only

1 REFERENCE

1.1	**Reference**	Hanstveit, A.O., Oldersma, H., 1992 Effect of L(+) lactic acid on the growth of the alga Selenastrum capricornutum (OECD 201). TNO, report nr. IMW-91-0076-05. 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 201
2.2	**GLP**	Yes
2.3	**Deviations**	The test was ended after 70,5 h instead of 72 h. Furthermore, no EC50 based on biomass was calculated, which was not common at the time the study was performed.

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	About 80% L(+) lactic acid
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	Not applicable
3.1.6	Method of analysis	Enzymic 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**	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

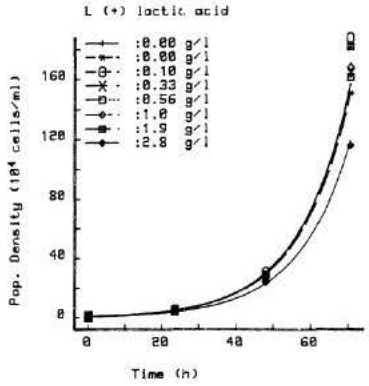
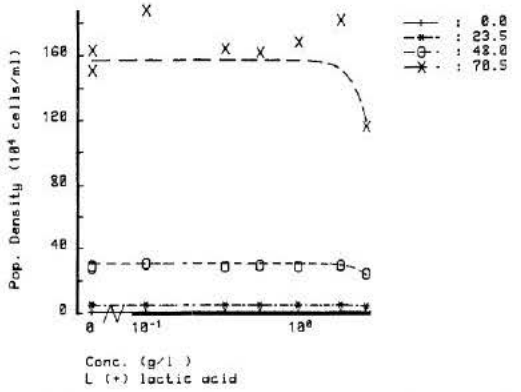
3.4.1	Culture medium	Medium was prepared from concentrated stock solutions in Milli-Q filtered water and sterilized by micropore filtration. $NaHCO_3$ content is 150 mg/L, $CaCl_2 \cdot 2H_2O$ content 18 mg/L, $MgCl_2 \cdot 6H_2O$ content 12 mg/L and $MgSO_4 \cdot 7H_2O$ content 15 mg/L.
3.4.2	Test organisms	For details see table A7_4_1_3-2.
3.4.3	Test system	For details see table A7_4_1_3-3
3.4.4	Test conditions	For details see table A7_4_1_3-4
3.4.5	Duration of the test	70.5 h
3.4.6	Test parameter	Growth inhibition
3.4.7	Sampling	One sample taken from each flask after 0, 23.5, 48 and 70.5 h.
3.4.8	Monitoring of TS concentration	Yes, at start and end of the test.
3.4.9	Statistics	EC values with respect to the area under the growth curve were calculated according to the method given in OECD 201. EC values with respect to the inoculum viability followed by exponential growth were calculated according to a parametric model developed by Kooijman et al., assuming an error proportional to the number of cells.

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			
4.2	**Results test substance**				
4.2.1	Initial concentrations of test substance	Nominal test substance	Nominal lactic acid	Measured lactic acid	
		0	0	<5	mg/L
		100	80	65	mg/L
		1000	800	620	mg/L
		2800	2240	1595	mg/L
4.2.2	Actual concentrations of test substance	Nominal test substance	Nominal lactic acid	Measured lactic acid	
		0	0	<5	mg/L
		100	80	12	mg/L
		1000	800	455	mg/L
		2800	2240	1645	mg/L

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

4.2.3	Growth curves	 ***Figure 1*** *Growth curves for Selenastrum capricornutum exposed to a range of concentrations of an 80% aqueous solution of L(+)lactic acid.*	
4.2.4	Concentration / response curve	 ***Figure 2*** *Concentration-effect curves for Selenastrum capricornutum exposed to a range of concentrations of an 80% aqueous solution of L(+)lactic acid.*	
4.2.5	Cell concentration data	See table A7_4_1_3-5	
4.2.6	Effect data (cell multiplication inhibition)	Effects on inoculum viability: E_eC_{50} >2.8 g/L (extrapolated E_eC_{50} 3.5 g/L) E_eC_{10} 2.3 g/L E_eC_{90} >2.8 g/L (extrapolated E_eC_{50} 5.4 g/L) Effects on area under the growth curve: E_bC_{50} >2.8 g/L E_bC_{10} 2.4 g/L E_bC_{90} >2.8 g/L Estimated NOEC: 1.9 g/L (by visual comparison of the measured and calculated growth curves of exposed and control algal suspensions)	x
4.2.7	Other observed effects	Microscopic examinations of the cells revealed the presence of many bacteria in the cultures exposed to 1.0, 1.9, and 2.8 g/L	
4.3	**Results of controls**	See table A7_4_1_3-5	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test performed according to OECD 201. A range-finding study was performed to determine the dose range in the study.	
5.2	**Results and discussion**	The range-finding test revealed that inhibiting effects could be expected at concentrations higher than 100 mg/L. Effects could be caused by the low pH level (pH 3.2 at 1008 mg/L). In the growth inhibition test, an effect on inoculum viability was observed. This effect may be expected when a test substance looses its toxicity during the test. The observed bacterial growth and the chemical analysis indicate degradation of the test substance during the test.	
5.2.1	NOE_rC	1.9 g/L (estimated by visual comparison of the measured and calculated growth curves of exposed and control algal suspensions)	x
5.2.2	E_eC_{50}	>2.8 g/L (extrapolated E_eC_{50} 3.5 g/L)	x
5.2.3	E_bC_{50}	>2.8 g/L	
5.3	**Conclusion**	Validity criteria not fulfilled, due to biodegradation of the test substance, the concentration was <80% during the test.	x
5.3.1	Reliability	1	
5.3.2	Deficiencies	The test was ended after 70,5 h instead of 72 h. Furthermore, no EC_{50} based on biomass was calculated, which was not common at the time the study was performed. Additionally, the concentrations tested were too low to derive a proper EC_{50}, however, from the range-finding test it was concluded that the observed toxicity of higher doses is probably caused by the low pH level.	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	2009/04/07
Materials and Methods	Applicant's version is acceptable

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3 *Selenastrum capricornutum*

Results and discussion	Applicants version can be adopted with the following remarks: To 4.26, 5.2.1- 5.2.3: The estimated EC_{50} value based on growth inhibition is higher than the highest test concentration. This value was obtained by extrapolation because no 50% inhibition was reached at the end of the test in the highest test concentrations. The re-calculation of EC_{50} values and NOEC value by RMS results in effect values in the same order of magnitude: E_rC_{50} = 5.39 g a.s./L (nominal) E_bC_{50} = 2.38 g a.s. /L (nominal) NOE_rC = 1.52 g a.s. /L (nominal) 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. Therefore a recalculation was conducted as follows: Because of the nominal E_rC_{50} (5.39 g a.s./L) is higher than the highest nominal test concentration (2.24 g a.s./L) for the calculation of the mean measured effect values the geometric mean from start to the end of the test from the highest test concentration of 2.24 g/L with 72.3 % will be used. **E_rC_{50} (70,5 h) = 3.90 g a.s./L** ***E_bC_{50} (70.5 h) = 1.72 g a.s. /L*** **NOE_rC (70,5 h) = 1.10 g a.s./L** 5.3.: As possible biodegradation or transformation from Lactic acid to Lactate could cause the decrease in test substance concentration the effect values were estimated based on the actual measured concentration. 5.3.2: In the test protocol it is stated that pH value was adjusted to pH 7.5-8 and that it remained constant during testing. In the presence of algae the pH value was found to increase just a little with algal cell density (pH 8.2-8.7).
Conclusion	Applicant's version can be adopted
Reliability	2, see remarks
Acceptability	acceptable
Remarks	Deficiencies: Instead of 3 proposed replicates according to the current OECD guideline 201 only 2 replicates were tested.

	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_3-2: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum*
Strain	ATCC 22662
Source	Culture was supplied by the "American Type Culture Collection", Rockville, Maryland, USA
Laboratory culture	Yes
Method of cultivation	Not reported
Pretreatment	According to OECD 201, a preculture of algae in the exponential growth phase was prepared in the medium used for the test.
Initial cell concentration	In the test flasks a mean inoculum cell density of 0.9 x 10^4 cells/mL was measured in the control cultures.

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	200 mL conical test flasks
Counting apparatus	Electronic particle counting with Coulter Counter model TAII
Light quality	Fluorescent lamps, light intensity within the standard range 120 ± 20% $\mu mol \cdot S^{-1} \cdot m^{-2}$
Procedure for suspending algae	Shaking (100 rpm in a Gallenkamp orbital shaker)
Number of vessels/ concentration	Duplicate, four controls with algae only and a single background series containing test substance without algae
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	23 ± 1°C
pH	Start of test: 7.5-8.0 End of test without algae: 8.0-8.1 End of test with algae: 8.2-8.7
Aeration of dilution water	No
Light intensity	120 ± 20% $\mu mol \cdot S^{-1} \cdot m^{-2}$
Photoperiod	Not reported

Table A7_4_1_3-5: Cell concentration data

Test-Substance **Concentration (nominal)** **[g/l]**	**Cell concentrations (mean values) [cells/ml]**							
	measured				**Percent of control**			
	0 h	**23.5 h**	**48 h**	**70.5 h**	**0 h**	**23.5 h**	**48 h**	**70.5 h**
0	0.9	5.3	28.5	157.0	100	100	100	100
0.10	0.9	5.1	30.3	188.0	100	96	106	120
0.33	1.0	5.0	28.6	164.3	111	94	100	105
0.56	0.9	4.9	29.4	161.8	100	92	103	103
1.0	1.0	5.1	28.7	168.4	111	96	102	107
1.9	1.0	5.0	29.2	181.9	111	94	102	116
2.8	0.9	4.1	24.0	116.0	100	77	84	74
Temperature [°C]	23 ± 1°C							
pH	7.5-8.0	8.2-8.7						

3. Tables for Applicant's Summary and Conclusion

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	**Not fullfilled**
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**x**	
Concentration of test substance ≥80% of initial concentration during test		**x**

Criteria for poorly soluble test substances	Not applicable	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

Official use only

		1 REFERENCE
1.1	**Reference**	L.M. Bouman, Activated sludge respiration inhibition test with PURAC HS 88, NOTOX project no. 483211, unpublished report, February 2007
1.2	**Data protection**	Yes
1.2.1	Data owner	Purac Biochem B.V. Arkelsedijk 46 4206 AC Gorinchem , The Netherlands
1.2.2		
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 Guidelines 209, ISO 8192
2.2	**GLP**	Yes
2.3	**Deviations**	Yes, a limited test with one concentration was carried out.
		3 MATERIALS AND METHODS
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	0602001247
3.1.2	Specification	As given in section 2.
3.1.3	Purity	88.2% activated substance in solution and 100% calculated on the dried basis.
3.1.4	Composition of Product	
3.1.5	Further relevant properties	
3.1.6	Method of analysis	Not relevant
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable
3.3	**Reference substance**	Yes: 3,5-Dichlorophenol
3.3.1	Method of analysis for reference substance	Not relevant
3.4	**Testing procedure**	
3.4.1	Culture medium	Synthetic sewage
3.4.2	Inoculum / test organism	Activated sludge from municipal sewage treatment plant that predominantly treats domestic sewage. See table A7_4_1_4-2.
3.4.3	Test system	see table A7_4_1_4-3.
3.4.4	Test conditions	see table A7_4_1_4-4

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

3.4.5	Duration of the test	3h	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen measurement	
3.4.8	Sampling	The oxygen concentration was measured continuously for 10 minutes.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	One control without test substance was tested at the start and one at the end of each test series (test substance and reference test), four activity control flasks with reference substance.	
3.4.11	Statistics	% inhibition= $(1 - \frac{2 * Rt}{Rc(start\ test\ series) + Rc\ (end\ series)}) * 100\%$ Rc = respiration rate of the control Rt= respiration rate of the test/ reference substance (mg O_2/l/hr)	
		4 RESULTS	
4.1	**Preliminary test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Effect data	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	100 mg/l (duplicate test flasks)	x
4.2.2	Actual concentrations of test substance	Not determined.	
4.2.3	Growth curves	Not determined	
4.2.4	Cell concentration data	Only given as 4.3 g/l MLSS	
4.2.5	Concentration/ response curve	Not relevant	
4.2.6	Effect data	EC50>100 mg/l. No toxic effects were found at the tested concentrations.	x
4.2.7	Other observed effects	None observed	
4.3	**Results of controls**	Oxygen consumption in blank control: 44 mg O_2/l/hr	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	1.0, 3.2, 10, 32	
4.4.2	Results	EC50=5.9 mg/l	x

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test performed according to OECD 209, with as deviation that a limit test with one concentration was carried out.	
5.2	**Results and discussion**	No inhibition of the respiration rate was observed for the test substance. The EC50 for the reference substance was 5.9 mg/l, which is within the accepted range. The variation within the controls was acceptable (<15%).	x
5.2.1	EC_{20}		
5.2.2	EC_{50}	>100 mg/l	
5.2.3	EC_{80}		
5.3	**Conclusion**	The test was valid, because the reference test resulted in an acceptable EC50 and the blank controls showed limited variation (<15%)	
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	2009/04/29
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is adopted with the following amendments: 4.2.1: A limit test was carried out with only one test substance concentration. 4.2.6: Test substance; Effect data: EC_{50} > 100 mg/L 4.4.2: Reference substance; Results: EC_{50} = 5.9 mg/L. This result is within the range of 5-30 mg/L, therefore validity criterion is fulfilled. 5.2: The difference between the respiration rates of the blanks is 5%. The value is less than 15 %, therefore validity criterion is fulfilled.
Conclusion	The applicant's version is adopted.
Reliability	1
Acceptability	Acceptable
Remarks	Deviation from Guideline OECD 209 – a limit test with one concentration was carried out.
	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*

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

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_4-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Unknown
Strain	Unknown
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	Waterschap de Maaskant, 's Hertogenbosch, The Netherlands
Laboratory culture	No
Method of cultivation	Not relevant
Preparation of inoculum for exposure	The sludge was coarsely sieved, washed and diluted with ISO medium. Total suspended solids content was set at 4.3 $g.l^{-1}$, pH 7.5. Sludge was kept aerated at test temperature until use.
Pretreatment	Before use 50 ml synthetic sewage was added to each litre of sludge at the end of the collection day.
Initial cell concentration	Cell concentration only given as 4.3 g/l mixed liquor suspended solids.

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	1 litre glass test bottles for the incubation and glass 300 ml oxygen bottles for the oxygen measurements.
Number of culture flasks/concentration	Two test flasks for the test substance, 1 litre flask per concentration for the reference substance, four (2x2) blank control flasks.
Aeration device	Pipette
Measuring equipment	O_2-electrode (WTW inolab Oxi 730 & WTW Cellox 325 oxygen electrode)
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	**Details**
Test temperature	18.0-18.6 (measured continuously)
pH	7.9
Aeration of dilution water	No
Suspended solids concentration	4.3 g/l

Section A7.4.2 Annex Point A7.4.2	Bioconcentration	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [x]**	
Detailed justification:	According to the Technical Guidance Document on data requirements, the intrinsic potential for bioconcentration in aquatic organisms may be based on physical and chemical parameters. An EPIWIN calculation was performed to estimate the BCF, based on the octanol-water partition coefficient (EPIsuite v3.12, EPA, 2000). The outcome is reproduced below. BCF Program (v2.15) Results: ========== SMILES : O=C(O)C(O)C CHEM : Propanoic acid, 2-hydroxy-, (S)- MOL FOR: C3 H6 O3 MOL WT : 90.08 ---------- Bcfwin v2.15 ------- --------- Log Kow (estimated) : -0.65 Log Kow (experimental): -0.72 Log Kow used by BCF estimates: -0.72 (user entered) Equation Used to Make BCF estimate: Log BCF = 0.50 (Ionic; Log Kow dependent) Estimated Log BCF = 0.500 (BCF = 3.162)	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009-04-07	

Section A7.4.2	**Bioconcentration**
Annex Point A7.4.2	
Evaluation of applicant's justification	Applicant's justification is acceptable with the following comment: The BCF for fish (Formula 74) and BCF for earthworm (Formual 82d) were also calculated according to Technical Guidance Document on Risk Assessment (TGD) by using the experimental log K_{ow} of -0.74 for lactic acid: Log BCF_{fish} = (0.85 * log K_{ow}) -0.7 **BCF_{fish} = 0.049** Log $BCF_{earthworm}$ = (0.84+0.012 * K_{ow})/$RHO_{earthworm}$ **$BCF_{earthworm}$ = 6.78** For risk assessment the values calculated by RMS were used.
Conclusion	Applicant's justification is acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE (specify)
Date	Give date of comments submitted
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Remarks	

Section A7 **Annex Point IIA7.4.3**	**Effects on aquatic organisms, further studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	According to the Technical Notes for Guidance on data requirements, this is an additional data requirement. As the substance is to be used in-house only, no exposure to the aquatic environment is expected, and no data are required.	x
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009/04/07	
Evaluation of applicant's justification	Applicant's justification is acceptable	
Conclusion	Applicant's justification is acceptable with the following comment: No direct exposure of the aquatic environment is expected. Exposure via STP is possible. In addition to this further testing on aquatic species for which no short-term toxicity has been demonstrated (i.e $L(E)C_{50}$ > 100 mg/L) would not be required. (TNsG on data requirements for active substances and biocidal products, chapter 3, part A, point 7.4).	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A7 **Annex Point IIA7.5**	**Effects on terrestrial organisms**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [x]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	According to the Technical Notes for Guidance on data requirements, this is an additional data requirement. As the substance is to be used in-house only, no exposure to terrestrial organisms is expected, and no data are required.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	2009/04/07	
Evaluation of applicant's justification	Applicant's justification is acceptable	
Conclusion	Applicant's justification is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		